

PL01

Enhancing student experience and graduate outcomes through inclusive physiology

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Across the United Kingdom, many level two and three learners are from regions with high social deprivation and poor progression to higher education. It is widely acknowledged that outreach activities can address this as they seek to ensure secondary school students do not make choices that limit access to university thence science-based careers simply because they do not know they exist. At The University of Salford, related initiatives such as the “Salford Schools Network” now mean 40 % of students are from low-income backgrounds and a significant proportion are from areas with the lowest progression to higher education *nationally* [1].

However, facilitating progression to higher education is only the first step towards improving inclusivity and graduate outcomes for all. The career achievement gap experienced by university students from disadvantaged or underrepresented backgrounds is well known and a barrier to employability or progression to PhD. Multiple and complex socioeconomic factors underpin this. Yet, it remains the case that while at university many disadvantaged students are - *for the first time* - made aware of thus develop ambitions to enter physiology-based research and related careers. However, this late realisation means they are often behind the curve in terms of engagement with extracurricular activities that broaden horizons and enhance the CV. In many cases this arises from a lack of opportunity rather than disengagement which is especially true if students come from communities with limited awareness of academia and related careers, or they are the first in their family to go to university. Despite being every bit as *academically* capable as students from more advantaged backgrounds, they do not have the same level of experience and insight. This frequently means they are less competitive at interview.

In my lecture I will cover the various extra-curricular initiatives I have put in place to level up disadvantaged students at The University of Salford. Examples include development of a career hub, launch of Salford's Research Career Working Group, facilitation of undergraduate engagement with research and The Physiological Society and the introduction of international mobility opportunities. Fundamentally, all seek to enhance widening participation in physiology-based and wider research thus provide the experience and insight that is often lacking but essential for progression to related careers.

I also hope to demonstrate that these initiatives are transformative. Resulting impact is not metric-based but tangible; the day a disadvantaged student holds their own at a scientific meeting or is offered a PhD position, a place at medical school or achieves any other career ambition. The added value is considerable, especially given that three years prior, most were completely unaware such career pathways existed.

[1]. Namvar S et al. (2019). Employability: breaking the mould, 81-87

PL02

Biophysical and molecular mechanisms of voltage-gated sodium channel gating: A quarter-century of resurgent sodium current

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Electrical signaling in most nervous systems depends upon sodium current, which flows through voltage-gated sodium channels. From their first voltage clamp measurements, Hodgkin and Huxley (1952) recognized the “dual effect” of voltage on the sodium conductance: In modern terms, depolarization first activates and then inactivates voltage-gated sodium channels, such that current flows only briefly at positive potentials and requires a recovery period at negative potentials before it can flow again upon subsequent depolarization. Both the voltage-dependence and time course of recovery from inactivation set the refractory period for action potential firing. Although tetrodotoxin-sensitive voltage-gated sodium currents show little heterogeneity across neurons, about 25 years ago, we found that cerebellar Purkinje neurons show a qualitatively distinct form of sodium channel gating. There, voltage-gated channels open briefly upon depolarization, permitting transient sodium current to flow, but the same channels reopen to pass a “resurgent” sodium current upon repolarization, indicative of a less stable form of inactivation. This component of sodium current is present in many other neurons typified by rapid or burst firing of action potentials. Changes in resurgent sodium current have been predicted to occur in disorders of excitability, e.g., in association with paroxysmal extreme pain disorder, epilepsy, paramyotonia congenita, long-QT syndrome, and neuropathy. A primary question relevant to the understanding of sodium channel gating, as well as the action potentials that result, is what the mechanisms of resurgent current are, both biophysically and molecularly. In early work, we proposed that sodium channels that generate resurgent current are subject to a rapid, voltage-dependent, open-channel block by an endogenous blocking particle. With depolarization, channels would open and, instead of inactivating in the usual manner, would rapidly become blocked by the native blocker. With repolarization, the blocker would unbind, briefly leaving the pore open to pass resurgent current before channels inactivated normally (at moderately negative potentials) or deactivated (at more negative potentials). Since the time that this mechanism was proposed, many electrophysiological as well as structural results have emerged, which have not only rendered this hypothesis more precise, but also linked it more clearly to research that preceded it. In this talk, I will place the idea of open-channel block as a mechanism for resurgent sodium current in the context of earlier and later studies of ion channel biology and discuss the implications for neural signaling, pathophysiology and drug targeting.

PL03

Kings and Queens of the mountains: human physiology at high altitude

Andrew Murray

undefined

As we ascend to high altitude, air pressure falls and our bodies experience low oxygen availability - a condition known as hypoxia. In response, our heart rate and breathing rate increase - an attempt to maintain the supply of oxygen to our vital organs. Over time, levels of oxygen-carrying red cells increase in our blood. Meanwhile, the cells of our bodies, and the oxygen-consuming mitochondria within, re-wire their metabolism. This serves to decrease our bodies' demand for oxygen and improve the efficiency at which we use this increasingly scarce but vital resource. Despite this process of acclimatisation, we remain limited by the low oxygen available to us, and this impacts our capacity to function, limiting our ability to exercise and think. Pregnancy at altitude poses a particular challenge, restricting growth of the developing fetus and potentially endangering the health of both mother and her offspring. In human populations that have spent thousands of years at altitude, including groups resident in the Himalayas and the Andes, there has been a selection of physiological traits, underpinned by genetic differences, which enable people to live, work and successfully reproduce. In this lecture, we will look at the responses of our bodies to altitude, and consider the different evolutionary strategies adopted by high altitude dwelling people. We will look at adaptations that support pregnancy at high altitude and will see how research into physiology at altitude is helping us to understand the condition of patients at sea level who experience hypoxia in common, but life-threatening contexts, such as complications of pregnancy or critical illness.

PL04

“The Place of Physiology in the Neuroscience of Memory”

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Historically, several biomedical disciplines have played a part in developing our understanding of memory, with physiology playing a key role. Best known is the discovery of long-term potentiation (LTP) in the hippocampus by Terje Lømo in Norway (Lømo, *Acta Physiologica Scand.* 1966) and the first full report of the phenomenon by Bliss and Lømo (*Journal of Physiology* 1973). From the outset, LTP was found to have properties desirable of a memory mechanism – that it long outlasts the duration of the initiating stimulus, is pathway specific, and apparently associative in character. Another key discovery was that of place cells in the hippocampus by O'Keefe and Dostrovsky (*Brain Research* 1971), a finding followed in the years afterwards by those of other spatially tuned cells such as head-direction units (Taube et al, *J. Neuroscience* 1990) and grid cells (Hafting et al, *Nature* 2005). Collectively, these helped build the idea of the hippocampus being key to spatial memory. An entirely different learning system, based in the striatum, is instrumental in the learning of actions and habits as established in physiological, human functional imaging data and computational models; it deploys an error-correcting learning rule (Schultz et al, *J Neuroscience* 1992; Montague et al, *J Neuroscience* 1996).

It would, however, be wrong to suppose that the significance of these findings rests solely on physiological data. Neuroanatomy has also played a key role, dating back to Cajal's Croonian Lecture to the Royal Society in 1894; likewise neuropsychology, as in Hebb's conjectures about cell-assemblies in the brain and his proposal for a simple synaptic learning rule, as exemplified by LTP (Hebb, *The Organisation of Behavior*, 1949); pharmacology also weighed in with the discovery that glutamate is the major excitatory transmitter of the brain and that selective glutamate antagonists such as D-AP5 blocks the induction of LTP without affecting baseline glutamatergic transmission (Collingridge et al, *Journal of Physiology*, 1983). Behavioral studies have also contributed by rigorously testing the idea that activity-dependent synaptic transmission is necessary for the formation of episodic and spatial memory traces (Morris et al, *Nature*, 1986).

Contemporary studies using several of the remarkable technological innovations of recent years (e.g. optogenetics, calcium imaging in awake animals) are building on these foundations in intriguing ways. This lecture, with its requested historical backbone, aims to outline progress over the years and the challenges that remain in understanding memory as a fundamental feature of higher cognitive function.

PL05

Store-operated calcium channels: from nano domains to in vivo pathophysiology

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Communication between and within cells is essential for the development and survival of any complex organism. Cells converse with each other through the judicious use of a complement of chemical messengers, including neurotransmitters, hormones, and paracrine factors. These molecules bombard the cell surface, generating further signals, or second messengers, within the cell that then trigger the appropriate responses. Although several hundred hormones, neurotransmitters, and other molecules can stimulate cells, the number of intracellular second messengers they activate is remarkably small. Perhaps the most widespread and versatile of these second messengers is the calcium ion (Ca^{2+}).

Store-operated Ca^{2+} channels are a universal way to raise cytosolic Ca^{2+} in eukaryotic cells. These channels are particularly important in electrically non-excitable cells and are indispensable for immune cell function.

Growing evidence shows that store-operated channels engage in private conversations with downstream targets, through the use of spatially restricted Ca^{2+} signals, called Ca^{2+} nanodomains, which build up rapidly near open channels. Scaffolding proteins juxtapose with store-operated channels and position Ca^{2+} -dependent signalling molecules within the nanodomain, forming a signalosome. One such signalosome, involving AKAP79, allows for local Ca^{2+} signals to activate transcription factors of the NFAT family which then regulate gene expression. In this talk, I will describe properties of store-operated channels, how they participate in a membrane-delimited signalling complex to activate nuclear gene expression and how targeting the signalosome might open up new approaches for treating human disease.

PL06

Neuropeptide-Y: being “unsympathetic” to the broken hearted

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William Bayliss and Ernest Starling are famous as pioneers in cardiovascular physiology but are also responsible the discovery of the first hormone (from the Greek “setting in motion”), the intestinal signalling molecule and neuropeptide secretin in 1902¹. My research group focuses on neuropeptides and neuromodulators that influence cardiovascular autonomic control as potential biomarkers in disease and tractable targets for therapeutic intervention. Acute myocardial infarction (AMI) and chronic systolic heart failure (CHF) result in high levels of cardiac sympathetic stimulation, which is a poor prognostic indicator. Whilst beta-blockers improve mortality in these conditions by preventing the action of the neurotransmitter noradrenaline, a substantial residual risk remains. Recently, we have identified the sympathetic co-transmitter neuropeptide-Y (NPY) as being released during AMI, leading to larger infarcts² and life-threatening arrhythmia³ in both animal models and patients. Moreover, in patients with severe CHF, local cardiac NPY levels correlate with mortality⁴. I will present recently published and unpublished data demonstrating that peripheral venous NPY levels are associated with heart failure hospitalisation and mortality after AMI⁵, and all cause and cardiovascular mortality in CHF, even when adjusting for known risk factors (including BNP). We have investigated NPY expression in human and rat stellate ganglion and cardiac tissue and used human induced pluripotent stem cell (hiPSC) cardiomyocytes to manipulate NPY neurochemistry using state-of-the-art imaging techniques, establishing the receptor pathways responsible for NPY signalling. We propose NPY as a new mechanistic biomarker in AMI and CHF patients and aim to determine whether specific NPY receptor blockers can attenuate the development of heart failure.

References: 1. Bayliss WM, Starling EH. (1902) The mechanism of pancreatic secretion. *J Physiol* 28:325-53. 2. Herring N, et al (2019) Neuropeptide-Y causes coronary microvascular constriction and is associated with reduced ejection fraction following ST-elevation myocardial infarction. *European Heart Journal* 40(24):1920-9 3. Kalla M, et al (2020) The cardiac sympathetic co-transmitter neuropeptide-Y is pro-arrhythmic following ST-elevation myocardial infarction despite beta-blockade *European Heart Journal* 41(23):2168-79 4. Ajijola OA, et al (2020) Coronary sinus neuropeptide-Y levels predict adverse outcomes in patients with stable chronic heart failure. *JAMA Cardiology* 5(3):318-325 5. Gibbs T, et al (2022) Neuropeptide-Y Levels in ST-Elevation Myocardial Infarction: Relationship with Coronary Microvascular Function, Heart Failure and Mortality. *JAHA* 11:e024850

SA01

A day-night rhythm in the heart including the sinoatrial node: an intrinsic mechanism and neurohumoral regulation

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In mammals, there is well known to be a day-night rhythm in the electrical activity of the heart (in heart rate, PR interval, QRS duration and QT interval) and arrhythmogenesis¹. This has previously been attributed to short-term regulation of ionic conductances in the heart by the autonomic nervous system, but this explanation has been challenged¹. Instead, recent studies of the mouse have provided an alternative explanation.

In the sinoatrial node, we have shown that there is an intrinsic mechanism that can explain or at least contribute to the day-night rhythm in heart rate: there is a day-night rhythm in ion channels, including the pacemaker channel HCN4, and block of HCN4 abolishes the day-night rhythm in heart rate *in vivo* and *in vitro*². We have shown that there is a functioning circadian clock in the sinoatrial node that could be driving the day-night rhythm in ion channel gene transcription: in the case of HCN4 at least, knockout of the clock gene *Bmal1* abolishes the day-night rhythm in *Hcn4*². However, data are emerging for a system of transcriptional 'combinatorial regulation' in which a specific combination of transcription factors is obligatory for gene transcription: there is a day-night rhythm in the sympathetic nervous system and chronic β -adrenergic receptor blockade also abolishes the day-night rhythm in ion channel transcripts (including *Hcn4*)³. For the atrioventricular node, a similar picture is emerging: there is day-night rhythm in ion channel transcripts and a functional circadian clock⁴ and genetic knockout of *Bmal1* blunts the day-night rhythm in the PR interval (unpublished data). Again the picture is similar for the ventricles with a day-night rhythm in ion channel transcripts and a functional circadian clock³; the day-night rhythm in ion channel transcripts is suggested to be responsible for the well-known vulnerability to ventricular tachyarrhythmias at the start of the awake period. Once again there is evidence of combinatorial regulation: genetic knockout of *Bmal1* abolishes the vulnerability to ventricular tachyarrhythmias at the start of the awake period⁵; chronic β -adrenergic receptor blockade abolishes the day-night rhythm in ion channel transcripts³; and RU486 (an antagonist to the glucocorticoid receptor, Nr3c1; of interest because there is a day-night rhythm in plasma corticosteroid) again abolishes the day-night rhythm in ion channel transcripts as well as the vulnerability to ventricular tachyarrhythmias at the start of the awake period (unpublished data). ATAC-seq has shown a day-night rhythm in accessibility to certain genes (chromatin has to be made accessible for transcription to take place) and in many cases of genes showing a day-night rhythm in accessibility there is a consensus binding site for Nr3c1 (a transcription factor as well as a receptor) (unpublished data). Genetic knockout of Nr3c1 also abolishes the

vulnerability to ventricular tachyarrhythmias at the start of the awake period (unpublished data). In summary, a new explanation of the day-night rhythm in the heart is beginning to emerge involving a summation of inputs from an intrinsic cardiac circadian clock and neurohumoral factors.

1. Black N et al. (2019). *Heart Rhythm* 16, 298-307. 2. D'Souza A et al. (2021). *Heart Rhythm* 18, 801-810. 3. Anderson C et al. (2022). *Philosophical Transactions of the Royal Society B* (in press). 4. Boyett MR et al. (2021). *Progress in Biophysics and Molecular Biology* 166, 61-85. 5. Hayter EA et al. (2021). *Nature Communications* 12, 2472.

SA02

Inflammatory Remarks: Targeting pro-inflammatory Galectin-3 prevents cardiac conduction system dysfunction in heart failure

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Background: In patients with heart failure (HF), concomitant cardiac conduction system (CCS) dysfunction is an important predictor of mortality. Despite this, the molecular mechanisms underlying HF-induced CCS disease are poorly understood. Inflammation is a hallmark and mechanistic proponent of ventricular remodelling in chronic HF but its involvement in CCS dysfunction is presently unknown.

Methods and Results: We assessed the global signature of HF-induced molecular remodelling of the sinoatrial (SAN) and atrioventricular node (AVN) in the mouse transverse aortic constriction model of HF. Transcriptomic analysis using RNAseq and mass spectrometry-based proteomics combined with single nucleus RNAseq data intersection determined that downregulated proteins were predominantly enriched for ion channels involved in pacemaking, whereas upregulated proteins annotated to the immune-inflammatory response. In particular, striking enrichment of the macrophage population was observed in the failing CCS alongside a significant increase in expression of the macrophage-secreted proinflammatory protein Galectin-3 (Gal-3), a biotarget and biomarker in human HF.

To investigate a functional role for Gal-3 in HF-induced CCS remodelling, sham-operated and HF animals were randomised into anti-Gal-3 treated and untreated groups. Animals in the anti-Gal-3 treated group received 100 mg/kg/day modified citrus pectin (MCP), a well-characterised and clinically utilized Gal-3 inhibitor, starting from the day of surgery and continuing for 8 weeks. At termination, the impact of Gal-3 inhibition on CCS electrophysiological parameters was tested *in vivo* and in Langendorff-perfused hearts: MCP treatment significantly blunted prolongation of sinus cycle length, corrected SAN recovery time and the rate-corrected PR interval seen in untreated HF animals, whereas the Wenckebach cycle length and AVN effective refractory period were unaffected. To further evaluate SAN remodelling, high resolution unipolar multielectrode array mapping was carried out on the endocardial surface of isolated SAN preparations from the four groups of animals. Analysis of activation maps demonstrated that HF SAN had an inferior leading pacemaker site as well as slower conduction than control animals, changes that were restored to control levels in the MCP treated TAC group. Unipolar fractionated electrograms - indicative of structural and electrical remodelling resulting in asynchronous activation of myocytes - were significantly more prevalent in untreated HF animals, and the incidence of complex fractionated electrograms were also restored to control levels in the HF group receiving MCP treatment. Finally, using sharp microelectrodes, intracellular action potentials were recorded from the compact AVN. Strikingly, MCP treatment abrogated the HF-induced reduction in the resting membrane potential, upstroke velocity, action potential amplitude and slope of diastolic depolarisation.

Conclusions: These data provide novel proof-of-concept that Gal-3 inhibition prevents CCS dysfunction in HF of a pressure overload pathophysiology. Studies incorporating precision transgenics to study the impact of CCS-specific inflammation, coupled with state-of-the-art imaging mass cytometry to characterise the precise Gal-3 secreting macrophage population infiltrating the failing human and mouse CCS are underway.

SA03

The switch from nonfiring to firing mode in cells of the sinoatrial node.

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The sinoatrial node in the heart is composed of pacemaker cells which generate the heartbeat. Individual pacemaker cells exhibit substantial heterogeneity in their electrophysiological properties. Recently, we discovered that sinoatrial node cells can switch abruptly between a firing mode, in which they regularly fire action potentials, and a nonfiring mode, in which they stop firing for a certain period of time. Within the sinoatrial node network, firing and nonfiring cells interact electrically via gap junctions. Nonfiring cells slow action potential frequency in cells with intrinsic automaticity and, conversely, firing cells recruit nonfiring cells to fire. This mechanism is termed tonic entrainment and is important for the ability of the leading pacemaker region to generate regular electrical discharges that control electrical activation of the entire heart. Most importantly, this mechanism can be tuned by the autonomic nervous system. We show that the proportion of firing cells can be increased by the sympathetic nervous system via cAMP-dependent regulation of the pacemaker ion channel HCN4, thereby stabilizing sinus node function. Lack of cAMP regulation of HCN4 in a genetic mouse model results in inappropriately increased SAN heart rate responses to vagal nerve activity in vivo, sinus bradycardia, dysrhythmia and chronotropic incompetence.

SA04

Transcriptomic responses to disuse muscle atrophy and exercise-induced muscle hypertrophy

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Background: Skeletal muscle atrophy is a prominent characteristic of many disease states, however, the extent of similarities and/or differences in the underpinning mechanisms between atrophying conditions is unclear. Two of the most prevalent and costly atrophic conditions are ageing and disuse, with resistance exercise training (RET) the most effective nonpharmacological countermeasure. We conducted gene-level and network-level meta-analyses to compare transcriptomic signatures of disuse and RET, plus young and older RET to establish the molecular features of, and therapeutic targets against, muscle atrophy in conditions of high socio-economic relevance.

Methods: Integrated gene- and network-level meta-analysis was performed on publicly available microarray data sets generated from young (18–35 years) *m. vastus lateralis* muscle subjected to disuse (unilateral limb immobilization or bed rest) lasting ≥ 7 days or RET lasting ≥ 3 weeks, and from older (≥ 60 years) *m. vastus lateralis* muscle subjected to RET (≥ 3 weeks).

Results: Disuse and RET displayed predominantly distinct transcriptional responses, and transcripts altered across conditions were mostly unidirectional. However, disuse and RET induced directly inverted expression profiles for mitochondrial function and translation regulation genes, with COX4I1, ENDOG, GOT2, MRPL12, and NDUFV2, the central hub components of altered mitochondrial networks, and ZMYND11, a hub gene of altered translation regulation. A substantial number of genes ($n=140$) up-regulated post-RET in younger muscle were not similarly up-regulated in older muscle, with young muscle displaying a more pronounced extracellular matrix (ECM) and immune/inflammatory gene expression response. Both young and older muscle exhibited similar RET-induced ubiquitination/RNA processing gene signatures with associated PWP1, PSMB1, and RAF1 hub genes.

Conclusions: Transcriptional signatures of disuse are not simply the converse of RET, with limited opposing gene profiles. Therefore, the mechanisms of atrophy cannot be derived from studying hypertrophy alone. Moreover, this provides a molecular basis for understanding why RET fails to target all transcriptional features of disuse. Loss of RET-induced ECM mechanotransduction and inflammatory profiles might also contribute to suboptimal ageing muscle adaptations to RET. Disuse and age-dependent molecular candidates further establish a framework for understanding and treating disuse/ageing atrophy.

SA06

Boosting nitric oxide bioavailability as a strategy for enhancing neurovascular coupling and preventing cognitive dysfunction

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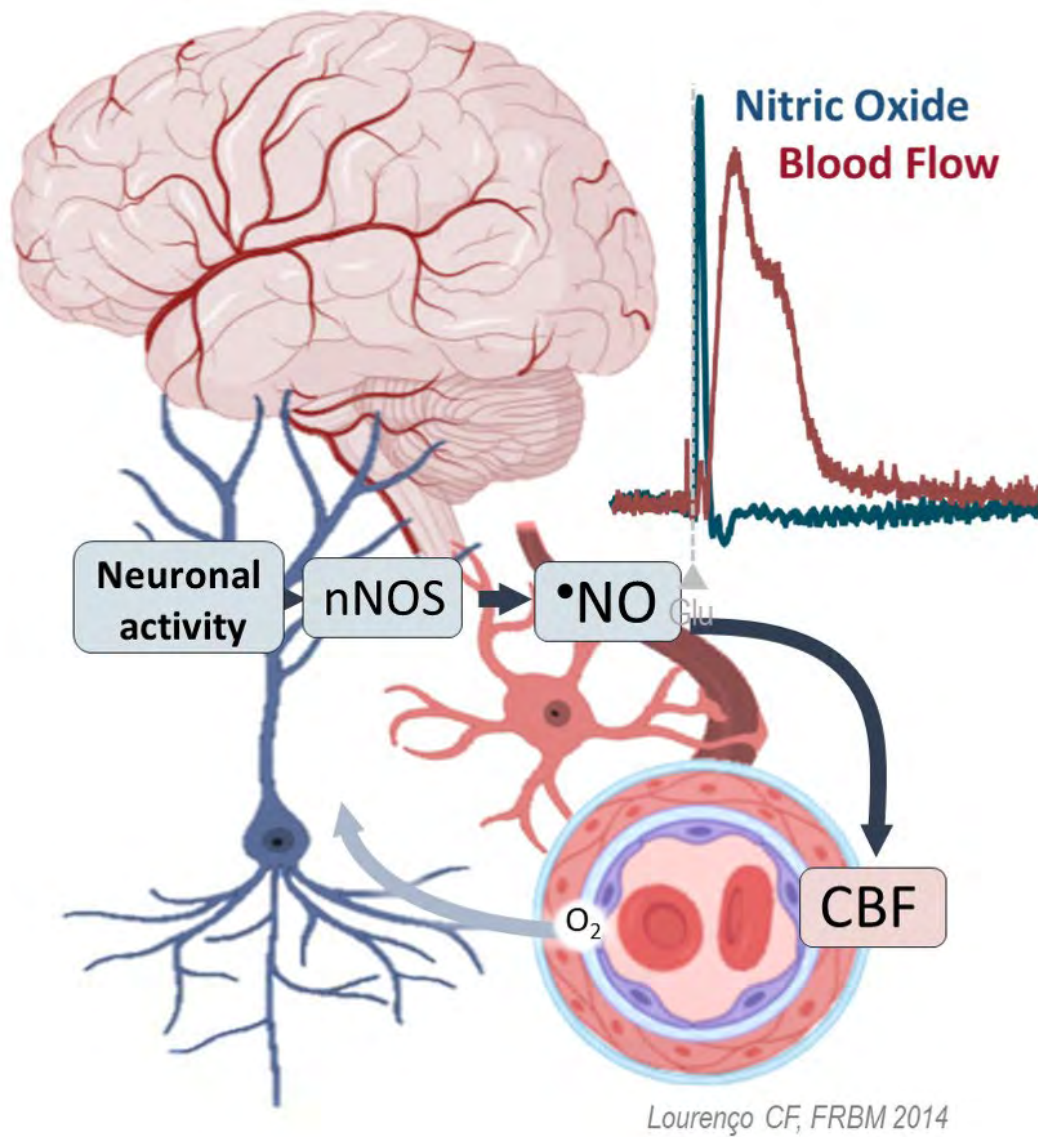
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The brain's function and structural integrity rely on a tightly regulated delivery of metabolic substrates (glucose and oxygen) matching the ongoing neuronal activity. This process – neurovascular coupling (NVC) – is critically orchestrated by nitric oxide (•NO) via the glutamate NMDAr-nNOS-sGC pathway, especially in the hippocampus, a brain region involved in memory processing (Lourenço et al., 2014, Figure 1). The dysfunction of NVC, linked to compromised •NO bioavailability and bioactivity, has been increasingly associated with neuronal dysfunction in several neurodegenerative conditions, such as Alzheimer's Disease and Vascular Cognitive Impairment and Dementia (VCID), being recognized as a relevant contributor to dysfunctional cascade leading to neurodegeneration and cognitive decline. In addition to the canonical enzymatic pathways, •NO can be produced upon the sequential reduction *in vivo* along the nitrate-nitrite-NO pathway. In this line, we hypothesized that dietary nitrate can be used as a strategy to foster •NO-dependent NVC under conditions of limited •NO bioavailability.

We tested our hypothesis in two rodent models mimicking specific features of VCID: 1) 2VO rats modeling cerebral hypoperfusion and 2) diabetic Goto-Kakizaki (GK) rats modeling microvascular dysfunction. Dietary nitrate intervention was achieved by providing sodium nitrate in water *ad libitum* for 8-12 weeks. The NVC functionality was accessed by measuring hemodynamic responses to glutamatergic activation in the hippocampus *in vivo* by laser Doppler flowmetry simultaneously with coupled •NO dynamics by electrochemical methods. The spatial working and reference memory dependent on hippocampal function was assessed in the Barnes maze paradigm. NADPH oxidase-mediated superoxide formation was detected by a lucigenin-dependent chemiluminescence assay. All the procedures were performed in compliance with the ethical regulations for animal-based research.

We found a compromised NVC in response to glutamatergic activation in both animal models (CBF changes were reduced to 41±3% in 2VO and 33±4% in GK as compared to their controls), which in GK rats were coupled with •NO transients with a shorter and faster profile. Of notice, in both GK and 2VO rats, these findings were coupled to a compromised spatial memory performance. As hypothesized, the intervention with dietary nitrate was able to counteract the spatial memory decline in both animal models which was correlated with an improvement in the NVC. Also, dietary nitrate reduced the NADPH activity in the hippocampus of both 2VO and GK rats.

Overall data support a close mechanistic association between hippocampal neuronal-triggered •NO concentration dynamics, hemodynamic responses, and cognitive performance, establishing the functionality of NVC as a critical early factor to consider in the cascade of events leading to cognitive decline in VCID that can be improved by dietary nitrate intervention.



Neurovascular coupling in hippocampus is mediated via diffusion by neuronal-derived nitric oxide. Lourenço CF, Santos RM, Barbosa RM, Cadenas E, Radi R, Laranjinha J. Free Radic Biol Med. 2014 Aug;73:421-9

SA07

Sympathetic neural responses and adaptation to the challenge of exercise, and to high altitude stress

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Sympathetic nerves play a pivotal role in control of systemic vascular resistance and autonomic regulation of arterial blood pressure. Furthermore, microneurography, the technique for recording neural activity directly from peripheral nerves innervating skeletal muscle vessels, is a fundamental tool for studying sympathetic modulation of vascular resistance and blood pressure during a variety of physiological and environmental challenges. During a bout of vigorous exercise, increased muscle sympathetic nerve activity (MSNA) and ensuing vasoconstriction in contracting muscle avert a drop in arterial blood pressure and vital perfusion when metabolic vasodilation would otherwise threaten to outstrip the pumping capacity of the heart. Notably, increased sympathetic outflow to skeletal muscle vasculature during exercise arises from integration of multiple neural inputs to the lower brainstem, including central command, afferent feedback from contracting muscle, and the arterial baroreceptors. In healthy individuals, it is apparent that exercise training can lead to heightened basal MSNA and resetting of the vascular sympathetic baroreflex, adjustments that might be important for maintaining arterial blood pressure in the face of cardiac and vascular adaptation induced by years of athletic training. Exposure to high altitude (HA) hypoxia is another physiological state in which skeletal muscle vasodilation challenges sympathetic modulation of vascular resistance and arterial blood pressure. Although relatively few in number, microneurographic studies indicate that heightened MSNA is a feature of HA exposure, not only in lowland natives, but also in highland populations who have generational exposure to ambient hypoxia. This invited talk will explore mechanisms underpinning the sympathetic neural adaptive responses to exercise in health and compare these with alterations in sympathetic outflow that are a feature of altitude acclimatization and adaptation.

SA08

Altered blood pressure regulation during simulated orthostatic stress in exercise trained premenopausal women with functional hypothalamic amenorrhea

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In premenopausal women, exercise training is commonly associated with menstrual disturbances, including functional hypothalamic amenorrhea (FHA). FHA is characterised by chronic estrogen deficiency similar to that observed in postmenopausal women. Despite being young and otherwise healthy, exercise trained women with FHA (ExFHA) demonstrate impaired endothelial function, increased regional vascular resistance, and decreased regional blood flow. Estrogen deficiency is thought to play an important role. Accordingly, similar findings have been reported in postmenopausal women. However, in contrast to postmenopausal women, ExFHA women demonstrate low, rather than elevated, resting arterial blood pressure. In postmenopausal women, estrogen deficiency due to menopause is associated with both increased sympathetic nervous system activity and augmented activation of the renin-angiotensin system. Our investigations of blood pressure regulation in young premenopausal women with ExFHA, compared with age- fitness- and body mass-matched eumenorrheic women, identified augmented lower limb skeletal muscle sympathetic nerve activity (MSNA) yet lower arterial blood pressure during simulated orthostatic stress using lower body negative pressure (LBNP). Further, in ExFHA, non-activation of the renin-angiotensin system despite increasing LBNP (0, 10, 20 and 40 mmHg) was also observed. Thus, otherwise healthy ExFHA women demonstrate low arterial blood pressure and disruption of the normal circulatory response to an orthostatic challenge: namely plasma renin, angiotensin II and aldosterone fail to increase and blood pressure is defended by augmented sympathetic vasoconstrictor responses. This invited talk will examine what is known about the uncoupling of the reflex sympatho-neural and renin-angiotensin system responses to a hypotensive stimulus in estrogen deficient physically active premenopausal women with FHA.

SA09

Blood pressure control during exercise: implications for hypertension

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An exaggerated blood pressure (BP) response to maximal exercise is an independent risk factor for cardiovascular events and mortality. People with hypertension have an elevated muscle sympathetic nerve activity (MSNA) at rest and during exercise, which is in part mediated by the metaboreflex. In people with hypertension, it was unclear if treating resting BP to guideline levels could reduce the activity of the metaboreflex and normalise the rise in BP during exercise. In our studies it was found that individuals with treated and controlled, treated and uncontrolled, and untreated hypertension have an exaggerated BP response to incremental exercise testing ($\dot{V}O_2$ peak testing) and metaboreflex isolation compared to age matched healthy controls. Heightened metaboreflex sensitivity in these individuals, could in part, be due to impaired functional sympatholysis during exercise. Dietary nitrate intervention lowers resting BP in hypertensive individuals, whilst also improving exercise performance, blood flow and exercise BP in healthy individuals. In our study, despite increased levels of plasma nitrates and nitrites in patients with treated-controlled hypertension, 4 weeks of dietary nitrate supplementation had no impact on the submaximal or maximal BP response to $\dot{V}O_2$ peak testing or metaboreflex isolation compared to a placebo. This invited talk will examine what is known about the abnormal metaboreflex during exercise in hypertension, potential treatments, and also future directions.

SA10

Abnormal reflex rise in sympathetic activity during exercise in heart failure and the impact of exercise training

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Patients with heart failure with reduced ejection fraction (HFrEF) are characterised by increased sympathetic nerve traffic directed to skeletal muscle and exercise intolerance; both associate with increased mortality. Our studies with direct microneurographic recordings of muscle sympathetic nerve activity (MSNA) revealed a qualitative difference in MSNA response during mild exercise in HFrEF patients compared with age-matched healthy controls: an increase in MSNA in patients vs. a drop in healthy controls. The elevation in MSNA at rest and during exercise in HFrEF relates inversely to peak oxygen uptake, supporting a neurogenic limit to exercise. The augmented exercise-induced sympathetic response is due partially to greater muscle metaboreflex activation and is exaggerated in those with low exercise capacity. When patients undergo 6 months of exercise training, MSNA burst frequency is lowered, peak oxygen uptake is improved and the autonomic benefit is particularly effective in those who can train at higher intensity. This sympathoinhibitory effect of training partially reflects a blunted muscle metaboreflex but little is known about the contributions of other reflexes. This invited talk will examine what is known about the abnormal reflex rise in sympathetic activity during exercise in HFrEF patients and the relative contributions of excitatory and inhibitory reflexes which may be modifiable by exercise training.

SA11

Viroporins: structure, function and potential as antiviral targets

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Ion channels are targeted by ~20% of currently licensed drugs, yet those encoded by viruses, “viroporins” are neglected by comparison. This is despite the clinical precedent of adamantane drugs targeting the influenza A virus (IAV) M2 proton channel. Viroporins play essential roles during the lifecycles of many pathogenic viruses yet, with few exceptions, precise understanding of their function within virion and/or host cell membranes remains limited. One major bottleneck has been limited usefulness of prototypic small molecule inhibitors.

Viroporins also perform non-channel related functions, confounding mutagenesis studies. Thus, we have focused upon improving small molecules as tools to investigate their properties. Druggable binding sites identified by prototypic drugs can be refined via an array of approaches yielding inhibitors with improved fidelity and utility. Iterative increases in both structural and functional understanding of viroporins can identify new biological roles, simultaneously forming a platform for future therapeutic discovery.

SA12

Role and purpose of microbial rhodopsins in giant viruses.

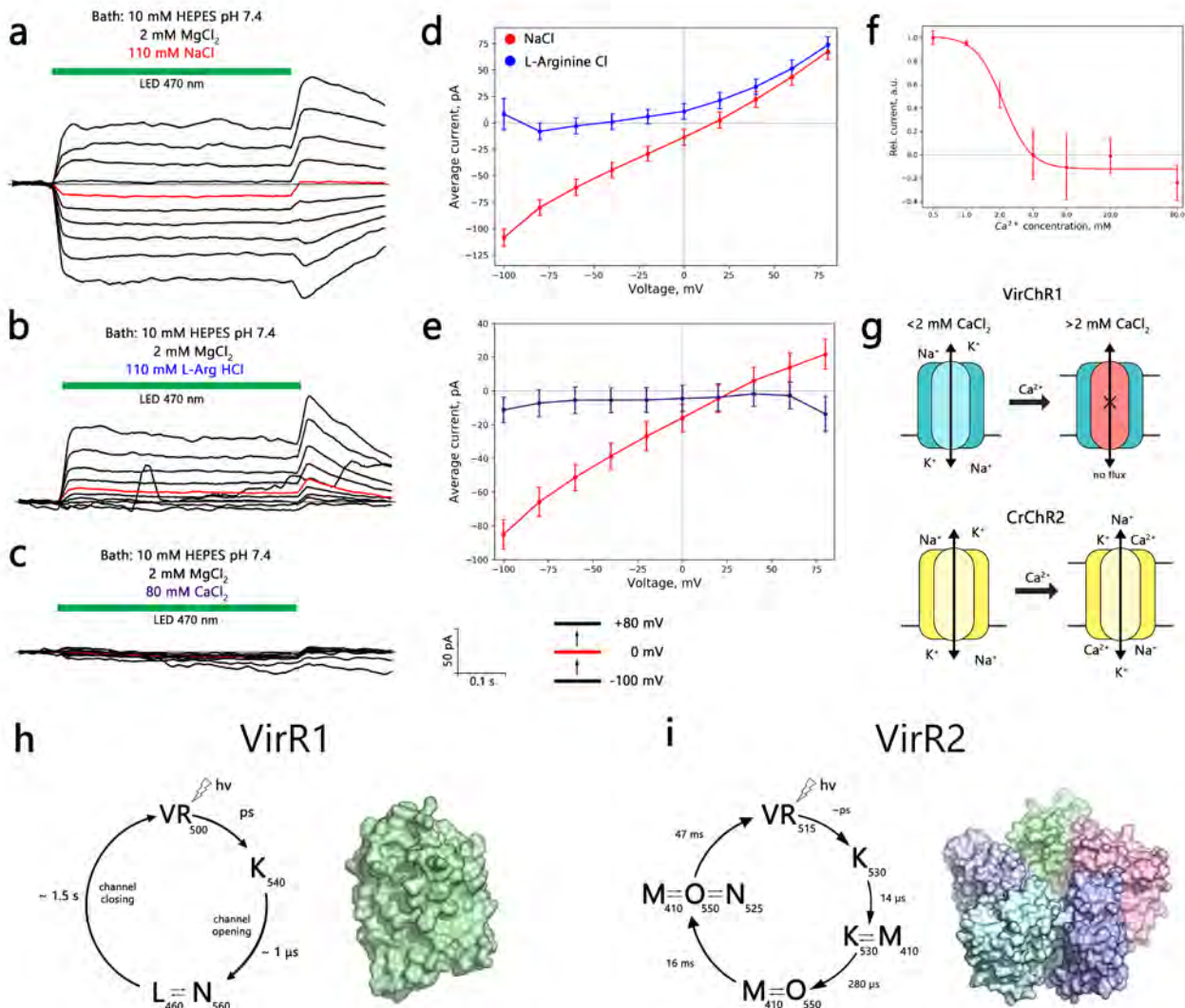
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Microbial rhodopsins are a family of light-sensitive membrane proteins that perform various functions upon light illumination. Recently, rhodopsin-like genes were found in genomes of nucleoplasmic large DNA viruses, which opened a discussion about the potential biological implications of the light-sensitive machinery of giant viruses. The viral rhodopsins family comprises two subgroups, namely group 1 (VirR1) and group 2 (VirR2), that differ phylogenetically from non-viral rhodopsins (Yutin and Koonin 2012). We studied members from both groups to shed light on their molecular and cellular functions. Using the patch-clamp method, we tested the electrophysiological properties of VirR-expressing human neuroblastoma cells (SH-SY5Y) in both light and dark conditions. We observed that viral rhodopsins expressed well, but showed strong retention in the cytosol. To address the plasma membrane localization issue, we tested multiple N- and C- terminal modifications of VirR constructs. When we supplemented one of the VirR1 proteins, VirChR1 with p2A self-cleavage peptide prior to fluorescence tag, we were able to get measurable photocurrents. The electrophysiological characterization revealed that VirChR1 is a Na⁺/K⁺ selective light-gated ion channel, which can be inhibited by moderate concentrations of Ca²⁺ ions (~ 2 mM) (Zabelskii et al. 2020). Besides that, we were able to demonstrate that, upon illumination, VirChR1 is able to drive neural firing. Our efforts in the electrophysiological characterization of the VirR2 group did not result in observing any measurable photocurrents.

In order to gain more insight into the molecular function of viral rhodopsins, we expressed, purified, and characterized OLPVR1 and VirChR1 rhodopsins from the VirR1 group, and OLPVR2 rhodopsin from the VirR2 group. Upon light illumination, OLPVR2 rhodopsin undergoes a photocycle with 70 ms duration, which indicates pump-like behavior, whereas, both OLPVR1 and VirChR1 have channel-like photocycle with a duration of around several seconds. OLPVR1 and OLPVR2 proteins were crystallized using *in meso* crystallization method and have yielded high-resolution structures of 1.4 Å and 1.9 Å respectively. Due to the conservativity of viral rhodopsins, the structures provide structural insight into their potential function. OLPVR2 forms a pentamer, with a symmetrical, bottle-like central channel with a narrow vestibule in the cytoplasmic part covered by a ring of 5 arginines, whereas 5 phenylalanines form a hydrophobic barrier in its exit (Bratanov et al. 2019). The putative central channel is blocked by a hydrophobic tail of lipid from the crystallization matrix that potentially prevents the channel-like function of the protein. OLPVR1 crystallizes as a monomer and shares many structural features with well-studied channelrhodopsin 2 from *Chlamydomonas reinhardtii* (Volkov et al. 2017). OLPVR1 has three consecutive constriction sites that facilitate ion transport upon photon absorption. The OLPVR1 protomer has short extracellular loops, which sharply differentiates it from other channelrhodopsins that typically have large N- and C-terminal domains (Ernst et al.

2014). We are currently looking for ways to improve the plasma membrane localization of viral rhodopsins that can help to understand the function of OLPVR11 and help viral rhodopsins to find their niche in optogenetics applications.



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SA13

The SARS-CoV-2 accessory protein Orf3a is not an ion channel, but does interact with trafficking proteins

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The severe acute respiratory syndrome associated coronavirus 2 (SARS-CoV-2) and SARS-CoV-1 accessory protein Orf3a colocalizes with markers of the plasma membrane, endocytic pathway, and Golgi apparatus. Some reports have led to annotation of both Orf3a proteins as viroporins. Here, we show that neither SARS-CoV-2 nor SARS-CoV-1 Orf3a form functional ion conducting pores and that the conductances measured are common contaminants in overexpression and with high levels of protein in reconstitution studies. Cryo-EM structures of both SARS-CoV-2 and SARS-CoV-1 Orf3a display a narrow constriction and the presence of a positively charged aqueous vestibule, which would not favor cation permeation. We observe enrichment of the late endosomal marker Rab7 upon SARS-CoV-2 Orf3a overexpression, and co-immunoprecipitation with VPS39. Interestingly, SARS-CoV-1 Orf3a does not cause the same cellular phenotype as SARS-CoV-2 Orf3a and does not interact with VPS39. To explain this difference, we find that a divergent, unstructured loop of SARS-CoV-2 Orf3a facilitates its binding with VPS39, a HOPS complex tethering protein involved in late endosome and autophagosome fusion with lysosomes. We suggest that the added loop enhances SARS-CoV-2 Orf3a's ability to co-opt host cellular trafficking mechanisms for viral exit or host immune evasion.

SA14

Autonomic control of body temperature and blood pressure in women: overlap of integrative mechanisms

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Ongoing concerns regarding climate change have increased questions regarding the potential for differences between men and women in the risk of heat illness during exertional heat stress. Interestingly, autonomic control mechanisms contributing to the regulation of body temperature and the regulation of arterial blood pressure have significant overlap in humans. This includes central autonomic control in the hypothalamus as well as peripheral control of blood flow. Mechanisms by which estradiol affects central and peripheral autonomic mechanisms result in conditions that favor both heat dissipation and lower arterial pressure. This is likely an adaptive effect in terms of maintaining low / normal resting blood pressure - that is, young women are less likely to become hypertensive compared to men. Similarly, conditions of high estradiol are often associated with increased heat dissipation (sweating / skin blood flow) and lower body temperature. However, conditions favoring lower blood pressure and increased skin blood flow can decrease orthostatic tolerance – which can also contribute to collapse in the heat. Menopause is associated with higher resting blood pressure and increased risk of hypertension. Older people also have increased risk of heat illness due to changes in thermoregulatory mechanisms, which, in women, are in part due to loss of circulating reproductive hormones. Some of the overlapping mechanisms associating estradiol with lower blood pressure and lower body temperature include beta-adrenergic receptors on peripheral blood vessels and increased nitric oxide-mediated vasodilation. Practical implications for women in a range of occupational settings are currently being investigated, including influences of common types of contraception which provide varying concentrations of exogenously administered estrogens and/or progestins.

SA15

Physiological adaptations to heat stress in women: potential “advantages” and “disadvantages” relative to men

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Sex differences in physiological responses to heat stress have been a “hot topic” over recent years, in particular with regard to risk of developing exertional heat illnesses and the important countermeasure of heat acclimation. Heat acclimation is the process through which the body garners adaptations from systematic, repeated heat exposure. These adaptations primarily include lower core body temperature (T_{core}), lower heart rate (at rest and during exercise), increased sweating rate, and increased plasma volume. Mechanistically, we know there are differences between men and women in thermoregulatory responses to exercise-heat stress. Some of these differences result from physical and anthropometric differences between the sexes. For example, men are often larger and thus have lower body surface area (BSA) to mass ratio ($BSA:mass^{-1}$). This is an important biophysical factor because in individuals with lower $BSA:mass^{-1}$, heat dissipation (i.e. via sweating from the skin surface) may be limited relative to heat production (i.e. heat produced from skeletal muscle mass contraction during exercise). This physical difference may benefit women in certain environments. Additionally, female sex hormones influence thermoregulation with progesterone increasing the thermoregulatory setpoint by $\sim 0.3-0.5^{\circ}C$, and estradiol increasing nitric oxide mediated vasodilation. This increase in T_{core} by progesterone, during acute heat stress, does not appear to be an obstacle for women, and may prove beneficial in the acclimation process (more research is currently needed to elucidate the possible impact). The estradiol-mediated increase in vasodilation is beneficial in terms of thermoregulation, allowing for increased heat dissipation during exercise and/or heat stress. Due to the increasing utilization of hormonal contraceptives, both short and long-acting, that exogenously supplement estrogens and progestins, more work is needed to evaluate the impact of such exogenous hormonal administration on thermoregulatory processes and adaptations, including heat acclimation.

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SA16

Using Exercise Physiology to address gender health inequalities in climate change and occupational health research.

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The health impacts of climate change are already being felt by vulnerable communities, particularly in the Global South. It is widely purported that women in the Global South will be more adversely affected by climate change than men, yet there remains a dearth of empirical evidence to confirm or challenge this idea. Somewhat similarly, in human performance and occupational health literature, women were assumed to be more susceptible to the ill effects of excessive heat exposure (i.e., exertional heat stroke) based on limited empirical evidence that is now being challenged. Using a multi-year cohort study on industrial agricultural workers in Central America (the Adelante Initiative) as a case study, this session will discuss how sex-related physiological differences and lessons learnt from exercise physiology research can inform occupational health outcomes in male and female working populations in the Global South.

Case study: The Adelante Initiative began in 2017 in response to the Central American epidemic of chronic kidney disease of non-traditional origin, which is highly prevalent in agricultural workers in this region. The primary aim of this Initiative was to assess how a rest, shade, hydration intervention programme impacted the health and work conditions of sugarcane workers at one of the largest sugarcane mills in Central America (Ingenio San Antonio). Heat stress, kidney health outcomes and workload in workers performing manual outdoor jobs (e.g., burned cane cutting, seed cutting, drip irrigation repair) were assessed longitudinally (2017-present). Very few females are currently employed as burned cane cutters at ISA. However, females are increasingly being employed in other strenuous outdoor work (i.e., seed cutting) and consequently are exposed to occupational heat stress and its associated health risks. Initial data indicates that females work at a higher physiological workload than their male counterparts. Due to a limited sample size, it is unclear if females in this work context suffer a higher incidence of kidney injury or other heat-related illness than men.

The introduction of women into a susceptible workforce such as industrial sugarcane workers, provides a unique opportunity to assess biological sex-differences in heat-related illnesses/injuries and thus gain further insight into the aetiology of diseases such as chronic kidney disease of non-traditional origin. In workforces exposed to occupational heat stress, population-level physical differences and biological differences between men and women should be factored into exposure assessments and workplace interventions. Male:female workforce ratios, particularly in jobs historically dominated by one gender, provides further information on who is at risk, what personal factors are most relevant and therefore, what interventions are the most practically beneficial. To address gender health inequalities in climate change and occupational health research it is imperative that we make every effort to include women in ongoing and future research.

SA17

Female thermal sensitivity across the life span: a hot journey

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Global warming is now the greatest threat to human prosperity and survival. Hot weather and heat extremes severely limit people's work and exercise capacity, with consequent detrimental effects on individuals' health, comfort, and productivity [1]. Undoubtedly, adjusting our thermoregulatory behaviour represents the most effective mechanism to maintain thermal homeostasis and ensure heat stress resilience [2]. Remarkably, our thermal behaviour is entirely dependent on the ability to detect variations in our internal (i.e., body) and external environment, via sensing changes in skin temperature and wetness.

In the past 30 years, we have seen a significant expansion of our understanding of the molecular, neuroanatomical, and neurophysiological mechanisms that allow humans to sense temperature and wetness [3]. However, we still lack a comprehensive understanding of how autonomic, perceptual, and behavioural responses to heat vary at an individual level, for example as a function of sex, age, and hormonal status.

Women are a group of individuals that undergo unique morphological, physiological, and hormonal changes across the lifespan. For example, consider the impact of the menstrual cycle, pregnancy, and menopause, all of which are accompanied by both short- and long-term effects on female body temperature regulation, heat tolerance, thermal sensitivity, and comfort. Surprisingly, women have been largely unrepresented in heat stress research. Indeed, a recent review highlighted that only 12-18% of participants in thermoregulation research were female over the last decade [4].

Empirical evidence indicates that innate differences in skin thermal and wetness sensitivity may exist between men and women, and this could underlie divergent behavioural responses to heat stress between these groups [5]. However, knowledge on how thermal and wetness sensitivity may vary across women's life cycle, and the implications that this may have for female thermal behaviours under heat stress, continue to be lacking. This knowledge gap provides a significant barrier to develop interventions (e.g. personalised cooling) and solutions (e.g. body-mapped sport garments) that meet the thermal needs of females across different life stages and facilitate the maintenance of an active lifestyle.

This symposium talk will review both established and novel evidence on the peripheral and central neurophysiological mechanisms underpinning skin thermal and wetness sensitivity in women, as well as their role in driving female thermal behaviours. It is hoped that this overview will stimulate the development of testable hypotheses to increase our understanding of the behavioural thermal physiology of women across the life span and at a time of climate change.

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Exercise Thermoregulation Research. Sports Med, 7 (43). [5] Greenfield et al (2023). Sex differences in thermal sensitivity and perception: Implications for behavioral and autonomic thermoregulation. Physiol Behav 263:114126.

SA18

Studies of the distribution of CFTR-rich Ionocytes in mouse airway epithelium

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Ionocytes are a new type of rare airway epithelial cells that express approximately 50% of *Cftr*-transcripts in the mouse airways. They are characterized by the expression of *Ascl3* and *Foxi1* transcription factors, which have been used to identify this cell type in the airway epithelium and submucosal glands of both humans and mice. However, their function and precise localization remain largely unknown. In this study, we aimed to investigate the distribution of ionocytes in the mouse airway epithelium.

Mice were bred in the C57Bl6/J background and maintained in the Specific Pathogen Free mouse facility of Centro de Estudios Científicos (CECs) with access to food and water ad libitum. We used 6 and 8-week-old wild-type and *Cftr*^{ΔF508/ΔF508} animals. The trachea was splitted in two sections: the upper trachea containing the submucosal glands and the rest of the lower trachea, down the cricoid cartilage, sliced in 5μm paraffin sections in the transverse and frontal plane respectively. Ionocytes were identified by immunofluorescence against the FOXI1 transcription factor. All values were expressed as mean±S.E.M. All animal procedures were approved by the institutional IACUC (CECS-2022-03).

We found that FOXI1+cells had a triangular shape with a basolateral process. In the lower trachea the number of cells decreased towards the distal part (proximal= 2.4 ± 0.3 vs distal= 0.8 ± 0.2 FOXI1+cells mm⁻¹ basal lamina, n=5, p=0.005, t-test), and were not found in the intrapulmonary airways. FOXI1+cells were more often observed in the epithelia around the collecting duct exit, in the collecting duct epithelium and in the serous acini of the submucosal glands. The total number of FOXI1+cells per millimeter of basal lamina was higher in the airway epithelium of upper trachea than in the lower trachea (6.5 ± 0.6 vs 4.0 ± 0.6 FOXI1+ cells mm⁻¹ basal lamina, respectively; n=3, p=0.004, Rank Sum test). In general, FOXI1+cells were often present in the epithelia on top of the annular ligaments ($61.7\% \pm 3.0$; n=5; p=0.008, Rank Sum Test).

Preliminary analysis of *Cftr*^{ΔF508/ΔF508} tissues indicated that there were no differences in the amount of FOXI1+cells when compared to wild-type lower tracheas (2.0 ± 0.7 vs 1.9 ± 0.1 FOXI1+cells mm⁻¹ basal lamina, respectively, n=2 each group). Unexpectedly, we observed that FOXI1+cells were lower in issues of 6-week-old than in those obtained from 8-week-old wild type mice (2.0 ± 0.5 (n=2) vs 4.0 ± 0.6 (n=5) FOXI1+cells mm⁻¹ basal lamina, respectively, p= 0.078; Rank Sum Test).

In conclusion, our study provides new insights into the localization and distribution of ionocytes in the mouse airway epithelium. Our results indicate that ionocytes may play a role in regulating mucus composition in upper airways. We suggest that age-dependent changes in cell quantity might reflect the need of increased CFTR function in adult stages. Further research is needed to fully understand the function of ionocytes in the airway and their potential role in respiratory diseases such as cystic fibrosis.

SA19

Morphological, molecular and functional analysis of airway epithelial cell types

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The airway epithelium, the first barrier against pathogens, is endowed with active antimicrobial mechanisms and consists of different cell types, from the most abundant ciliated, goblet and basal cells to rare pulmonary neuroendocrine and tuft cells. Recently, different studies have revealed the existence of a new rare cell population, named ionocytes (1-2), characterized by a high expression of the CFTR chloride channel and the transcription factor Foxp1 (FOXI1). In cystic fibrosis (CF), impaired CFTR function results in dehydration of airway surface, mucus accumulation and bacterial colonization. The airway epithelium is also the entry site for a variety of viruses, like SARS-CoV-2, who enters the cells by binding to the angiotensin-converting enzyme 2 (ACE2) (3). Interestingly, CF patients, who are particularly sensitive to respiratory viral infections, do not seem to be at risk of severe COVID-19.

Our general aim is to investigate the composition of the airway epithelium under health and disease. Specifically, we are investigating 1) expression and role of ionocytes in transepithelial ion transport; 2) ACE2 expression in CF and non-CF cells to understand whether a different expression explains the apparent resistance of CF patients to SARS-CoV-2.

Our molecular and functional studies are based on nasal and bronchial cells from CF patients and control individuals, freshly collected or cultured as differentiated epithelia.

We analyzed by immunofluorescence nasal cells from a broad panel of CF and non-CF patients. Ionocytes were easily detected in both sample types as FOXI1-positive cells and appeared more abundant in the nasal (3-5%) compared to bronchial epithelium. CFTR expression at the plasma membrane correlated with the type of CF mutation: patients with severe mutations (affecting CFTR synthesis or trafficking), showed absent or markedly decreased expression in the plasma membrane. In contrast, patients with milder mutations exhibited a clear CFTR signal in the apical membrane. In general, we found no enhanced abundance of ionocytes in CF individuals with severe CFTR mutations, which could be expected as a compensatory mechanism for the defect in CFTR function. We conducted similar analysis in patients with primary ciliary dyskinesia, another genetic disease with defective mucociliary clearance and susceptibility to bacterial infection. We detected no significant differences compared to control individuals.

Regarding ACE2, we found a substantial higher expression in nasal vs. bronchial cells. Interestingly, ACE2 appeared to be specifically localized on the apical membrane of ciliated cells, at the base of cilia. Furthermore, we found no different ACE2 expression between CF and non-CF samples, thus in contrast with the results of a recent study that reported a decreased ACE2 expression in CF epithelia (4).

The role of ionocytes in airway epithelia is still unclear. The high expression of CFTR may imply a prevalent role in chloride secretion. However, since other more abundant cell types in the

epithelium also express CFTR, it is possible that CFTR in ionocytes have a more specialized function. The higher resistance of CF patients to severe forms of COVID19 does not correlate with lower ACE2 expression. Further studies are needed to clarify the underlying mechanism.

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SA20

Pulmonary Neuroendocrine Cells: Rare, but not Dispensable

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Pulmonary neuroendocrine cells (PNECs) represent less than 1% of airway epithelium. Aside from being precursors for small lung cancer cells, whether they play a role in normal lung function remains poorly understood. Our findings show that PNECs are essential airway sensors that perceive and respond to aerosol signals. They are essential for amplifying allergen-induced asthmatic response. When increased in number, they produce excess neuropeptides which disrupts endothelial barrier, resulting in accumulation of fluid in lung and respiratory distress. These findings illustrate the multiple facets of PNEC function in homeostasis and disease.

SA21

Calcium Cycling in the Avian Heart: the missing link in vertebrate cardiac evolution

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Bird cardiomyocytes are long, thin and lack t-tubules, similar to ectothermic non-avian reptiles. Yet, birds achieve greater contractile rates and developed pressures than mammals, whose wide cardiomyocytes contain a dense transverse (t)-tubular network allowing for uniform excitation-contraction coupling and strong contractile force. To address this apparent contradiction, this talk will link recent electrophysiological studies on bird cardiomyocytes with ultrastructure measurements and computational approaches. Data will show that the strong transsarcolemmal Ca^{2+} influx via the L-type Ca^{2+} current (I_{CaL}) and the high gain of Ca^{2+} -induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR), coupled with the internal SR Ca^{2+} release relay system, facilitates the strong fast contractions in the long thin bird cardiomyocytes, without the need for t-tubules. The significance of this in relation to the evolution of the vertebrate heart and the evolution of endothermy will be discussed.

Shiels HA. Avian cardiomyocyte architecture and what it reveals about the evolution of the vertebrate heart. *Philosophical Transactions of the Royal Society B*. 2022 Nov 21;377(1864):20210332.

SA22

Pacing intracellular Ca²⁺ signals in exocrine cells

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The major physiological function of exocrine acinar cells from the pancreas and salivary gland is the secretion of proteins and fluid which are initiated by changes in cytosolic Ca²⁺ following neurotransmitter or hormone exposure. It is established that the spatiotemporal characteristics of the Ca²⁺ signal are vitally important for the appropriate stimulation of secretion and these properties are often disrupted in disease states. Experiments performed in isolated tissue have documented the complexity of these signals including sub-cellularly restricted signals, intra and inter-cellular Ca²⁺ waves, and apparent pacing of signals within individual acinar clusters by initiator cells. Whether these characteristics are mirrored *in vivo* was not known. To address this question, we have generated mice expressing the genetically encoded Ca²⁺ indicator GCaMP6f specifically in acinar cells and developed an imaging platform to study the characteristics of Ca²⁺ signals *in vivo* in anesthetized mice by multi-photon microscopy. In submandibular salivary acinar glands (SMG), we show that stimulation of intrinsic nerves to the gland result in rapid oscillatory Ca²⁺ signals following ACh release. These events are positively correlated with fluid secretion. These signals appear to initiate in specific cells within individual acini and propagate to neighboring cells. In pancreas, Ca²⁺ signals were observed following neural stimulation that were dependent on ACh release. Ca²⁺ signals as a function of elevated serum cholecystokinin were observed in fasted animals that were augmented in terms of the number of responding cells and peak response following feeding. Both nerve stimulation and CCK induced Ca²⁺ oscillations in pancreatic acini, but with markedly distinct temporal and spatial characteristics. We speculate that initiating cells in each gland are more directly stimulated, either by direct neural innervation or by proximity to the vasculature or alternatively represent cells most sensitive to secretagogue by virtue of receptor number. In total, these studies define the physiological characteristics of Ca²⁺ signals *in vivo* and the platform will be useful in future investigation of disruption of Ca²⁺ signaling in disease states of exocrine tissue.

SA23

Origin of rhythmicity in the bladder and urethra

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The smooth muscle organs of the lower urinary tract comprise the bladder detrusor smooth muscle (DSM) and internal urethral sphincter, which have a reciprocal contractile relationship during urine storage and micturition. As the bladder fills with urine, DSM remains relaxed to accommodate increases in intravesical pressure while urethral smooth muscle cells (USMC) generate sustained tone to occlude the urethral orifice, preventing leakage. Upon onset of micturition, this contractile behaviour reverses, as USMC relax, allowing passage of urine from the bladder, which contracts to expel urine via the now open urethra. While neither of these organs displays uniform coordinated regular contractions, similar to phasic tissues such as the small intestine, lymphatics or renal pelvis, they do exhibit certain patterns of rhythmicity at cellular and tissue levels which underly their physiological function. In rabbit and guinea-pig urethra, regular electrical slow waves are recorded from circular USMC. This activity is linked to specialized populations of pacemaker cells expressing vimentin, c-kit and Ca^{2+} -activated- Cl^- channels, like interstitial cells of Cajal (ICC) in the gastrointestinal (GI) tract. While contractions of urethral muscles do not manifest as coordinated phasic contractions, in these species ICC-like cells might pace individual USMC bundles (through activation of voltage-gated Ca^{2+} channels) to contract asynchronously, with contractions of multiple bundles summing as tone. In mice, USMC are indeed rhythmically active (firing propagating Ca^{2+} waves linked to contraction), and this rhythmicity is asynchronous across the tissue, summing to form tone. However, experiments in mice have failed to demonstrate a voltage-dependent mechanism for regulating this rhythmicity or contractions *in situ*, suggesting that urethral tone results from intrinsic abilities of USMC to 'pace' their own Ca^{2+} mobilization to generate Ca^{2+} waves required for contraction. During the filling phase, animal and human bladders exhibit small transient increases in intravesical pressure, brought about by locally propagating transient contractions of the bladder wall. These transient contractions are critical in regulating sensory afferent activity – relaying sensations of bladder fullness to the CNS. *Ex-vivo* DSM strips exhibit spontaneous rhythmic contractions, mimicking transient concentrations observed during filling *in-vivo*. While DSM spontaneous contractions appear to an intrinsic myogenic property, they are regulated by autonomic nerves and urothelium. Action potentials and associated rises in DSM cytosolic Ca^{2+} are essential for generating these contractions, with this activity appearing to be voltage dependent. The presence of putative 'pacemaker' interstitial cells in the DSM layers have been controversial. Similar, to the GI tract and urethra, Kit^+ cells are present in the DSM layer, however, unlike these other organs these Kit^+ cells are almost exclusively mast cells and thus unlikely to serve as pacemakers. However, another interstitial cell with immunopositivity for antibodies against PDGFR α , has recently been suggested to regulate DSM excitability by potentially serving as mechano and neural transducers, through activation of inhibitory purinergic-SK3 pathways. While the mechanics of rhythmic or tonic contractions in both bladder and urethra is myogenic, there are clear disparities in the cell types, molecular pathways and mechanisms of coordination that lead to these physiological behaviours in both organs.

SA24

Peristaltic pacemakers of the upper urinary tract

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Urine expulsion from the upper urinary tract is a necessary process that eliminates waste, promotes renal filtration, and prevents nephron damage. To facilitate the movement of urine boluses throughout the upper urinary tract, smooth muscle cells that line the renal pelvis contract in a coordinated effort to form peristaltic waves. Resident pacemaker cells in the renal pelvis are critical to this process and spontaneously evoke transient depolarizations that initiate each peristaltic wave and establish rhythmic contractions. This talk will discuss the mechanisms responsible for pacemaking and our current methods to improve the identification of pacemaker cells. Until recently, renal pacemakers have been termed "atypical smooth muscle cells" due to their low expression of smooth muscle myosin and poor organization of myofilaments compared to "typical smooth muscle cells" that perform peristalsis. Our group discovered that pacemaker cells also express the tyrosine kinase receptor PDGFR α , enabling their identification and purification amongst other renal pelvis cell types. Employing our improved identification methods, we have determined that the calcium-activated chloride channel, ANO1, is expressed by pacemaker cells and may contribute to spontaneous depolarization. A greater understanding of pacemaker and peristaltic mechanisms is warranted since aberrant contractile function may underlie diseases such as hydronephrosis, a deleterious condition that can cause significant and irreversible nephron damage.

SA25

The use of ex vivo human serum to study age and chronic inflammatory disease related muscle cell atrophy

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Cell-based models of ageing and disease provide an important platform to probe and extend the mechanistic insights of muscle cell atrophy from invasive and logistically challenging human trials. While cell-based models provide useful insights into the role of metabolic pathways, translation from cell to human can be limited by non-physiological culture conditions, supraphysiological treatment dosages and experimental conditions targeting a single protein, or receptor. Over recent years, there has been an increase in research aimed at improving the physiological relevance of in vitro work, including the use of human plasma and serum. The ex vivo co-culture model has the potential to create a systemic environment representative of ageing and chronic inflammatory disease states. Recent advancements in the application of the ex-vivo co-culture model have highlighted the capacity of human serum to induce atrophy in relation to its host environment. We, and others have utilised the human serum and plasma from young and old males, to investigate ageing-related cellular atrophy (Kalampouka *et al.*, 2018; Allen *et al.*, 2021) and chronic liver disease patients to investigate disease related atrophy (Allen *et al.*, 2022). This talk will describe the effects of young and old ex vivo serum on cellular growth and protein stasis, before outlining the utility of the ex-vivo model to investigate muscle atrophy in chronic inflammatory disease conditions e.g., chronic liver disease and rheumatoid arthritis.

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SA26

The effect of female sex hormones on human skeletal muscle metabolism – an ex vivo/in vitro approach

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In vitro models have long been used to further our understanding of skeletal muscle metabolism. Using these methods, researchers can study the mechanistic response to various stimuli (e.g., nutrient exposure/withdrawal, electric stimulation etc) that may be difficult to achieve or isolate in large human trials. Supraphysiological treatment conditions are often used on immortalised cells (often not derived from humans) and create an even more artificial milieu – rendering it difficult to translate *in vitro* findings to humans.

In recent years, there has been an ever-growing focus on the potential presence of sexual dimorphism in various aspects of muscle physiology, however, to date there has been little specific focus on female sex hormones. Female physiology is characterized by fluctuations in hormone levels throughout the menstrual cycle, such that oestrogen levels peak during the late follicular phase while progesterone levels are highest during the mid-luteal phase. Skeletal muscle expresses both oestrogen and progesterone receptors (1), and oestrogen has been purported to 'protect' muscle from exercise induced muscle damage via several different mechanisms in animal models (2). Moreover, progesterone treatment impairs insulin stimulated glucose metabolism in rodents implying a direct effect of progesterone on skeletal muscle that warrants further investigation (3).

In this talk, I will describe the findings from recent experiments whereby immortalised human skeletal muscle cells (4) were utilised to understand the effects of female sex hormones on skeletal muscle anabolism. To further enhance the translatability of such findings, serum samples obtained at different phases of the menstrual cycle (early follicular phase - low oestrogen/progesterone, late follicular phase - high oestrogen and mid luteal phase - high oestrogen/progesterone) were applied to cells to allow physiologically relevant concentrations/ratios of these hormones to be studied. Such a model can therefore provide a greater depth of understanding of the role of female sex hormones on human skeletal muscle anabolism.

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SA27

A translational model of muscle protein synthetic bioactivity using ex vivo human serum

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Background:

In vitro models provide an important platform for the investigation of skeletal muscle growth to inform and extend mechanistic insights in invasive and often logistically challenging human trials. Although these models allow for greater understanding of the mechanistic underpinning of adaptation in skeletal muscle, many models involve supraphysiological dosages and non-physiological conditions which limit translation of findings to humans. The aim of this research was the development and validation of a translational model for the evaluation of sustainable protein sources in stimulating muscle protein synthesis (MPS) and skeletal muscle anabolism using ex vivo human serum. To achieve this overall aim, three primary objectives had to be realised: (i) Development of an *in vitro* skeletal muscle cell bioassay to measure muscle growth and MPS; (ii) Development of an ex vivo model to evaluate the humoral effect on MPS in response to protein feeding; (iii) Use of a stable isotope technique to evaluate MPS in response to protein feeding *in vivo*.

Methods:

Changes in cell behavior and adhesion properties were monitored by measuring impedance via interdigitated microelectrodes using the xCELLigence system. MPS was measured by puromycin incorporation using the SUnSET technique, intracellular signalling measured by western blot, and myotube thickness by microscopy. To establish the ability of the bioassay to measure the humoral effect of MPS in response to protein feeding, media was conditioned by ex vivo human serum from fasted, and protein-fed conditions. To evaluate MPS in response to protein feeding *in vivo*, acute MPS (5 h) was assessed by measuring stable isotope deuterium oxide (D₂O) incorporation into m. vastus lateralis skeletal muscle following consumption of sustainable proteins compared with a non-essential amino acid (NEAA) formulation.

Results:

In this presentation we will demonstrate the ability to monitor changes in cell behaviour, cell size and intracellular signalling when conditioning media with ex vivo human serum in response to feeding with alternative proteins. Proteins containing essential amino acids, known regulators of MPS and muscle anabolism, display greater anabolic qualities than isonitrogenous NEAA formulations. We also confirm translation of this in a human *in vivo* model using stable isotope tracers.

Conclusion:

We have developed a translational model of muscle protein synthetic bioactivity using ex vivo and *in vivo* methodologies. We have shown that we can impact MPS *in vitro* using ex vivo human serum to condition media, that MPS *in vitro* is differentially regulated by ex vivo serum

from alternative proteins compared with an isonitrogenous NEAA control, as well as translation of these findings *in vivo* using stable isotope technology.

SA28

SPOCs, video feedback, and finding benefits from ed tech where others do not look

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Distant and online education has been around since long before the pandemic forced every teacher to consider this form of delivering education. Therefore, we can draw upon existing evidence to determine what works and what doesn't, from long before 2020. In the hectic of the pandemic, less informed choices led to very different experiences with online education of both teachers and students. During this presentation we will move from MOOCs to SPOCs (Small Private Online Courses). We will delve into scalability and focus on deep learning, and social presence as ways to make online education a rich experience. Forms of providing feedback in an online environment will be discussed, including different actors in feedback, and the learnings from both receiving and providing feedback. From feedback, we will naturally move towards assessment. We will discuss assessment *for* learning, assessment *of* learning and assessment *as* learning in the context of online education. Also here, students' new best friend ChatGPT will be included in our discussion on online education and online assessment.

Technologies in Biomedical and Life Sciences Education
(<https://link.springer.com/book/10.1007/978-3-030-95633-2>)

SA29

Pandemic positives - how technology changed how we teach and assess, and what does the next challenge look like.

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In March 2020 the COVID-19 pandemic led to a huge shift in teaching and assessment across the HE sector. Instead of in class sessions, institutions around the world moved teaching online. For lectures, this typically meant delivering content via asynchronous resources such as videos, supplemented by synchronous, online active learning sessions, which allowed students to consolidate knowledge and develop problem solving skills. Assessments moved away from invigilated examinations, to online, open book exams, and this meant a change in the types of assessments being used as well (away from recall and towards application). Many of the changes implemented during this time had positive benefits for students, and as the sector transitioned back to in class, these inclusive approaches were retained. Practitioners retained the use of flipped learning, supported by active sessions. Many assessments did not go back to invigilated exams, but stayed online and open book. All of these approaches provide a more inclusive approach, recognising that our student cohorts are diverse. However, the sector is about to undergo yet another shift, with the increased prominence of AI technology. While not the size of shift we saw with COVID-19, it is clear that if we are to retain the inclusivity benefits of having a range of assessments, then we are going to have to look carefully at how we design those assessments. We are also going to have to evaluate how we prepare our students for future career pathways where the targeted use of AI is becoming more frequent.

SA30

Non-invasive evaluation of skeletal muscle oxidative function in vivo in health and disease: an exercise physiology perspective by near-infrared spectroscopy.

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In most activities related to work or leisure the energy for muscle work substantially comes from oxidative metabolism. Impairments of this metabolism can significantly affect exercise tolerance and performance, may significantly affect the patient's clinical picture and quality of life, and represent an important predictor of mortality. Near-infrared spectroscopy (NIRS) can offer insights into the physiological and pathophysiological adaptations to conditions of increased O₂ needs which involve, in an integrated manner, different organs and systems of the body. In terms of patient evaluation, NIRS allows to determine the evolution of the functional impairments, identifies their correlations with clinical symptoms, evaluates the effects of therapeutic or rehabilitative interventions, and allows to gain pathophysiological insights^{1,2}.

Strengths and limitations of NIRS have been discussed in recent reviews^{1,3}. Skeletal muscle fractional O₂ extraction (SMFOE), the main variable evaluated by NIRS, is conceptually homologous to arterial-venous O₂ concentration difference, and is the result of the dynamic balance between O₂ utilization and O₂ delivery in the tissue under consideration^{1,3}. The reduced peak SMFOE during an incremental exercise identified and quantified the incapacity to increase O₂ extraction (one of the key pathophysiological mechanisms of these diseases) in patients with mitochondrial myopathies or McArdle disease². SMFOE allowed, in these patients, insights into the mechanisms responsible for the positive effects of exercise training,² and in McArdle patients into the pathophysiology of the “second wind”². Impairments of oxidative metabolism, expressed as reduced SMFOE peak, were described in several other pathological conditions¹ and after exposure to bed-rest/microgravity and/or hypoxia⁴.

The slope of the linear SMFOE increase at intermediate work rates, during an incremental exercise, allowed inferences in the adequacy of O₂ delivery in patients with chronic heart failure⁵ or in heart transplant recipients⁶. The plateau of SMFOE at high work rates has been the object of active research in terms of its associations with variables such as critical power, maximal lactate steady state, respiratory compensation point⁷.

SMFOE during the rest-to exercise transition was utilized to evaluate the adequacy of the adjustment of microvascular O₂ delivery vs. that of O₂ uptake, which was impaired in patients with chronic heart failure¹, metabolic myopathies², in subjects exposed to microgravity/bed-rest⁸.

During a transient muscle ischemia obtained by cuff inflation, the rate of deoxygenation determined by NIRS indicates muscle V'O₂³. By adopting rapid inflation-deflation protocols during the recovery from exercise, NIRS allowed to determine muscle V'O₂ off-kinetics, mirror image of [PCr] kinetics and a classic index of functional evaluation of oxidative metabolism⁹; studies have been performed in patients⁹, healthy subjects⁹⁻¹⁰, subjects exposed to microgravity/bed-rest¹¹. A modification of the rapid inflation-deflation protocol allowed to specifically investigate peripheral O₂ diffusion¹². The rate of reoxygenation following a transient muscle ischemia evaluates the microvascular response to an ischemic stress (“reactive hyperemia”)³. Insights into peripheral O₂ diffusion can be obtained by analysis of changes of the

total (oxygenated + deoxygenated) Hb signal, reflecting changes in capillary hematocrit³. Exciting new perspectives (simultaneous measurements of microvascular blood flow, SMFOE and regional oxidative metabolic rate) have been raised by diffuse correlation spectroscopy¹³.

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SA31

Measuring cerebrovascular function in humans in response to dietary interventions

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Flavonoids are small molecules that can be found ubiquitously in plants (e.g. cocoa, berries, grapes, apples) and can protect humans against vascular disease, as evidenced by improvements in peripheral endothelial function, likely through nitric oxide (NO) signalling. Emerging evidence also suggests that diets rich in these compounds may protect against cognitive decline later in life, but the underlying mechanisms are not well established.

We have conducted randomized, counterbalanced, double-blind, placebo controlled, within-subject acute studies in healthy young adults to investigate the effects of one single dose of cocoa flavonoids (flavanols) on prefrontal cortical oxygenation using Near Infrared Spectroscopy (NIRS). In our first study (N=18), we showed that flavanol intake leads to faster (approx. 1 min; $p < 0.001$) and greater brain oxygenation ($p=0.030$ for *Oxygenated Haemoglobin, O₂Hb*) in response to hypercapnia (5% CO₂), as well as higher performance in a Stroop Task, only when cognitive demand is high ($p=0.045$). We further observed that only participants who benefitted from flavanol intake during hypercapnia, also demonstrated improvements during cognitive performance (1). More recently we have also shown that cocoa flavanols might be beneficial in the context acute mental stress. During periods of stress, individuals often increase their consumption of unhealthy foods, especially high fat foods, and it is well established that both fat and mental stress alone can negatively impact peripheral vascular function. However, their effects on the cerebral vasculature are less understood. We have firstly demonstrated that a high-fat breakfast (56.5 g fat) impaired prefrontal cortical oxygenation ($p<0.05$ for *O₂Hb* and *Tissue Oxygenated Index, TOI*) during the mental stress episode in healthy young volunteers, in comparison to a low fat-breakfast (11.4 g fat) (N=19). In a follow-up study (N=23), we have further investigated whether a high-fat breakfast administered with an acute dose of cocoa flavanols may prevent fat-induced impairments in cortical oxygenation during stress. We are currently analysing this set of data and will be able to share at the meeting.

Together our data suggests that flavonoid-rich foods might be an effective dietary strategy to improve cortical oxygenation in young healthy adults and that might be important in the context of high cognitive demand and during periods of stress. These findings will have important implications for future research to explore the relationship between food choices and cerebral haemodynamics during cognitive performance/mental stress. Our data further suggests that flavonoids might exert similar actions on the cerebral vasculature as they do in the peripheral vasculature.

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SA32

Advancing clinical physiological monitoring with state-of-the-art diffuse optics techniques

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Diffuse optics (DO) has been widely used for physiological monitoring for over 40 years [1]. Near-infrared spectroscopy (NIRS) is a well-known DO technique that measures changes in light attenuation due to changes in tissue chromophore concentrations. While NIRS instruments based on continuous waves (CW) have primarily been used to monitor muscle and brain oxygenation changes, recent methodological advancements have increased the accuracy and robustness of NIRS measurements and enabled the monitoring of metabolism and the development of new biomarkers. These advances have pushed the use of NIRS, and more generally DO, in the clinic.

During my presentation, I will showcase the work I have been conducting at the University College London (UCL) to advance the capabilities of DO and extend its use in various fields, such as neuroscience, physiological research, and particularly in a clinical environment.

Indeed, our group, led by Prof. Tachtsidis, is focused on developing optical methodologies to measure brain oxygenation and metabolism. To do so, our primary focus has been on the development of broadband NIRS infrared spectroscopy, an extension of the standard NIRS technique that uses hundreds of wavelengths to acquire more information about the brain. This technique allows us to monitor, on top of the traditional oxygenation parameters monitored with NIRS, the concentration changes in cytochrome-c-oxidase (oxCCO), which is a marker of metabolism. We have shown that monitoring both oxygenation and metabolic changes concurrently could have significant impact for clinical monitoring [2].

Secondly, I will show more recent work that has focused on combining diffuse correlation spectroscopy (DCS) with our broadband NIRS device to further extend the capacities of the system, in an instrument called FLORENCE [3]. Indeed, DCS is an established optical modality that enables non-invasive measurements of blood flow in deep tissue by quantifying the temporal light intensity fluctuations generated by dynamic scattering of moving red blood cells. Thus, the addition of DCS gives us access to blood flow information, enabling us to extract information about the cerebral metabolic rate of oxygen (CMRO₂). I will show the benefits of accessing all of this information in a clinical context.

Finally, I will briefly talk about the most advanced instrument that we have been developing, called MAESTROS, which is based on time-domain NIRS (TD-NIRS) and measures the arrival time of photons [4]. This is the most advanced form of NIRS and can unlock new possibilities. It is notably the best technique to enhance the depth sensitivity of NIRS measurements, enabling us to overcome the most significant issue with NIRS, i.e., superficial tissue contamination [5]. I will show an example of a clinical use of this system, together with the new possibilities that it offers.

In conclusion, my talk will provide an overview of the work done at UCL to advance the state-of-the-art of DO and promote its usefulness and adoption in a clinical context.

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SA33

Dual slopes in diffuse optics: Applications to the brain and skeletal muscle

Sergio Fantini, Angelo Sassaroli, Giles Blaney, Cristianne Fernandez, Jodee Frias, Fatemeh Tavakoli

undefined

Introduction: Near-infrared spectroscopy (NIRS) and diffuse optical imaging (DOI), in the wavelength range 600-1000 nm, have been used for non-invasive optical studies of biological tissues for a long time. Some notable applications include tissue oximetry, pulse oximetry, assessment of blood flow and oxygen consumption in skeletal muscles, functional brain imaging, and optical mammography. Besides continuous-wave (CW) methods that use constant illumination, time-resolved methods either in the time domain (TD: pulsed illumination and time-resolved detection) or in the frequency domain (FD: intensity-modulated illumination and phase-resolved detection) have been introduced. Furthermore, slope methods based on the collection of data at multiple source-detector distances have been proposed, especially with CW and FD methods, to perform absolute measurements of tissue optical properties or to minimize sensitivity to superficial tissue layers. Slope techniques are typically based on either a single source (and multiple detectors) or a single detector (and multiple sources), in which case they may be termed “single-slope” methods. A “dual-slope” approach, identified as “self-calibrating,” was introduced to perform slope measurements that are insensitive to instrumental and optical coupling effects, resulting in calibration-free measurements.

The motivation of this work is to achieve quantitative optical measurements and preferential sensitivity to deep tissue using frequency-domain NIRS (FD-NIRS) in dual-slope configurations. This is important for non-invasive optical measurements to achieve preferential sensitivity to brain and muscle tissue underneath scalp/skull and skin/adipose layers, respectively.

Methods: Theoretical calculations based on diffusion theory were first run to characterize the spatial region of sensitivity of the dual slope technique implemented with FD-NIRS and two illumination points and two collection points that realize source-detector distances of 2.5 and 3.5 cm. Homogeneous and heterogeneous media were considered, with special emphasis on two-layered media. *In vivo* results were then obtained on human subjects from the primary visual cortex during visual stimulation, and from the forearm muscle during either venous occlusion or arterial occlusion in the upper arm.

Results: In both theoretical simulations and *in vivo* measurements with FD-NIRS, we consistently found enhanced depth sensitivity using phase vs. intensity data, and using dual-slope vs. single-distance data. We also found that the relative scattering properties of superficial and deeper tissue affect the depth sensitivity achieved by different optical measurements. In the case of brain measurements, we observed the lowest sensitivity to cortical hemodynamics using single-distance intensity, intermediate sensitivity using single-distance phase or dual-slope intensity, and maximal sensitivity using dual-slope phase. In the case of muscle measurements, the different hemodynamics and oxygen metabolic rates in superficial adipose tissue and deeper muscle tissue result in quantitatively and qualitatively different dynamics observed with different data types.

Conclusions: Dual-slope measurements feature desirable aspects of practical and conceptual significance that can help advance a number of spectroscopy and imaging applications in the field of non-invasive diffuse optics. Specifically, they can provide more specific measurements of cerebral hemodynamics in functional brain imaging, and more detailed characterization of skeletal muscle hemodynamics and oxygenation during vascular occlusion and exercise protocols.

SA34

The relationship between respiratory mechanics and neural control of respiratory muscles

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The diaphragm is the major inspiratory muscle, but the intercostal and scalene muscles also generate inspiratory pressure to draw air into the lungs. There is synergistic activation of the inspiratory muscles to ventilate the lungs, but coordinated activity across these muscles is also important to reduce the work of breathing. In recent history, the mechanics of the inspiratory muscles for breathing is quantified as a fractional change in muscle length per passive increase in lung volume, i.e. mechanical advantage (De Troyer *et al.*, 2005). The relative contribution of the inspiratory muscles, and even different regions within each muscle, to generate inspiratory pressure differs. How does the central nervous system deal with the diversity and redundancy in respiratory muscle mechanics?

In humans, single motor unit studies have demonstrated that the parasternal intercostal muscles are activated in a precise pattern, with earlier and greater activity in the rostral interspaces compared to the caudal spaces during eupnoea. This finding is robust, having been replicated twice (Hudson *et al.*, 2019). This rostrocaudal pattern of neural drive mirrors the relative inspiratory mechanical advantages of these muscles. Similarly, a rostrocaudal gradient of neural drive parallels that of mechanical advantage in the external intercostal muscles. In addition, in the external intercostals, there are *within* interspace differences in neural drive and mechanical advantage, both being greatest in the dorsal portion of muscle and declining along the interspace. The coefficient of correlation between neural drive and mechanical advantage is 0.99 for both intercostal muscle groups (Hudson *et al.*, 2019). This remarkable relationship between neural drive and mechanics is a strategy that minimises the metabolic cost of muscle activation (De Troyer *et al.*, 2005) and led to the discovery of the “principle of motor unit recruitment by neuromechanical matching” (Hudson *et al.*, 2019).

For the diaphragm, neural drive is greater to the costal than the crural portion, with increases in either voluntary or involuntary drive to breathe (Nguyen *et al.*, 2020). Given the costal portion generates more thoracic expansion, via the zone of apposition (Domnik *et al.*, 2020), this suggests motor unit recruitment according to neuromechanical matching occurs across portions of the major inspiratory muscle in humans.

The ‘respiratory muscles’ have other motor functions, and their neural control adapts according to their mechanics in the motor task. In targeted voluntary breaths, where the mechanics of the intercostal muscles are comparable to eupnoea, the rostrocaudal pattern of neural drive is maintained (see Hudson *et al.*, 2019). However, in ipsilateral trunk rotation, for populations of the same intercostal motor units, the pattern of recruitment across interspaces is reversed compared to the rostrocaudal gradient during eupnoea. This is likely to reflect different mechanics for the parasternal intercostal muscles in these tasks (Hudson *et al.*, 2017).

Motor unit recruitment by neuromechanical matching is the most efficient way to recruit the respiratory muscles for breathing and other tasks in health. Non-invasive methods to assess

patterns of inspiratory muscle activity will facilitate discoveries on neuromechanical matching in clinical populations and is the focus of new research.

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SA35

Respiratory mechanics, breathlessness and exercise limitation in health and disease

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Breathlessness has been defined as “a subjective experience of breathing discomfort that consistent of qualitatively distinct sensations that vary in intensity”. It is a complex phenomenon, which is influenced by both neurophysiological and psychological factors. Breathlessness impacts upon individuals' capacity to undertake physical activity. This can limit exercise capacity and, in severe cases, restrict the ability to mobilise, undertake daily activities and independently self-care, thereby deleteriously impacting upon health-related quality of life.

The sensation of breathlessness arises as a consequence of respiratory muscle motor activity through proprioceptive pathways. Inspiratory muscle activity increases in health during exercise, and in disease states where there is imbalance in the loads and capacity of the respiratory muscle pump. This imbalance leads to increased neural respiratory drive in the medulla. Conscious awareness of this ventilatory drive is perceived as breathlessness. It has been proposed that breathlessness intensity increases when there is mismatch between sensory afferents and efferent neural respiratory drive.

Disease states leading to load-capacity-drive imbalance can broadly be considered under the classifications of obstructive airways disease, neuromuscular and chest wall disease and obesity. In obstructive lung disease, most commonly chronic obstructive lung disease (COPD), airway inflammation, bronchospasm and sputum impose resistive loads, loss of alveolar fibroelasticity leads to elastic loading, and expiratory flow limitation with consequent intrinsic positive end expiratory pressure (PEEP_i) imposes a threshold load. Capacity of the respiratory muscle pump is reduced in COPD due to hyperinflation, which impairs force generating capacity. In neuromuscular and chest wall disease, respiratory muscle weakness reduces pump capacity, and upper airway obstruction, secretions and stiff lungs. In obese subjects, upper airways obstructive imposes resistive loading, reduced lung compliance contributes to elastic loading and threshold loading arises through early airway closure leading to PEEP_i. Capacity may be impaired through reduced functional residual capacity and ventilation:perfusion mismatch.

It is not possible to directly quantify central ventilatory drive, therefore surrogate indices are utilised. Inspiratory muscle activity increases in response to increased neural respiratory drive, and thus represents a measurable and potentially clinically valuable objective physiological marker of neural respiratory drive. Electromyography (EMG) has been implemented using invasive and non-invasive techniques amongst healthy subjects and in patients with load-capacity imbalance. This talk will provide an overview of these approaches and clinical applications of respiratory muscle EMG.

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Home parasternal electromyography tracks patient-reported and physiological measures of recovery from severe COPD exacerbation. ERJ Open Res 2021;7

SA36

The effects of ageing on the respiratory physiology of exercise

William Sheel

undefined

Normative aging of the respiratory system involves significant structural changes leading to a progressive decline in pulmonary function. Age-related structural changes include decreases in lung elastic recoil, airway size, respiratory muscle strength and chest wall compliance. Declines in pulmonary function are especially critical when considering the significant demands placed on the lung, airways, and respiratory musculature during conditions of dynamic exercise. Moreover, biological sex has historically not been considered within those studies designed to examine the interaction between age-related changes to the structure and function of the respiratory system and the effect on the integrated response to exercise. This presentation will first summarize changes to the respiratory system that are associated with healthy ageing. With this framework in mind, two inter-related questions will be addressed. First, what is the metabolic cost of exercise hyperpnea and how does this differ on the basis of age and sex? We have recently quantified the metabolic cost of breathing of exercise ventilations through voluntary hyperpnoea in healthy younger (23 ± 3 y) and older (63 ± 6 y) males and females. We found that both younger and older females have a higher cost to breathe than their male counterparts during moderate and high-intensity exercise. In addition, older individuals incur a higher cost to breathe than younger individuals for a given absolute ventilation. Second, is the pressor response during high levels of inspiratory work heightened in older adults and is there an effect of circulating ovarian hormones. Healthy, normotensive young (26 ± 3 y) and older (64 ± 5 y) males and females performed inspiratory pressure threshold loading to task failure. Consistent with previous reports, younger females had a lower blood pressure response to high respiratory work relative to young males. Older adults had a greater mean arterial pressure compared to young however, the sex difference was absent in older individuals. Our observations in young adults are analogous to previous work where metaboreflex responses differed by sex when limb work is performed. Likewise, we interpret the similar blood pressure response between older males and females to be related to the reduced concentration of sex hormones. Our findings point to independent effects of ageing and sex on the respiratory muscle metaboreflex responses. Collectively, our recent work speaks to the need to further understand the demands placed on the respiratory system during exercise of healthy older adults and how this may differ on the basis of sex. Understanding what constitutes 'normal' is especially important from a clinical perspective given that many diseases of the heart and lungs occur in older individuals.

SA37

The mechanisms of emmetropization

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One of the basic questions of myopia development is why it does not inhibit itself but rather tends to progress. This is in apparent contradiction to results of animal models, where myopia can be simulated by positive lenses and, in fact, inhibits eye growth, leading to shorter eyes and hyperopia. Together with Barbara Swiatczak at IOB, we found that also the human retina can detect myopic defocus and trigger shorter eyes, but this was possible only in emmetropic eyes while myopic eyes had apparently lost this ability. A big question is then how the emmetropic retina can detect positive defocus (note that we don't need such a function for vision). A great signal would emerge from longitudinal chromatic aberration which makes image focus more myopic in the blue, compared to the red. Comparison of focus in both planes could provide the sign of defocus. We have developed software to present movies with simulated chromatic defocus. We found that the emmetropic retina responded exactly as expected. When blue was calculated unsharp, eyes became shorter but when red was calculated unsharp, eyes became longer. Again, myopic eyes had lost this ability and only became longer. There appears to be a fundamental functional change in the myopic retina - we need to find out what it is and when it happens. An emerging question is then why various novel spectacle designs can slow myopia progression - as we found that the myopic retina does not respond to positive defocus, these glasses must stimulate the retina in a different way, other than imposing positive defocus.

SA38

Aspects of Retinal Signalling in the Myopic Eye

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Introduction: It is well accepted that reduced visual function occurs in physiological (non-pathological) myopia. However, it is less well understood whether this is due to true dysfunction in the retinal cells, or simply a result of reduced density of functionally normal cells secondary to myopic eye growth and retinal stretch. To investigate this, we considered spatial and temporal summation characteristics in physiological myopia, and compared this to our previous work in glaucoma, a pathological eye condition that causes reduced cell density (cell death) and cell dysfunction. Spatial and temporal summation are core visual functions which refer to how the perceptive fields of the visual system summate light over space and time respectively.

Aim: To investigate spatial and temporal summation in physiological myopia

Method: Spatial summation was investigated in 24 myopes (mean: -4.14DS, range: -0.50DS, -9.75DS) and 20 non-myopic controls (mean: +0.71DS, range: +1.75DS, -0.25DS) by measuring achromatic contrast thresholds for six stimuli varying in area (0.01–2.07 deg², 200ms). The effects of refractive error induced variations in retinal image size (RIS) were considered by correcting refractive error separately with (i) trial lenses placed at the anterior focal point (constant RIS in mm for all participants), and (ii) contact lenses (RIS increases in line with eye length). Temporal summation was investigated in a similar cohort (24 myopes, mean: -4.65DS, range: -1.00DS, -11.25DS; 21 controls, mean: +0.87DS, range -0.25D, +2.00D) by measuring achromatic contrast thresholds for six stimuli varying in duration (1.1 – 187.8ms, 0.43° diameter). RIS was kept constant. The upper limit of complete summation ('Ricco's Area' (RA) and 'Critical Duration' (CD) for spatial and temporal summation respectively) was estimated from the data with iterative two-phase regression analysis. Retinal temporal summation was also measured objectively using electrophysiology and analysing how the amplitude of response changed for stimuli of constant energy (3cd/m²) but varying duration (0.5-100ms).

Results: With spectacle correction, RA was significantly larger ($p=0.02$, Mann Whitney U-test) in the myopes compared to controls (myopes median: -0.92 log deg², IQR: -1.10, -0.78; controls median: -1.14 log deg², IQR: -1.29, -1.07). However, for contact lens correction, there was no significant difference in RA ($p=0.44$) between groups (myopes median: -1.19 log deg², IQR: -1.28, -0.96; controls median: -1.14 log deg², IQR: -1.24, -0.87). There was also no significant difference in CD between groups measured psychophysically (myopes median: 44.3ms, IQR: 26.5, 51.2; controls median: 41.6ms, IQR: 27.3, 48.5), nor objectively with the electroretinogram.

Conclusions: The area of complete spatial summation was altered in myopia when differences in projected RIS were accounted for, likely a compensation for reduced cell density secondary to axial elongation. However, when RIS was allowed to increase in line with axial length, there was no measurable difference in spatial visual function in myopia. In addition, temporal

summation was unchanged in myopia. This contrasts to glaucoma, where both spatial and temporal summation are altered. Taken together, these results suggest that reduced visual function in myopia is due to reduced cell density, rather than dysfunction in the cells themselves.

SA39

Associations between myopia risk polymorphisms and retinal electrophysiology

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Myopia, most often caused by elongation of the eye, is a leading, and increasing, cause of vision impairment globally. Existing evidence indicates retinal signalling has a key role in driving ocular growth. Genome-wide association studies have identified many genetic polymorphisms associated with myopia in the general population. Several of these are near genes expressed in the retina and involved in retinal physiology. The common variant most strongly associated with myopia is at locus rs524952 near the *GJD2* gene, which encodes the Connexin-36 protein, forming retinal gap junctions. The electroretinogram (ERG) represents the electrical response of the retina to light stimuli, and it can be recorded non-invasively from the living human eye. Here, we present findings from our studies investigating associations between myopia risk polymorphisms and ERG parameters. TwinsUK comprises several thousand adult twins who have volunteered to participate in research studies based at St Thomas' Hospital in London. Initially, dark-adapted and light-adapted ERGs were recorded in response to international standard and experimental stimulus protocols in over 200 twins, following pharmacological pupil dilation (1.0% tropicamide supplemented in most cases with 2.5% phenylephrine), using a conductive fibre electrode placed in the lower conjunctival fornix. Stimuli were delivered using the Colordome (Diagnosys UK, Cambridge, UK). In genotyped individuals (n=186), we specifically investigated associations between allelic dosage at rs524952 and ERG parameters (amplitudes and peak times of a-waves, b-waves and 30 Hz flicker responses), using a mixed linear model, adjusting for age, sex and familial relatedness. We found significant associations with parameters relating to cone-driven retinal signals. Taken together with findings in patients with selective loss of post-receptor signals, we found evidence of a specific association with cone-driven OFF bipolar cell signals. Subsequently, we analysed ERG recordings from over 1000 twins, made with a portable device (RETeval system, LKC technologies, Gaithersburg, MD) and using skin electrodes. These were responses to 30 Hz flickering stimuli delivered through natural pupils, but with stimulus and background strength adjusted according to pupil diameter to deliver retinal illuminance equivalent to international standards. In genotyped individuals (n=895), we explored associations between 334 known myopia-risk loci and ERG flicker peak times. Although no association achieved statistical significance after correction for multiple testing, one of the top loci attaining nominal significance was rs13268738, within the *CNGB3* gene (which encodes a subunit of the cyclic nucleotide-gated channel in the outer segments of cone photoreceptors). This specific locus was then examined in the group of participants who had undergone mydriatic recordings with the conductive fibre electrode: allelic dosage was found to be significantly associated with flicker peak times in this group also, thus replicating the association found in the larger group. Overall, our findings highlight possible pathways through which these particular myopia risk loci might be acting, supporting a role for alterations in retinal cone-driven signalling in myopia development.

SA40

The landscape of genetic variants conferring susceptibility to myopia in the general population

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Genome-wide association studies have transformed our understanding of the pathophysiology of common diseases in the last 15 years, and risk prediction through polygenic risk scores is now a reality. For myopia, the first two common genetic variants were described in 2010, and within ten years almost 450 genetic loci were identified, with the power obtained through increasingly large studies, the most recent including over half a million participants. Refractive error, and myopia in particular, is not dissimilar to height genetically, in that the trait is highly heritable but that risk is transmitted through many risk variants all of relatively small effect size. In keeping with our understanding of the physiology of emmetropisation being driven by the retina sensing defocus and regulating eye growth, stimulating axial elongation where there is hyperopic defocus and stopping growth when emmetropia or myopia is reached, many genes involved in retinal signalling have been associated with myopia. Where mutations in genes cause often devastating retinal dysfunction and impair vision, the common variants identified may cause (or are markers of) relatively minor functional changes, resulting in disturbance of the normal physiological processes. In common with many other GWAS studies, there is still a limited knowledge of the mechanisms involved. One of the commonest risk variants of strongest effect is on chromosome 15, near the *GJD2* gene which encodes the Connexin-36 protein, forming retinal gap junctions. Other examples include *KCNQ5*, *GRIA4*, *CACNA1D*, *RGR*, *RDH5*, *GNB3* and *RORB*. Bioinformatic tools (eg DEPICT) show the most significant gene sets associated with myopia are 'abnormal photoreceptor inner segment morphology', 'thin retinal outer nuclear layer', 'detection of light stimulus' and 'nonmotile primary cilium'. Other genes that appear related to refractive error include those associated with circadian rhythm and pigmentation.

Hysi PG et al (2020) Nat Genet 52:401-407 Tedja MS et al (2018) Nat Genet 50:834-838

SA41

Structural insights into the mechanism of glycine transport and inhibition

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Glycine transporter GlyT1 (encoded by *SCL6A9*) is the main regulator of neuronal excitation and inhibition mediated by neurotransmitter glycine in the brain. Prolonging glycinergic signalling through selective inhibition of GlyT1 has been pursued extensively over the past two decades as a key strategy for the treatment of a broad range of neurological/psychiatric disorders including schizophrenia. GlyT1 inhibitors achieve antipsychotic and pro-cognitive effects against many symptoms of schizophrenia, however a successful drug candidate has to come. To elucidate structure-based mechanisms for inhibition and transport in GlyT1, we have investigated its complexes with a benzoylpiperazine chemotype inhibitor and substrate glycine. Using an inhibition state-specific sybody and a serial synchrotron crystallography (SSX) approach, we have determined the structure of GlyT1 at 3.4 Å resolution to reveal the selective inhibitor-bound state, adopting an inward-open conformation. More recently, we have determined the cryo-electron microscopy (cryo-EM) structure of GlyT1 at 3.3 Å resolution showing the glycine-bound inward-facing occluded conformation. The data unveil a dual nature of non-competitive inhibitors of functional transport exhibiting also competitive binding to the substrate binding site of glycine. The results provide detailed insight into the mechanism of glycine transport and reuptake inhibition and help re-evaluate efforts for the development of efficacious GlyT1 inhibitors.

SA42

The extended SLC Atlas: towards a unified view

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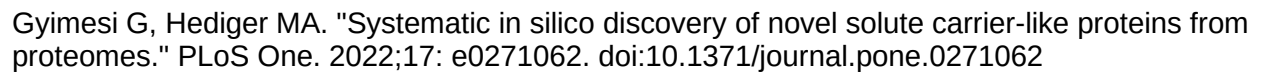
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Background and aims: Transport of solutes across various biological membranes is essential to maintain cellular homeostasis and metabolism, and its dysfunction plays a pivotal role in the development of various diseases. The Solute Carrier (SLC) superfamily represents the largest and most diverse group of membrane transporter proteins, raising significant challenges in their identification, classification and annotation. Heterogeneity of SLC members also manifests at the level of protein structures, leading to several distinct structural folds among SLC transporters. Due to the absence of conserved sequence or structural signature motifs, we hypothesized that yet unidentified SLC transporters could exist in the human genome. In our work, we have undertaken a systematic meta-analysis of available data and literature in order to discover SLC-like proteins not yet in the official nomenclature. Contrary to similar analyses, we have strived to also find SLC-like proteins that are markedly dissimilar in sequence to the currently annotated ones, as well as use available structural information to define SLC superfamilies. A complete view of the human SLC-ome will play an instrumental role in understanding human physiology and can potentially be exploited for therapeutic benefits.

Methods: As a basis of our analysis, the Transporter Classification Database (TCDB), Protein families (Pfam), Uni-Prot, Protein Data Bank (PDB) databases have been used. Sequence similarity search was carried out using sequence profile hidden Markov-models (HMMs), using either models built by ourselves for individual TCDB protein families, or models obtained from Pfam.

Results: In order to perform a top-down search of SLC-like proteins, we have derived a set of eight criteria defining “SLC-likeness” in terms of properties that can be extracted from available databases. Manual curation of TCDB protein families and corresponding Pfam models was carried out based on the textual description of the families at the TCDB and Pfam web sites, respectively, in order to filter proteins and Pfam models that violate any of our SLC-likeness criteria. The remaining 166 protein families and 217 Pfam models were then used in sequence similarity searches against the proteomes of seven clinically relevant organisms, including human, rat, and mouse. The resulting 3669 proteins, including 520 from human, have subsequently been classified into families based on their pattern of similarity (fingerprint) to individual HMMs used in the search. Our analysis gave ~120 additional (“novel”), potentially SLC-like proteins compared to previously annotated SLCs, as well as ~40 additional protein families. Subsequent literature search on the found human proteins revealed that 53 of the “novel” SLC-like proteins could be assigned a small-molecule substrate.

Conclusions: The “newly” found transporters represent proteins that might have received less attention from the scientific community due to being missing from the official SLC nomenclature. In addition, several other putative SLC-like transporter proteins have been found. Subsequent analysis of structural homologs or predicted structures can identify further evolutionary relationships between the newly defined protein families. In summary, our results pave the way



SA43

Predicting the physiological function of SLC nutrient transporters in models of symbiosis

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Our current knowledge of solute carrier (SLC) transporter proteins comes from decades of detailed functional studies and more recent structural analyses. However, it remains problematic to accurately predict function of uncharacterised SLC proteins from across all kingdoms of life. Many animals have nutritional symbiotic partnerships with microbes which are intrinsically dependent on directional transport of nutrients between species. Such microbial symbionts can aid food digestion or synthesise nutrients missing from the host animal's diet. For example, sap feeding insects such as aphids, and blood feeding insects such as lice, have intracellular symbionts who provide their host insect with essential amino acids and vitamins. For these relationships to be maintained, specific transporters must function at multiple membranes in both species, yet little is known as to the identity and function of the proteins involved. We have used knowledge of bacterial and mammalian SLC transporters to accurately predict the function of aphid transporters involved in facilitating nutritional symbiosis. By doing so we are not only advancing knowledge of the molecular mechanisms central to a fundamental aspect of invertebrate pest biology but also of how an archetypal transporter binding pocket has evolved to produce a multitude of protein functions.

SA44

The Role of SLC transporters in Host-Tumour Metabolic Interactions

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Cancer is a systemic disease that is associated with host metabolic changes, including obesity, diabetes, and cachexia/muscle wasting syndrome, each of which alters the host's metabolic and nutritional environment. Cancer cells actively acquire nutrients from the extracellular space to support their growth, but how they sense and respond to changes in systemic nutrient availability remains incompletely understood. To explore host-tumour metabolic and nutritional interactions, we use the fruit fly *Drosophila melanogaster* as a model system. Our studies have started to uncover how tumours modulate the expression of SLC transporters to respond to systemic metabolic changes. These findings provide insight into the mechanisms by which tumours adapt to changes in nutrient availability and offer a potential therapeutic strategy to target transporters for cancer treatment.

C01

Cardiovascular effects of in utero Angiotensin II exposure in a rat model of superimposed preeclampsia

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Preeclampsia is a hypertensive disorder of pregnancy that affects up to 8% of women worldwide. Preeclampsia is one of the main causes of maternal and neonatal deaths and has shown to increase the risk for the development of cardiovascular disease in the offspring later in life. This effect is worsened when preeclampsia takes place on a hypertensive background, known as superimposed preeclampsia. The association between hypertension during pregnancy and the detrimental cardiovascular impact in the offspring remains to be determined. For this reason, the development of an animal model of preeclampsia is needed to understand the deleterious effect throughout the offspring's lives. The aim of this study was to investigate the cardiovascular impact of a hypertensive *in utero* environment in the offspring of a rat model of superimposed preeclampsia.

Pregnant stroke-prone spontaneously hypertensive rats (SHRSP) were implanted with an osmotic minipump for the continuous delivery of 0.9% saline (control) or 660-700 ng/kg/min angiotensin II (ANGII) at gestational day 10.5 (n=6/group). Pregnancies progressed until birth and offspring were weighed between 1 and 7 days of age. Echocardiography and systolic blood pressure (SBP) measurements were carried out regularly on the offspring (n=16-19/group) between 5 and 17 weeks of age (W5-17), and tissues were harvested for wire myography at sacrifice (W18-19). Animal procedures were performed according to regulations established in

the Animals (Scientific Procedures) Act 1986, under the Home Office approval of Project Licence PP0895181.

Exposure to ANGII infusion during pregnancy caused significant growth restriction in the offspring (7.2 ± 2.4 (control) v. 5.9 ± 1.6 g (ANGII); Welch's t-test, $P=0.003$). Regular phenotyping of the offspring between W5 and 17 showed no significant differences in SBP between groups (151.5 ± 24.7 (control) v. 147.2 ± 22.2 mmHg (ANGII); repeated measures two-way ANOVA, $P=0.14$). In contrast, early indices of increased left ventricular mass index were apparent at 9 weeks of age in the ANGII-exposed offspring (2.0 ± 1.5 (control) v. 3.1 ± 0.5 (ANGII); repeated measures two-way ANOVA, $P=0.04$), in parallel with an elevated cardiac output compared to the control offspring (40.1 ± 24.6 v. 62.2 ± 16.1 ml/min; repeated measures two-way ANOVA, $P=0.02$), which may be part of a protective mechanism in response to the limited cardiac development in an adverse *in utero* environment. Evidence of systolic dysfunction in the offspring exposed to ANGII was observed at W17 by a reduction in fractional shortening (53.5 ± 6.9 (control) v. $45.9 \pm 6.9\%$ (ANGII); repeated measures two-way ANOVA, $P=0.01$). In addition, the E/A ratio was increased in the ANGII-exposed offspring between W5-17 (1.52 ± 0.08 (control) v. 1.94 ± 0.1 (ANGII); repeated measures two-way ANOVA, $P=0.0006$), which is evidence of diastolic dysfunction. Finally, mesenteric arteries from the offspring exposed to *in utero* ANGII showed a trend towards increased contraction in response to noradrenaline (150.7 ± 83.1 (control) v. 215.8 ± 115.9 mN·M (ANGII); Welch's t-test, $P=0.08$).

In conclusion, the offspring of the pregnant SHRSP females exposed to ANGII present a worsened cardiovascular phenotype compared to controls, evidenced by both systolic and diastolic cardiac dysfunction. The causes of this cardiovascular effect are yet to be elucidated, however, these results allow an exploration of potential links between preeclampsia and the detrimental developmental programming of the offspring.

C02

Ventricular repolarization abnormalities and arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia

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Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic cardiomyopathy most commonly caused by mutations in the ryanodine receptor (RyR2) Ca^{2+} release channel resulting in a hyperactive or “leaky” phenotype. Patients typically have normal resting ECGs but experience dangerous ventricular tachycardias during exercise- or emotional stress. Delayed afterdepolarizations (DADs) are believed to be the underlying mechanism of arrhythmia in CPVT and occur as a result of increased spontaneous Ca^{2+} release in cardiac myocytes in the form of Ca^{2+} waves during diastole that produces an inward current via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. Repolarization abnormalities are critical in other arrhythmogenic diseases, such as long QT syndrome, however this has received less attention in CPVT. This work aimed to investigate whether altered repolarization contributes to arrhythmogenesis in CPVT.

Methods

All experiments were conducted with UK Home Office and local ethical approval. Adult male and female mice with CPVT-causing heterozygous RyR2-R420Q mutation (R420Q; N=9) or wildtype littermate controls (WT; N=10) were killed by stunning and cervical dislocation. Hearts were perfused with physiological Tyrode's solution +/- isoproterenol (100 nM) using a Langendorff apparatus. Hearts were electrically paced using a pair of platinum electrodes positioned on the right ventricle. Monophasic action potentials (MAP) were recorded from the left ventricle apex using custom-made Ag/AgCl electrodes and signals digitized and recorded at 5kHz.

Results

Mean action potential duration at 90% repolarization (APD90) during steady state (sinus rhythm) was not different between WT and R420Q mice in normal Tyrode's solution ($P=0.90$). Isoproterenol shortened APD90 in WT ($P<0.05$) but not R420Q hearts. Hearts were subjected to a short burst (1s) of rapid electrical pacing then the APD was measured during subsequent sinus beats. The first APD following cessation of pacing was prolonged in R420Q hearts compared to WT ($P<0.05$). Hearts were paced to steady state at 10 Hz then an extrastimulus (S2) was delivered during repolarization. S2 elicited runs of premature ventricular complexes in 40-75% of R420Q hearts in normal Tyrode's and isoproterenol solutions, whereas these did not occur in any WT hearts. Similar electrophysiological abnormalities were observed in whole cell patch clamp recordings from isolated ventricular myocytes from R420Q mice compared to WT. Confocal Ca^{2+} imaging revealed abnormal patterns of spontaneous Ca^{2+} release occurring in R420Q myocytes that could contribute to the altered repolarization at the single cell and whole heart levels.

Conclusion

Our findings suggest that repolarization abnormalities during dynamic changes in heart activity may occur in and contribute to CPVT pathology. The functional consequences on arrhythmogenesis in the heart are currently under investigation.

C03

Blunted cAMP signalling is ameliorated by Fibroblast Growth Factor 1 in a model of diabetic cardiomyopathy using human induced pluripotent stem cell-derived cardiomyocytes

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Diabetic Cardiomyopathy (DCM) is a subset of heart failure that is a consequence of diabetes mellitus¹. The contractile dysfunction of heart failure is characterised by blunted beta-adrenergic-cAMP signalling². Insulin is known to reduce cAMP via phosphodiesterase (PDE)³, which experiences dysfunction in the diabetic state, owing to insulin resistance. Recently insulin and Fibroblast Growth Factor 1 (FGF1) signalling has been shown to be convergent⁴ and appears to be protective against DCM⁵. We tested whether cAMP signalling is impaired in a model of DCM using human induced pluripotent stem cell (hiPSC) derived cardiomyocytes, and investigated whether cAMP kinetics is altered by FGF1.

Healthy hiPSC (OX1-19, obtained from The James Martin Stem Cell Facility, Oxford) were differentiated into cardiomyocytes and cultured in either normal or high-glucose, high-fatty acid media. Cytoskeletal and nuclear staining were used to assess cell and nuclear area. ATP based cell viability and glucose reuptake were assessed using a luminescence-based assay. BNP release was measured by a NT-proBNP ELISA kit and gene expression levels were detected by bulk RNA-sequencing. Real time cAMP kinetics were investigated with a fluorescence resonance energy transfer (FRET) based sensor Epac-S^{H187}. DCM model cells expressed phenotypic changes consistent with the disease. Cells cultured in either 11 mM (n=122 cells, 2 wells) or 25 mM glucose (n=102 cells, 2 wells) had a significant increase in cell area (μm^2) compared to control (n=236 cells, 2 wells), ($p<0.0001$, Kruskal-Wallis). DCM cells (n=7 separate wells) had a significant increase in the concentration of NT-proBNP, a key hypertrophic marker, in the media assay compared to control cells (n=7 separate wells, $p<0.0001$, one-way ANOVA). DCM cells (n=7 separate wells) had a significantly lower cell viability compared to control (n=7 separate wells, one-way ANOVA, $p<0.001$). RNA-seq informed the KEGG pathway analysis were enriched in PI3-Akt, cAMP signalling, Ras-Raf (ERK-MAPK), and hypertrophic cardiomyopathy pathways. Hypertrophic genes, collagens, and ANP and BNP genes, both of which are released in failing heart, were upregulated, as was PDE4DIP. cAMP signalling in response to forskolin was lowered in the DCM model cells (n=21, $7.21\pm0.05\%$) compared to control (n=20, $9.53\pm0.12\%$, $p<0.05$). This was reversed when DCM cells were treated with FGF1 (1 ng/ml, n=19, $13.65\pm0.09\%$, $p<0.0001$).

hiPSC-derived cardiomyocytes cultured in high-glucose, high-fatty acid media produce characteristics of DCM. cAMP signalling was blunted in DCM cells. This response was ameliorated by increasing FGF1. Gene expression changes point to a novel type of FGF1 signalling pathway in the heart, which might lead to improved contractility, as postulated in Figure 1.

Figure 1 - A model of the convergent signalling of insulin and FGF1. The red arrow indicates a hypothetical pathway where FGF1 may be blunting PDE4 activity that is responsible for the increase in cAMP.

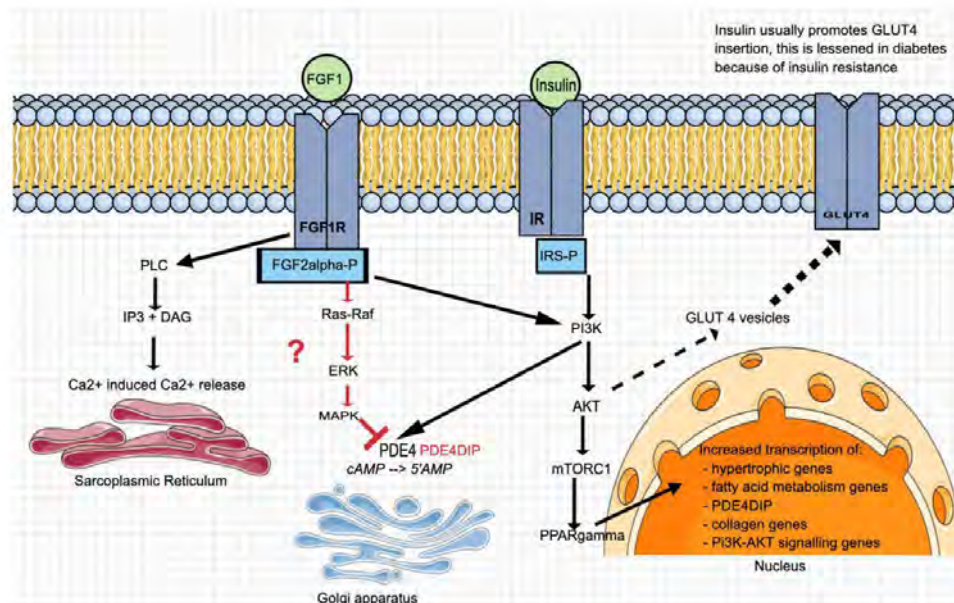


Figure 1 - A model of the convergent signalling of insulin and FGF1. The red arrow indicates my hypothesis: FGF1 may be blunting PDE4 activity, responsible for the increase in cAMP.

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C04

Neuropeptide Y signalling in human induced pluripotent stem cell derived cardiomyocytes

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Background: Acute myocardial infarction and chronic systolic heart failure result in heightened cardiac sympathetic activation¹, driving release of the co-transmitter neuropeptide-Y (NPY)², with circulating levels correlating with morbidity and mortality in both conditions³. The acute physiological effects of NPY on cardiac excitability are well studied⁴, but the effects of chronic NPY exposure, which may be important for subsequent ventricular remodelling, are poorly characterised. We therefore investigated this using human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs).

Methods and Results: Immunohistochemistry and western blot analysis of hiPSC-CMs (SFC845, obtained from The James Martin Stem Cell Facility, Oxford) demonstrated NPY1R, NPY2R and NPY5R expression in these cells, receptors which were also found in ventricular biopsies from human patients undergoing valve surgery using qPCR (n=5). Incubation with NPY over 2 days significantly (Kruskal-Wallis, $p < 0.0001$) increased cell area (following cytoskeletal immunofluorescent staining) at 1 nM (1669 [1242-1669] μm^2 , n=98 cells), 10 nM (2163 [1531-2657] μm^2 , n=94 cells) and 100 nM (1780 [1294-2399] μm^2 , n=60 cells) compared to control (1126 [842.7-1603] μm^2 , n=96 cells). NPY (10nM) also significantly increased ANP and BNP expression assessed by qPCR (unpaired t-test, $p < 0.05$, n=6 wells) and reduced luciferase-based ATP cell viability (unpaired t-test, $p < 0.05$, n=6 wells). NPY-mediated cell enlargement and cell viability reduction could be blocked with a NPY1R antagonist (BIBO 3304, 1 μM : 1414 [873.6-2387] μm^2 , n=33 cells vs NPY+BIBO: 1167 [761.5-1659] μm^2 , n=125 cells) and reversed with a NPY5R antagonist (CGP 71683A, 1 μM : 1488 [1051-2224] μm^2 , n=49 cells vs NPY+CGP: 1222 [688.4-2158] μm^2 , n=73 cells, MWU test, $p < 0.05$), which did not occur in the presence of an NPY2R antagonist (BIIE 0246, 1 μM : 1047 [699-1686] μm^2 , n=75 cells vs NPY+BIIE: 1517 [891.2-2287] μm^2 , n=76 cells, $p < 0.05$). The Forster resonance energy transfer-based sensor Epac-S^{H187} was expressed in hiPSC-CMs to monitor the cyclic adenosine 3',5'-monophosphate (cAMP) response to NPY. 1 and 10 nM NPY reduced intracellular cAMP levels, whilst higher doses of NPY (100 nM) slightly increased cAMP (n=65 cells). An NPY5R antagonist most effectively inhibited these cAMP changes ($p < 0.0001$, n=40 cells).

Conclusions: In hiPSC-CMs, chronic NPY exposure promotes cellular hypertrophy and reduces cell viability via NPY1R and NPY5R signalling, associated with G_i-coupled pathways. NPY1R and NPY5R may represent potential drug targets to treat chronic heart failure.

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2. Tan, C. M. J. et al. The Role of Neuropeptide Y in Cardiovascular Health and Disease. *Frontiers in physiology* **9**, 1281 (2018).

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1. Borovac, J. A., D'Amario, D., Bozic, J. & Glavas, D. Sympathetic nervous system activation and heart failure: Current state of evidence and the pathophysiology in the light of novel biomarkers. *World journal of cardiology* **12**, 373-408 (2020). 2. Tan, C. M. J. *et al.* The Role of Neuropeptide Y in Cardiovascular Health and Disease. *Frontiers in physiology* **9**, 1281 (2018). 3. Gibbs, T. *et al.* Neuropeptide-Y Levels in ST-Segment–Elevation Myocardial Infarction: Relationship With Coronary Microvascular Function, Heart Failure, and Mortality. *Journal of the American Heart Association* **11**, e024850 (2022). 4. Kalla, M. *et al.* The cardiac sympathetic co-transmitter neuropeptide Y is pro-arrhythmic following ST-elevation myocardial infarction despite beta-blockade. *Eur. Heart J.* **41**, 2168-2179 (2020).

C05

ST-elevation myocardial infarction (STEMI) alters iron status

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Introduction-HePCidin is the homeostatic hormone that controls iron status. Its regulation by inflammation, primarily interleukin IL-6, underpins iron deficiency of chronic disease. Iron deficiency is a recognised co-morbidity in chronic and acute heart disease. While hePCidin and iron status have been characterised in chronic heart failure, little is known about how they change in acute heart events. To address this gap in knowledge, we conducted a sub-study of ASSAIL-MI: a randomised double-blinded placebo-controlled trial of the effect of IL-6R antagonist tocilizumab in patients with acute ST-elevation myocardial infarction (STEMI)¹.

Methods- 200 patients with first-time STEMI presenting within 6 hours of the onset of chest pain were randomised to receive tocilizumab or matching placebo prior to percutaneous coronary intervention (PCI). Plasma hePCidin, IL-6, serum iron, transferrin saturation (Tsat) and Haemoglobin were measured at baseline, then 1 day, 3-7 days, 3 months and 6 months post infusion. Mixed effect modelling was implemented, controlling for the multiple measurements by patient as random intercept, and examining up to three-way interactions.

Results- STEMI was followed by a rapid rise from baseline in plasma hePCidin in the placebo group, whereas hePCidin levels decreased in the Tocilizumab group during the same interval. In both groups, plasma hePCidin levels returned to baseline values by 3 months. Plasma hePCidin levels correlated with IL-6 and the size of the myocardial area at risk in the placebo but not the tocilizumab group. STEMI was immediately followed by a decrease in serum iron, Tsat and haemoglobin, but only in the placebo group.

Conclusions- STEMI causes an acute rise in hePCidin, and corresponding decrease in plasma iron availability. IL-6 and myocardial injury likely drive these changes. The impact of these changes on clinical outcomes, on the benefits or otherwise of tocilizumab and on the management of iron status must be examined.

C06

Relaxation of cardiac pericytes contributes to cardioprotection mediated by remote ischaemic preconditioning

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Introduction: Pericytes are contractile cells wrapped around capillaries. They can evoke capillary constriction and dilation. We have previously demonstrated the crucial role that cardiac pericytes play in ischaemia-induced no-reflow following coronary artery block (O'Farrell et al., 2017). Here, we report improvement of no-reflow after ischaemia following remote ischaemic preconditioning (RPC). RPC relaxes cardiac pericytes via a GLP-1R-mediated mechanism.

Methods: Pentobarbital anaesthetized Sprague-Dawley rats were subjected to 45 min LAD occlusion followed by 15 min reperfusion. RPC was induced by 15 min occlusion of both femoral arteries. Systemic GLP-1R blockade was achieved by i.v. injection of the specific antagonist Exendin(9-39) (50µg/kg, Tocris) prior to RPC. At the end of the experiment, rats were perfused with FITC-albumin, and cardiac capillary perfusion was analysed using FIJI software (ImageJ 1.53c, NIH) following immunostaining. For dissection of molecular mechanisms, live cardiac tissue from NG2-DsRed mice (right free ventricular wall) was perfused intralumenally with modified Tyrode's solution via a glass cannula introduced into the right coronary artery, and imaged using a Zeiss confocal LSM780 microscope. The GLP-1R agonist Exendin-4 (Ex4, 100nM, Tocris), and/or the ATP-sensitive K⁺ (K_{ATP}) channel inhibitor glibenclamide (20µM, Insight Biotechnology) were applied in oxygenated Tyrode's solution. Oxygen/glucose deprivation was achieved by replacing glucose with sucrose in the Tyrode's solution and bubbling with 95% N₂/5% CO₂. Capillary diameters were measured using FIJI software.

Results: LAD occlusion-reperfusion (n=5 rats) blocked 69±4% of cardiac capillaries near pericyte somata vs 9±4% in sham controls (n=6; p<0.001, Tukey multiple comparisons test), which resulted in a 40% reduction of perfused blood volume within the affected region. RPC prevented pericyte constriction and capillary blockage (reduced to 28±8%, n=5; p<0.01). Ex(9-39) prevented RPC-mediated relaxation of cardiac pericytes (64±10%, n=5). Ex vivo, 25 min of OGD resulted in cardiac pericyte constriction and a decrease of capillary diameter to 84.6±1.3% (n=24 capillaries in 7 mice) compared to the baseline diameter. GLP-1R activation with Ex4, applied from 25 min of a 50 min period of OGD, relaxed pericytes and dilated capillaries back to the baseline diameter (98.4±2.0%, n=9 capillaries in 4 mice, p<0.0001). In the presence of glibenclamide, GLP-1R-mediated pericyte relaxation was abolished, and capillaries remained constricted at 87.3±2.2% of their original diameter (n=13 capillaries in 3 mice, p<0.01).

Conclusion: Cardioprotective effects of GLP-1 agonists are mediated, at least in part, by relaxation of cardiac pericytes. The downstream molecular mechanism of GLP-1R activation involves opening of K_{ATP} channels. Cardiac pericytes are therefore a novel therapeutic target in ischaemic heart disease.

O'Farrell FM, Mastitskaya S, Hammond-Haley M, Freitas F, Wah WR & Attwell D (2017) Capillary pericytes mediate coronary no-reflow after myocardial ischaemia. *Elife* 6: e29280.

C07

Ultrastructural dynamics of contracting cardiomyocytes

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Introduction: The structure and function of cardiomyocytes (CM) are tightly interlinked. However, ultrastructural dynamics of CM during the cardiac action potential, including mechanical organelle deformation, are poorly understood. Dynamics of contracting CM are conventionally resolved using light microscopy, a modality with orders of magnitudes lower spatial resolution than electron-based imaging.

Aims: The aim of this study is to develop and apply a methodology for the acquisition and analysis of ultrastructural dynamics of CM under control conditions and following microtubule (de-)stabilisation. Through this study, we aim to gain a better understanding of the ultrastructural dynamics of CM during cardiac contraction, which may have important implications for our understanding of cardiac function and mechanosensitivity.

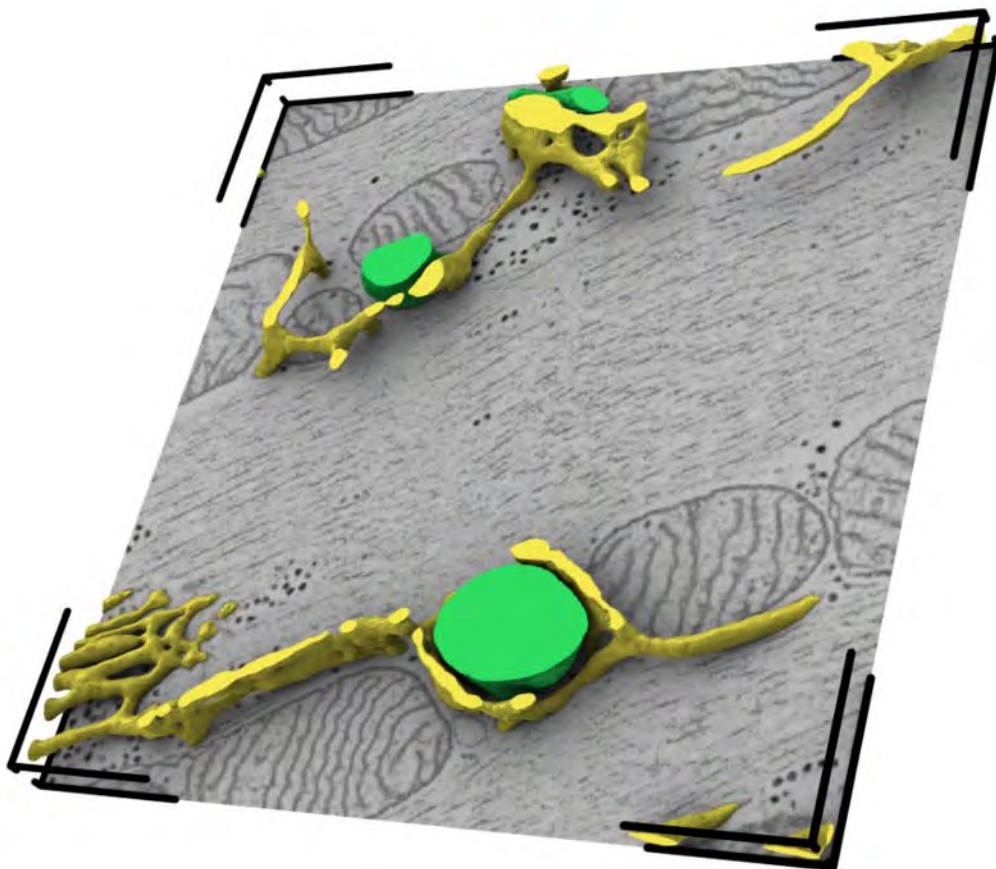
Methods: Here, we use action potential-synchronised high-pressure freezing to assess the ultrastructural dynamics during CM contraction with dual-axis electron tomography, resulting in a spatial resolution of (1.2 nm)³ and millisecond temporal resolution. CM were isolated from precision-cut left-ventricular New Zealand white rabbit tissue slices (N=8 animals). We used pharmacological interventions (paclitaxel and colchicine) to stabilise and destabilise microtubules. CM were high-pressure-frozen at time intervals corresponding to rest and peak contraction, freeze-substituted, heavy metal-stained, resin-embedded, and cut into 300 nm sections. Then, CM fragments were imaged using electron tomography on a 300 kV transmission electron microscope. The resulting images were reconstructed and segmented utilising fully convolutional neural networks into 3D organelle models. We developed custom software ('SegmentPuzzler') to proofread and correct automatic segmentations. We developed a portable, interactive browser-based visualization tool to foster a deeper comprehension of the otherwise unwieldy (TB-sized) image data and reconstructions. Statistical significance was assessed using the Kruskal-Wallis test and Dunn's posthoc test. All investigations reported in this article conformed to the German (TierSchG and TierSchVersV) animal welfare laws, compatible with the guidelines stated in Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, and they were approved by the local Institutional Animal Care and Use Committees in Germany (Regierungspräsidium Freiburg, X-16/10R).

Results: Using this workflow, we generated and visualised 353 3D reconstructions of CM organelles, including the sarcoplasmic reticulum and the transverse-axial tubular system (Figure 1). We analysed dyad dimensions, coupling distances, and deformation of the t-tubular geometry. In control conditions, we observed a t-tubular squeezing effect ($p < 0.0001$), which could not be detected after the acute (de-)stabilization of microtubules ($p > 0.05$). The presence

or absence of microtubules had no significant acute effect on dyad proximity ($p>0.05$).

Conclusion: Our proof-of-principle study resolves the structural dynamics of CM in a nanoscopic, 3D, and millisecond-accurate manner. Precisely understanding the ultrastructure and its modulation, ultimately in human CM under both physiological and pathophysiological conditions, is expected to advance our current understanding of the ultrastructural foundations of cardiac diseases, and their diagnosis and treatment.

Figure 1: Time-resolved 3D organelle model of the sarcoplasmic reticulum (yellow) and transverse-axial tubular system (green). Cardiomyocytes in this reconstruction were high-pressure-frozen in a relaxed state (0 ms offset to the action potential initiation). The reconstruction has a dimension of $3.14\ \mu\text{m} \times 3.14\ \mu\text{m} \times 180\ \text{nm}$ and a voxel size of $(1.2\ \text{nm})^3$.



C08

Characterisation of a 3D placenta-on-a-chip model utilising trophoblasts differentiated from human induced pluripotent stem cells

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Motivation

Many of the diseases relating to placental dysfunction occur in early or mid-gestation, yet our ability to study placenta dysfunction often relies on sourcing trophoblasts from either term-placenta or trophoblasts cell lines. Alternately we rely on large or small animal models to measure placental function. Arguably, all lack physiological relevance to human placental-dysfunction. Some advances have been made in placenta-on-a chip and organoids but so far, the technology has not advanced to accommodate high throughput studies required for drug discovery. We aimed to develop and validate a 3D placenta-on-a-chip model that can be used in high-throughput system using a methodology to derive-trophoblast from human induced pluripotent stems cells (HiPSC).

Methods

A commercially available HiPSC line (ChiPS4) were cultured on a ECM-collagen scaffold and seeded into Petri dish or one lane of a 3-lane OrganoPlate (Mimetas) both pre-coated with Geltrex for 2D and 3D culture respectively. A differentiation protocol was adapted from Amita et al (1). Organoplates were rocked to induce perfusion and tube formation, and the parallel lane was coated with collagen-1. Trophoblast and pluripotent markers at the RNA (PCR, RNAseq) and protein level (immunoblot, immunohistochemistry) were investigated over differentiation days(D)0-6. The integrity of the POC barrier was assessed daily (D0-6) by adding 10kDa or 155kDa- dextran linked to a fluorescent dye to the perfusable channel and imaged (Incucyte) over 20mins.

Results

In both 2D and 3D HiPSC culture differentiation protocol induced up regulation of trophoblast markers (KRT-7, GATA3, PGF ,HLA) from D2-6 of differentiation, as determined by qRT-PCR (n=4 repeats)) and immunoblotting (n=4). In accordance, down-regulation of pluripotent markers (Nanog, POU51, TBXT) at day 2 compared to day 0. In 2D culture, immunohistochemical staining of KRT7 was absent at D0 and present on D2, whereas the reverse was observed for Nanog, clearly showing trophoblast development. The HiPSC-trophoblast differentiated in the OrganoPlate formed a hollow tube-structure from D3, against the parallel channel containing ECM-collagen. RNAseq (n=4) at D2 was used to observe the changes in clusters of genes (>20) associated with different trophoblast types. At D4 differentiation there was an increase in syncytiotrophoblast whereas down-regulation of extra-villainous trophoblasts gene-clusters. Interestingly, immunochemistry showed a defined area in close proximity to the ECM appeared as a preferential site for cell fusion and β -hCG production. The placenta-on-a-chip formed a leak tight barrier from D4 differentiation which retained the 155kDa and 10kDa-dextran in the placenta-on-a-chip. Comparison of 2D and 3D culture by RNAseq showed good similarity,

but interestingly in 3D culture there were enhanced representation of total genes in GO terms and Reactome pathways associated with ECM, growth factor and interferon signalling pathways.

Conclusion

We successfully developed and characterised a 3D placenta-on-a-chip model using HiPSC derived trophoblasts in perfusable multi-chip OrganoPlates. Our placenta-on-a-chip model provides an exciting potential to replace animal studies to measure maternal-fetal barrier. Moreover, the current system provides the ability for robust high throughput studies for 40-POC per plate, in a more physiologically relevant system, than current 2D- primary trophoblasts or cell lines.

1. Amita M, Adachi K, Alexenko AP, et al. Complete and unidirectional conversion of human embryonic stem cells to trophoblast by BMP4. *Proc Natl Acad Sci.* 2013;110(13):E1212-E1221. doi:10.1073/pnas.1303094110

C09

The Impact of Eicosapentaenoic and Docosahexaenoic Acid on Arterial Pressure: A meta-analysis of Randomised Controlled trials

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Introduction: Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are Omega-3 fatty acids which have been shown to reduce the risk of cardiovascular disease in humans primarily by reducing blood pressure.

Aims: to investigate the impact of EPA and DHA on arterial pressure in healthy adults, and identify factors which may cause variation in this relationship

Methods: a random effects meta-analysis was carried out to establish the impact of EPA and DHA on Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Mean Arterial Pressure (MAP) and Pulse Pressure (PP). A systematic search was carried out in PubMed, Embase and the Cochrane Library to identify studies which;

1. Were conducted as a single- or double-blind, randomised, placebo-controlled trial
1. Used EPA and/or DHA as a nutritional supplement
1. Lasted a minimum of 42 days
1. Used healthy adults as participants (>18 years old, and free of metabolic and cardiovascular disease with the exception of hypertension)
1. Measured SBP and DBP at both baseline and at the end of the study

On instances when data was not available from the published manuscript, a data request was made by contacting the corresponding author. Where MAP and PP were not recorded, they were calculated through the following formulas;

$$\text{MAP} = (\text{SBP} + \text{DBP} \times 2) / 3$$

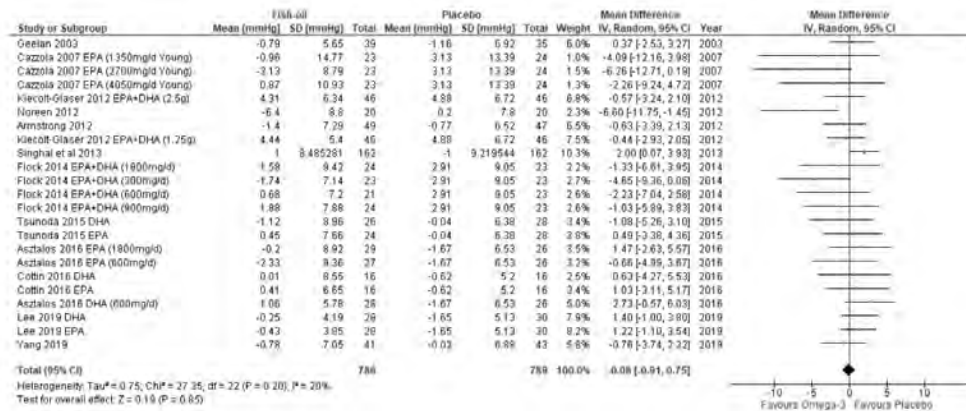
$$\text{PP} = \text{SBP} - \text{DBP}$$

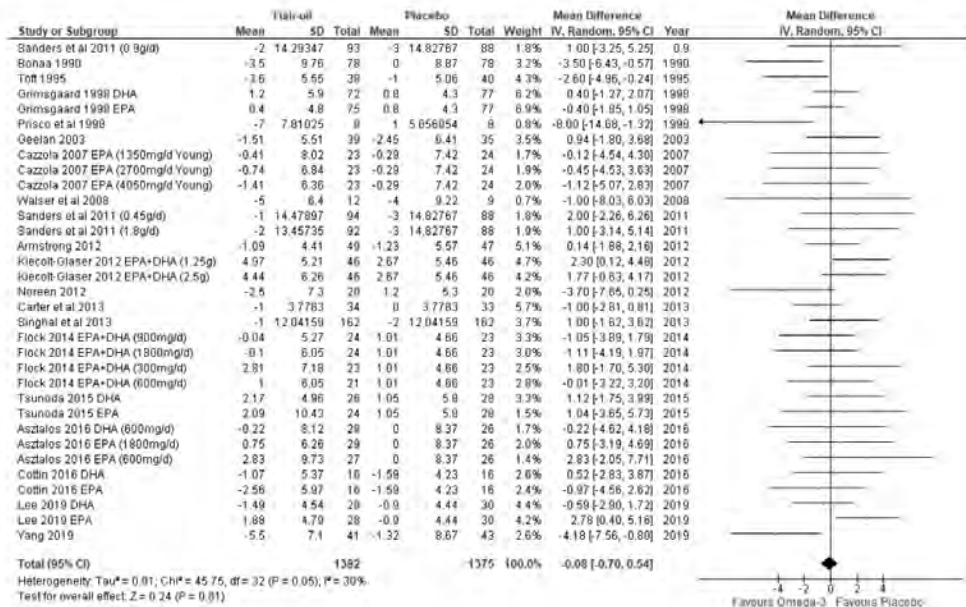
All articles were dual screened by two independent researchers with disputes being resolved by a third if necessary. Bias was assessed through visual inspection of a funnel plot, followed by the performing the Cochrane RoB2 assessment on each trial.

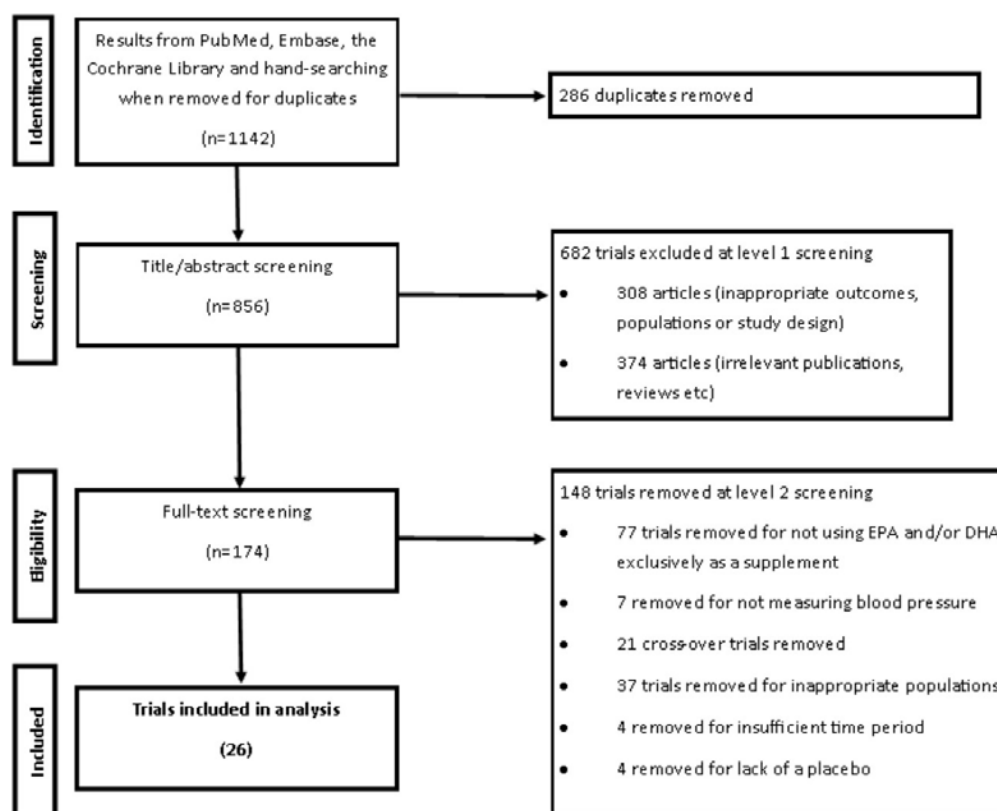
Results: a total of 26 studies were included between the years of 1989-2020 with a total of 3081 participants. There were non-significant reductions in SBP of 0.71 (95% CI's, -1.47 to 0.06; P=0.07), DBP of 0.25mmHg (95% CI's, -1 to 0.51; P=0.052), MAP of 0.08 (95% CI's, -0.7 to 0.54; P=0.81) and PP of 0.08 (95% CI's, -0.91 to 0.75; P=0.85). Subgroup analysis revealed that supplements using EPA or DHA as the predominant fatty acid had no significant impact in

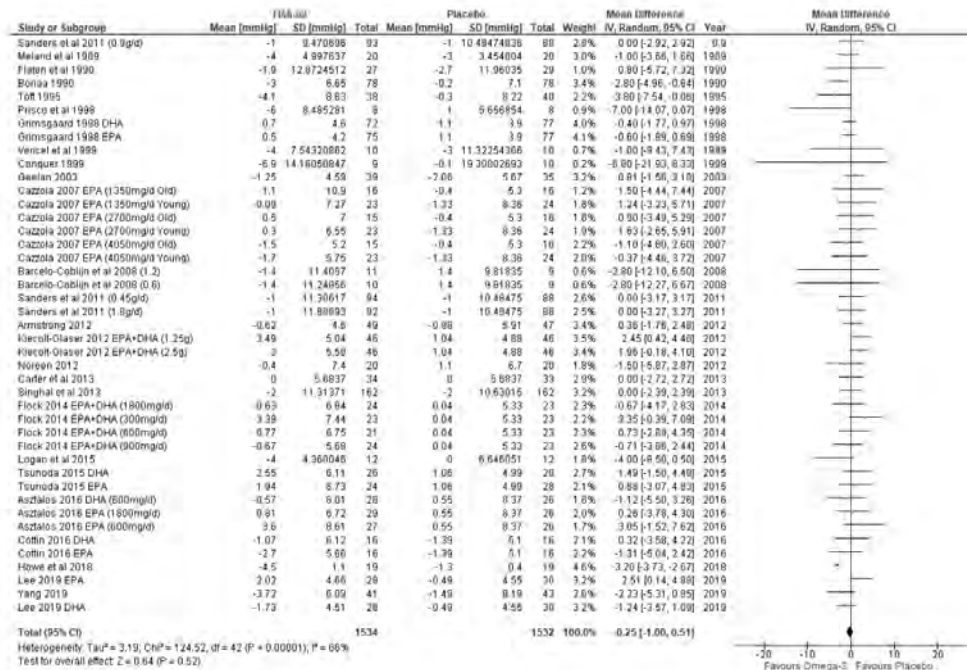
any of the outcomes, whereas supplements using similar concentrations of EPA and DHA had a significant reduction in SBP of 1.84 (95% CI's, -3.11 to -0.57; $P=0.004$). Furthermore, studies using corn oil as a placebo were significantly more likely to show a reduction in SBP ($\Delta=-1.82$; CI's, -2.62 to -1.03; $P<0.0001$) and DBP ($\Delta=-1.23$; 95% CI's, -2.43 to -0.02; $P=0.05$).

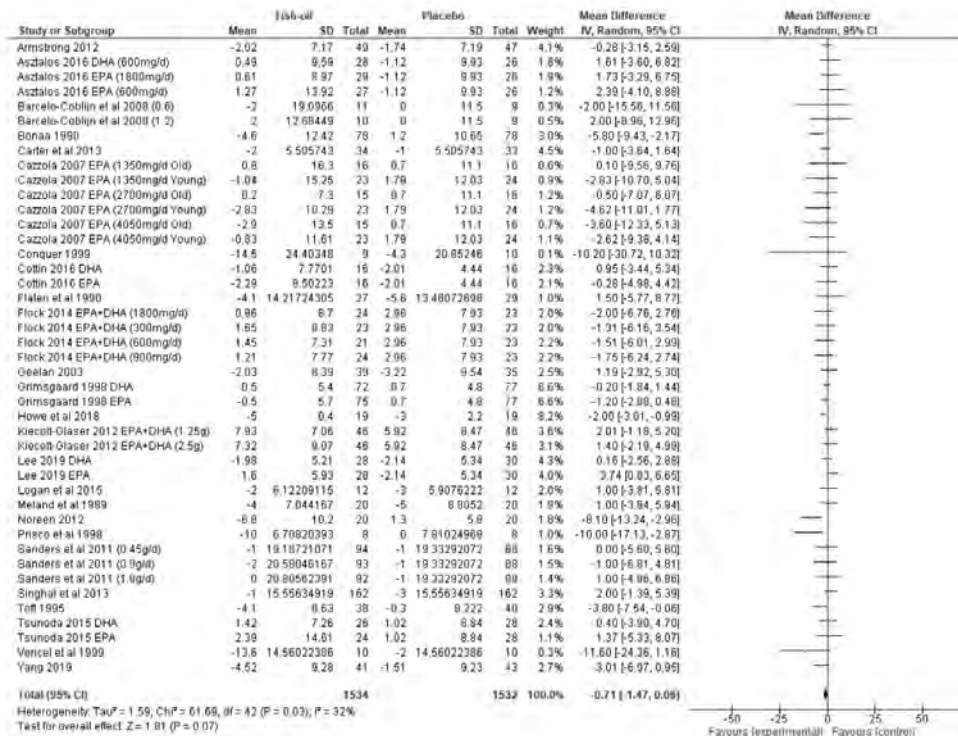
Conclusions: as most previous meta-analyses have generally found significant reductions in blood pressure following supplementation with EPA and DHA, it is possible that the lack of significance in this study could be due to the exclusion of participants with cardiovascular or metabolic disorders. It is also possible that the exclusion of studies using dietary interventions may have had an impact. Finally, it appears that the results of studies examining the impact of EPA and DHA on arterial pressure are dependent on the ratio of EPA to DHA, as well as the placebo supplement used.











C10

A novel patch-clamp based method for stimulating monolayers of human induced pluripotent stem cell-derived atrial-like cardiomyocytes reduces electrophysiological heterogeneity and promotes consistent responses to SK channel inhibition

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Introduction: Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) represent a useful *in vitro* model of cardiac function. Isolated iPSC-CMs, however, exhibit significant electrophysiological heterogeneity which hinders their utility as a model system for the study of certain individual cardiac currents [1]. Differentiation of iPSC-CMs using conventional methodologies produces cells which exhibit a ventricular-like phenotype, but the inclusion of retinoic acid (RA) during differentiation produces iPSC-CMs with an atrial-like phenotype [2, 3]. In the adult heart, the current mediated by small conductance, calcium-activated potassium channels (I_{SK}) is an atrial-selective current [4, 5]. Functional expression of I_{SK} within atrial-like iPSC-CMs has not been well investigated.

Aims: The present study therefore aimed to investigate atrial-like iPSC-CMs as a model system for the study of I_{SK} .

Methods: iPSC-CMs were differentiated in the presence of RA or a DMSO control in order to generate cells with a more atrial- or ventricular-like phenotype respectively. Following differentiation, iPSC-CMs were dissociated and plated sparsely as isolated cells (RA- or DMSO-iPSC-CMs) or plated densely to promote reformation of a confluent monolayer (RA- or DMSO-iPSC-MLs). All data are presented as mean $\pm$ S.E.M and statistical comparisons represent Student's t-tests (with Welch's correction where appropriate), unless otherwise stated.

Results: Although isolated RA-iPSC-CMs exhibited an atrial-like phenotype, they responded poorly to SK channel inhibition by UCL1684, with only 17.6% of cells exhibiting I_{SK} ($n = 17$). Isolated RA-iPSC-CMs exhibited substantial heterogeneity of spontaneous action potential (AP) duration (APD). APD heterogeneity was significantly smaller ($p < 0.001$; F-test) when spontaneous APs were recorded from *in situ* RA-iPSC-MLs, demonstrating that maintenance of the monolayer reduces electrophysiological variability.

A method for simultaneous electrical stimulation of iPSC-MLs and whole-cell recording has not previously been published to the best of our knowledge. Accordingly, we have developed a novel method for localized stimulation of iPSC-MLs which allows concurrent whole-cell patch clamp recordings to be made at a user-defined stimulation rate. Using this method >95% of RA-iPSC-MLs and DMSO-iPSC-MLs could be paced at 1 Hz. RA-iPSC-MLs ($n = 53$) paced at 1 Hz exhibited a more atrial-like phenotype than DMSO-iPSC-MLs ($n = 45$) as characterised by abbreviated repolarisation at APD₃₀ (40.5 ± 4.0 [RA] vs. 128.8 ± 4.6 ms [DMSO]; $p < 0.0001$) and APD₉₀ (220.8 ± 13.3 [RA] vs. 283.4 ± 10.2 ms [DMSO]; $p < 0.001$); and a lower AP and plateau amplitude (101.6 ± 1.6 mV and 82.2 ± 2.8 mV respectively) than DMSO-iPSC-MLs (113.0 ± 2.1 mV and 110.0 ± 2.2 mV; $p < 0.0001$ for both). Prolongation of APD₅₀ by application of UCL1684 was significantly larger in RA-iPSC-MLs ($18.7 \pm 3.0\%$; $n = 12$) than in DMSO-iPSC-

MLs ($4.2 \pm 2.6\%$; $p < 0.01$; $n = 12$). In contrast to data from isolated RA-iPSC-CMs, 100% of RA-iPSC-MLs responded to SK channel inhibition.

Conclusions: These data demonstrate that RA-iPSC-MLs represent a useful model for the study of I_{SK} . Moreover, this novel method of iPSC-ML stimulation may be of wider value in the study of other ion channels that are inconsistently expressed in isolated iPSC-CMs.

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C11

Effect of altered lipid trafficking on the modulation of vascular tone by the TMEM16A chloride channel

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Background The TMEM16A Ca²⁺-activated Cl⁻ channel plays a key role in the control of vascular tone and blood flow. TMEM16A has a pore with sections exposed to plasmalemmal lipids (1-3). This structural arrangement may confer the channel sensitivity to plasmalemmal lipids, including phosphatidylinositol 4,5-bisphosphate (PIP₂) (4). The lysosomal NPC1 protein regulates cellular distribution of lipids (5). Loss-of-function mutations in *NPC1* lead to Niemann-Pick disease Type C (NPC) a prematurely fatal neurodegenerative disorder with a range of systemic alterations including vascular (5). Here, we ask whether TMEM16A is modulated by NPC1 and examine the impact of this modulation on the tone of isolated systemic arteries, where TMEM16A is highly expressed.

Methods Whole-cell patch-clamp recordings of native and cloned TMEM16A currents, isometric tension recordings, confocal imaging and Förster Resonance Energy Transfer (FRET) were used in this study. For patch-clamp recordings, the external solution contained (mM): 150 NaCl, 1 CaCl₂, 1 MgCl₂, 10 glucose, 10 D-mannitol, and 10 HEPES (pH 7.4); the pipette solution contained (mM): 130 CsCl, 10 EGTA, 1 MgCl₂, 10 HEPES and 8 CaCl₂ (pH 7.3). The tone of isolated artery rings obtained from mice carrying *Npc1* deletion (*Npc1*^{-/-}) before and after 5-week treatment (4g/kg/week) with 2-hydroxypropyl-β-cyclodextrin (bCD), was assessed via wire myography. Expression of mRNA for phospholipase C (PLC) isoforms was conducted via quantitative RT-PCR (qRT-PCR). Data are given as mean±SEM alongside the number of independent experiments.

Results Heterologously expressed TMEM16A currents were enhanced by 2.3±0.3 fold (n=14) during pharmacological inhibition of NPC1 or by 2.6±1.0 fold (n=15) as a consequence of genetic deletion of the *Npc1* gene (knockout). These increases were prevented by treatment with b-cyclodextrin or re-introduction of *Npc1* gene in the knockout cells. The activation of cloned TMEM16A currents by NPC1 inhibition was independent on KCNE1, a proposed TMEM16A auxiliary subunit. Depletion of plasmalemmal PIP₂ or an inactivating mutation in the channel PIP₂ binding site (TMEM16A-R482A), prevented TMEM16A activation during NPC1 inhibition. Artery (aorta and mesenteric) rings obtained from *Npc1 null* mice showed increased contractility in response to phenylephrine, which was prevented by Ani9, a selective TMEM16A inhibitor and enhanced by increasing the depolarising Cl⁻ gradient. The underlying mechanism involves augmented plasmalemmal PIP₂ levels during NPC1 inhibition, assed via genetically-encoded PH-PLCδ domains and FRET imaging. The plasmalemmal PIP₂ level was increased by 1.2 ±0.6 fold (n=67) during pharmacological inhibition of NPC1 and was rescued by treatment with b-cyclodextrin. This change in PIP₂ homeostasis was presumably caused by reduction in the expression of phospholipase C during NPC1 inhibition, assessed using qRT-PCR.

Conclusions PIP₂-dependent changes in TMEM16A activity may form the basis of vascular overactivity during pathology caused by loss of NPC1 function and establish a role for the lysosome in the control of cell excitability and vascular tone.

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C12

Systemic shear stress sensing and coronary microvascular network are altered in a mouse model of heart failure with preserved ejection fraction

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Heart Failure with preserved Ejection Fraction (HFpEF) is a cardiovascular disease characterized by diastolic dysfunction and microvascular rarefaction. Affecting more women than men, its main risk factors include advanced age and comorbidities like obesity, type 2 diabetes, and renal dysfunction. Shear stress (SS) homeostasis, i.e., maintenance of SS value upon hemodynamic change, contributes to vascular network structural and functional efficiency. It is achieved through SS sensing by endothelial cells (EC), a process involving EC planar cellular polarity (PCP). We hypothesized that systemic alteration of SS sensing and SS homeostasis disruption are involved in HFpEF, and associated with architectural impairment of the coronary microvasculature.

Using a mouse HFpEF model, the aim of this study was to determine the systolic (SS_{sys}) and diastolic (SS_{dia}) SS of the carotid, the carotid EC orientation and polarity, and the coronary capillary network density and connectivity.

All procedures were done accorded with current national and European legislation, and agreed by the local ethical committee. 14-week-old female C57BL/Ks mice, a genetic background predisposing to renal dysfunction, and deficient for leptin receptors, inducing obesity and type 2 diabetes, were used as HFpEF model. 8-week-old C57BL/6J mice, lacking HFpEF risk factors, were used as healthy controls with 50% sex ratio, since no difference were found between sex for the studied parameters. SS_{sys} and SS_{dia} were calculated from the vascular diameter and maximal blood velocity (MBV) measured on the right common coronary artery (RCCA) by ultrasound imaging on anesthetized mice. After sacrifice, RCCA EC were labelled for the nucleus (DAPI) and the Golgi apparatus (Golp4) and imaged by confocal microscopy. EC orientation was measured as the angle of the nucleus-Golgi vector with blood flow direction, and classified as dromic (0° - 60°), lateral (60° - 120°), or antidromic (120° - 180°). EC polarity was defined as nucleus elongation (major/minor axis ratio). Volumic (per mm^3) vascular density (VD), segment number (SN), node number (NN), and total capillary length (TCL) of the left ventricle capillary network were calculated on processed light-sheet 3D microscopy images of lectin-labeled optically cleared hearts. Quantitative data are expressed as mean $\pm$ standard deviation and compared using Student *t* test. EC orientation angle distributions were compared by chi2 test. Results were considered statistically significant for $p < 0,05$, either non-significant (ns).

Compared to healthy mice (n=14), HFpEF mice (n=8) showed no significant changes in systolic and diastolic MBV nor SS_{dia} , but significant SS_{sys} decrease (Table). In healthy (566 cells, 14 mice) vs HFpEF (243 cells, 7 mice) RCCA, EC orientation was 23 vs 33 % dromic, 22 vs 27 % lateral, and 55 vs 40 % antidromic, respectively, and the distribution statistically different ($p=0.0006$), whereas EC nucleus elongation ratio was 1.83 ± 0.2 vs 1.97 ± 0.2 (ns). In healthy (n=14) vs HFpEF mice (n=7), VD was 46.6 ± 9 vs $39.6\pm6\%$ ($p = 0.048$), SN was $495,000\pm92,000$

vs $402,000 \pm 68,000$ ($p = 0.019$), NN was $265,000 \pm 49,000$ vs $220,000 \pm 39,000$ ($p = 0.039$), and TCL was 10 ± 1.3 vs 8.3 ± 1.3 m ($p = 0.008$), respectively.

In conclusion, HFpEF mice exhibited systolic SS homeostasis disruption associated with EC PCP alteration and coronary capillary network pattern impairment.

Table : Systolic (sys) and diastolic (dia) maximal blood velocity (MBV) and shear stress (SS) values in the right common coronary artery of healthy control (n=14) and HFpEF (n=8) mice.

	Healthy (mean±SD)	HFpEF (mean±SD)	
MBV _{sys} (mm.s ⁻¹)	967±244	841±234	ns
MBV _{dia} (mm.s ⁻¹)	177±64	158±15	ns
SS _{sys} (Pa)	29±7	23±5	<i>p</i> =0.021
SS _{dia} (Pa)	6.7±2.7	5.4±2.7	ns

C13

Escape “The Emergency Department”: design and evaluation of a digital escape room to encourage medical students to apply their knowledge of cardiac electrophysiology

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Physiology is a vital component to medical curricula, but to succeed as future doctors, medical students must learn to *apply* their knowledge of physiology to clinical scenarios. Although teachers can design classroom sessions that incorporate this critical skill, many students seek online resources to supplement their studies, and the majority of these resources focus on passive learning (e.g. watching videos). Therefore, there is a need for interactive physiology revision/consolidation resources that facilitate application of knowledge and that can be accessed by students in their own time.

The aim of this study was to design a digital escape room on the topics of cardiac electrophysiology and arrhythmias and evaluate whether the resource can support learning and enjoyment of physiology outside of the classroom.

The digital escape room – “*The Emergency Department*” – was created on a freely-accessible WordPress site (<http://activephysiology.com>) as a series of interactive puzzles (e.g. H5P). The escape room incorporated a countdown timer (Hurrytimer) and password protected elements (Passster) for a more realistic gaming experience. The activity was timetabled as a synchronous, remote, group learning activity for second year medical students (Keele University) as part of their cardiovascular pathophysiology module. Student engagement was evaluated by comparing the number of puzzle page views vs. exits and puzzle difficulty was quantified using the average time spent on each puzzle page (Google Analytics). Students’ perception of the digital escape room’s difficulty, functionality, and usefulness as a revision/consolidation exercise was assessed using a feedback questionnaire (Microsoft Forms). Ethical approval for the study was granted by The Keele Institute for Innovation and Teaching Excellence Educational Research Ethics Committee (KIITE EREC; Keele University).

Of the 48 groups of students (n=172 individuals) that were invited to play the escape room, a total of 58 unique page views were recorded for Puzzle 1, suggesting that either students split themselves into smaller groups or more than one student per group accessed the game. Analysis of Google Analytics data suggests that puzzles were created across a range of difficulties, with the amount of time spent on each puzzle ranging from 3 min and 50 s to 11 mins and 11 s. Furthermore, most groups (74.1%) were able to complete the exercise. All students who filled in the feedback questionnaire (n=11) found the escape room “Engaging” or “Very Engaging”. 82% of participants thought the escape room helped them to practice applying their knowledge, 64% thought it helped them to consolidate their knowledge, 91% said that they enjoyed playing the digital escape room and 91% said that they would recommend the resource to their peers.

In conclusion, digital escape rooms may provide a fun and engaging alternative to passive revision resources more commonly found online. While medical students felt that this new resource helped them to practice applying their knowledge of cardiovascular physiology, future studies will aim to recruit students studying physiology as part of other (non-medical) courses.

Horn MA (2023). Adv Physiol Educ 47: 82–92.

C14

“What is Physiology?” – interview insights straight from the physiologists’ mouths

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Physiology is often described as the ‘science of life’, which is a good headline, but without further definition is perhaps too simplistic an explanation of the complexity, fascination and importance of physiology. Since 2021’s iteration of Physiology Friday, we have tasked our physiology and biomedical undergraduate students with gathering perspectives on what physiology is, and what physiologists do, through recorded interviews with peers and academic staff across several UK universities. These are all hosted on an open access e-repository⁽¹⁾. The intentions of these activities are to (i) gather current views of physiology from academic researchers and teachers and (ii) increase public awareness of the importance of physiology as a scientific discipline – for teaching and learning; for the fundamental understanding of all aspects of human biology; for the improved treatment and diagnoses of diseases; and for informing public health policies.

Following success of voluntary involvement from two BSc Physiological Sciences students from Newcastle University across Physiology Friday in 2021 and 2022, we secured £1500 funding from Newcastle University’s Jobs on Campus to recruit two student interns to (i) develop our e-repository further by conducting further interviews with physiology-related staff and students within and outside of Newcastle; (ii) transcribe and upload existing and new interviews and related content to the repository; (iii) thematically analyse such content to identify key themes; and (iv) investigate historical comments on the role and purpose of physiological sciences.

We are currently at stages (i) and (ii) with 23 recordings to date of staff ($N=17$) and student ($N=6$) interviewees from 6 institutions across the UK. A total of 293.8 minutes’ worth of footage has been recorded and transcribed for thematic analysis, the results of which will be presented. Furthermore, the project has presented an excellent opportunity for students to enhance their communication, organisational and teamworking skills, while developing their global citizenship, broadening their physiological network and enhancing their understanding of physiology as a discipline, of help for their future academic studies and career decisions.

C15

Science Travels: Reaching out to Gypsy, Traveller, Roma, Showman and Boater Communities through Physiology

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The impact of outreach isn't measured just in footfall or statistical analysis of questionnaires. The qualitative aspects are key, as highlighted in this case study. Science Travels led by Marie Bowers, a Romany woman, and Technician in Physiology Teaching at University of Glasgow (UoG) aims to use physiology to engage with Gypsy, Traveller, Roma, Showman, and Boater (GTRSB) schoolchildren with STEM and education. GRT communities are some of the most marginalised minorities in the UK and have the worst educational outcomes of any ethnic group. In England in 2020/21, 9.1% of Gypsy/Roma and 21.1% of Travellers of Irish decent achieved grade 5 or above in English and Maths. The national average was 51.9%. ^[1] Showman and Boaters are not protected ethnicities and no data is specifically collected about them.

UoG Honours project students and academic staff were recruited, with Marie leading a hard-hitting cultural awareness session: *'The presentation made me more aware of how serious some of the issues facing GTRSB students in the education system are...'* ^[2]

Culturally appropriate outreach activities were designed and delivered 'live from the lab in Glasgow' to our first partner school in Wiltshire: *'It was lovely to see the undergraduate's passion for their subjects and their ability to pass on their knowledge to a much younger audience. All three students are a real credit to the university.'* ^[3]

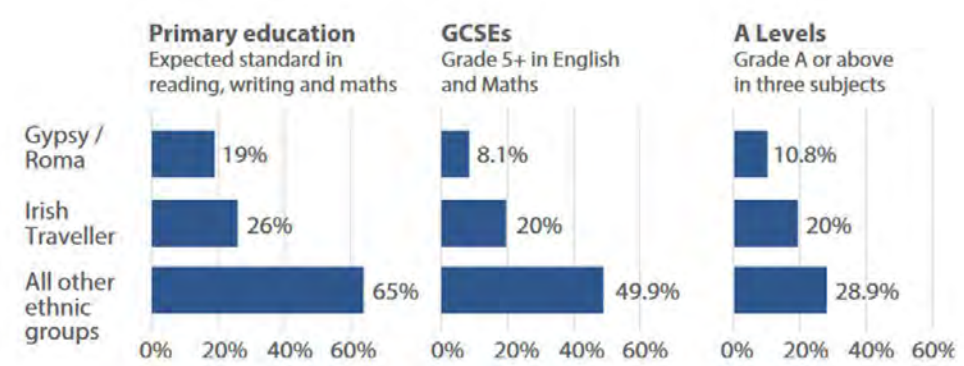
Blogs based on the experiences brought support from Widening Participation (WP) and the Equality and Diversity Unit (EDU) at UoG via funding for a GTRSB summer studentship: *'I learned new things and was able to earn some money before starting my BTec in Sports Science. It was fun.'* ^[4] This was developed further by a GRT student into a Lt (AD Instruments) biosensors lesson on the effects of physical activity on heart rate for primary school children.

Science Travels received an email from a Roma Marine Biology student at the University of Hull: *'I was so happy to see another Roma person in STEM as I felt so alone with such imposter syndrome.... I loved reading [your blog] and it is inspiring.'* ^[5] This student developed a 'Hook-A-Marine Animal' game via a further GTRSB summer studentship that then was turned into a real-life game for young children as part of community -designed activities at a GTRSB event held at the public opening event for the UoG Mazumdar-Shaw Advanced Research Centre. Science Travels is now embedded in Honours project options and contributes to Science Communication sessions with L3 UoG Life Science students.

Finally, in terms of policy engagement Science Travels was included as a case in the 'Report on the Contribution of Physiology Education and Training to the UK Economy' launch at Westminster and 'Physiology in Scotland: Achieving the Sustainable Development Goals' at Holyrood and was mentioned by an MSP in a debate on women in science in the Scottish

Parliament. These experiences indicate that when assessing impact of physiology in outreach, the qualitative as well as the quantitative outputs should be included.

Figure 1: Figure 1: Educational attainment among Gypsy / Roma, Irish Traveller, and pupils from all other ethnic groups, as of 31 January 2022^[1]



1. L. Brassington (2022) Gypsies, Roma and Travellers: The ethnic minorities most excluded from UK education. Higher Education Policy Institute Report 151. 2. Personal Communication with L4 Zoology undergraduate at University of Glasgow. 3. Personal Communication with Science Lead, Broad Town C of E Primary School. 4. Personal Communication with GTRSB summer student at University of Glasgow. 5. Personal Communication with GTRSB summer student at University of Glasgow.

C16

Evaluating the implementation of journal clubs into the biomedicine curriculum to promote physiological research and increase graduate capital.

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Introduction

Journal clubs are routinely used within academic research institutes and allied health professions to boost critical thinking and disseminate knowledge of novel research concepts (Honey & Baker, 2011). These group-based discussions regarding scientific literature have been shown to build internal knowledge, transferable skills and allow for the sharing of expertise across disciplines (Wenke et al., 2019). Many of these factors align with those of the graduate capital model and are highly desired for post-graduation employability (Clarke, 2018; Tomlinson, 2017). However, limited research has been conducted to evaluate the effectiveness of journal clubs in developing these skills at an undergraduate level, and specifically in biomedicine-aligned degree programmes. These degrees contain vast amounts of physiology; yet, a limited number of graduates seek to pursue physiological research following graduation from these programmes. This may be due to a lack of awareness regarding physiological research and/or limited opportunities to engage with research in a guided and structured manner. This study aims to determine if the use of structured journal clubs can promote an interest in physiological research and boost key transferable skills associated with increasing graduate capital.

Methods

This study was ethically approved by the University of Salford's ethical review board. All students from biomedicine-based degree programmes were invited to participate. Student feedback was assessed by the completion of an anonymous survey following the completion of each journal club. All questions were scored on a 5-point Likert scale including negative, neutral, and positive options. The responses to all questions were optional. Survey questions related to student demographics, career aspirations and feedback on the impact of journal clubs to boost key metrics of graduate capital.

Results

A total of 24 out of 41 (58.5 %) students responded to the survey. Of the 23 respondents who provided answers regarding gender 21.7 % identified as male, 73.9 % as female, and 4.4 % as non-binary. All 24 respondents identified their ethical background with 29.2 % identifying as White, 45.8 % as Asian/Asian British, and 25.0 % as Black, African, Caribbean, or Black British.

A total of 22 (87.5 %) respondents stated that participation in the journal club increased their interest in pursuing a physiological research career following the completion of their degree. Of all respondents, 87.5 % also stated that these activities significantly increased their knowledge of physiological research methodologies and its associated ethical considerations. All respondents stated that journal club attendance positively impacted their understanding of scientific writing, their ability to critically analyse scientific research articles and benefit their

wider degrees. The survey respondents also stated that journal clubs improved key graduate capital metrics including team working (91.6 %), communication (79.2 %), and confidence (66.7 %).

Conclusion

These data indicate that the majority of students who engaged with journal clubs increased their interest in pursuing physiological research-based opportunities post-graduation. They also highlighted that journal clubs had a positive effect on key transferable skills linked to improving their overall graduate capital and academic proficiency.

Clarke, M. (2018). Rethinking graduate employability: the role of capital, individual attributes and context. *Studies in Higher Education*, 43(11), 1923-1937.
<https://doi.org/10.1080/03075079.2017.1294152> Honey, C. P., & Baker, J. A. (2011). Exploring the impact of journal clubs: a systematic review. *Nurse education today*, 31(8), 825-831.
<https://doi.org/10.1016/j.nedt.2010.12.020> Tomlinson, M. (2017). Forms of graduate capital and their relationship to graduate employability. *Education + Training*, 59(4), 338-352. <https://doi.org/10.1108/ET-05-2016-0090> Wenke, R., O'Shea, K., Hilder, J., Thomas, R., & Mickan, S. (2019). Factors that influence the sustainability of structured allied health journal clubs: a qualitative study. *BMC Medical Education*, 19(1), 6.
<https://doi.org/10.1186/s12909-018-1436-3>

C17

What do students want from practical classes?

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Although the inclusion of learning outcomes for individual classes is ubiquitous, little attention seems to have been paid in the literature to the overall purpose of experimental practical work within undergraduate physiology courses. The enforced move to online practical classes in the academic year 2020-1, as a result of the SARS-CoV-2 pandemic, led us to wonder what, if anything, students at the University of Cambridge felt they were missing out on. Towards the end of that year, we asked our year 1 medical, veterinary and natural science students what they felt in-person practical classes would have offered them, and to what extent this had been replicated through the online format. The detailed questionnaire sent to the students included a list of 17 possible benefits of in-person practical classes; the students were asked to rate the importance of each on a Likert scale, and suggest any further benefits that we might have overlooked. We subsequently sent a very similar questionnaire to the same cohort of students when they had reached their third years. This second questionnaire was intended to assess whether opinions had changed, after these students had actually experienced a year of in-person practical teaching.

In year 1 we received 145 responses and in year 3, 43 responses, from around 600 students. Despite the low response-rate, the results of both questionnaires showed some striking consistencies. Of the possible benefits of practical classes, "Working in an 'active' way" achieved the highest importance rating in year 1, followed by "Becoming familiar with basic laboratory equipment and techniques", "Developing problem-solving skills" and "Discussing scientific questions with the academic staff". The bottom four were "Preparing you for the exam", "Having the opportunity to test your own ideas, experimentally", "Gaining experience in performing calculations" and lowest of all, "Thinking about the ethical aspects of scientific research". Although the order was different, the four top-rated and four bottom-rated items were the same in year 3, except that "Gaining experience in performing calculations" was replaced in the bottom four by "Developing a professional identity". Further benefits that emerged from open-ended student comments included the importance of being able to make mistakes without serious consequences, and the opportunity to experience what research science might be like as a career.

Year 3 students retrospectively recognized the convenience of the online format, and noted that such classes provided a more standardized experience, often coming with supporting material that was more accessible for revision purposes. The online format was seen to lend itself to certain types of classes, such as coding, bioinformatics and histology. However, students overall did not feel that online classes were good replacements for in-person practicals, which were felt to be more engaging and allowed for social interactions that were sorely missed during the pandemic. It was clear from the results of our study that students particularly value the opportunities to develop hands-on practical skills and ask questions of academic demonstrators, in the context of a live experimental class.

C18

A multifaceted approach to building more employable Biomedicine graduates.

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It is widely documented that students from underrepresented backgrounds often achieve lower outcomes at university. We set out to tackle gaps in achievement and employability through interventions that enhance student sense of belonging, support confidence development, and provide opportunities for students to build the many forms of graduate capital. Many of the projects described herein have been co-designed with students as partners. Our approach involved the re-design of skills modules, bespoke career mentorship groups and the launch of large careers events. Alongside this, we worked collaboratively with students to design and deliver a range of extracurricular opportunities to build employability, including an annual Ted-style public speaking competition (Salford PassionFlash), a BioArt competition and a student magazine.

Of those who belonged to a career mentorship group, 83% agreed that this helps them plan their careers. Similarly, 86% of students felt that taking part in the annual careers festival had a positive impact on their career planning. Over the last four years, more than 70 students have taken part in TED-style public speaking. When surveyed, 87% strongly agreed/agreed that the competition is a means to help students realise their potential, over 91% agreed it helps raise aspirations, whilst 70% felt that the competition enhances employability. The student-led Bioscientist Magazine team has grown immensely over the course of three years to include over 80 student publications. Skills obtained include communication, writing, teamwork, digital literacy etc., which are all of course highly transferable to the world of work. Similarly, the annual BioArt competition has attracted many students. When asked to rate the impact of these co-created extracurricular activities designed to build graduate capital, 83% felt that taking part enhanced employability, 90% felt it had improved their communications skills, 76% reported enhanced student satisfaction, whilst 79% reported growth in confidence. These results demonstrate that our interventions are having a positive result from the students perspective. Collectively these activities have been integral to the growth of on-campus community and the development of graduate capital. We have seen significant positive developments in student engagement and graduate outcomes, which may relate to the interventions outlined.

Tomlinson, M. (2017). Forms of graduate capital and their relationship to graduate employability. *Education + Training*, 59(4), 338-352. doi:10.1108/ET-05-2016-0090

C19

Addressing problem-solving in exams with an optional classroom session on lung function tests

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Based on audits, year 1 medical student performance on exams has been dropping since before the time of Covid, when our exams went from short answer to single best answer (SBA) multiple choice format. Restoring face-to-face classes has not returned student exam performance back to the levels of 5 years ago. The cause of these low single best answer exam performances could be due to

- (A) more demanding exam questions
- (B) students who are less studious
- (C) students who have weaker academic reserve
- (D) weaker teaching that is unmatched to the exams, or
- (E) an increase of taught material and a dearth of time on the exam.

Although option (E) is the most likely because we are aware of the creeping increase in material being taught, in this work we consider option (A), and show that the problematic SBA questions are often "vignette questions" that require problem solving in order to successfully answer them. As our lecture-based course does not teach year 1 students problem solving *per se*, one possible cause of examination issues is problem solving in year 1. In an audit of the previous year's performance, we find that purely factual exam questions are answered correctly, whereas a range of SBA questions requiring problem-solving have created difficulties for students. We have attempted to address this by teaching problem-solving with lung function tests (LFTs). Traditionally the problem-solving aspect of LFTs are taught by summarising with ATS/ERS flow charts, which most students find difficult to follow or memorise. We taught this topic with a supplementary and optional hour-long problem-solving session working through each individual LFT case. Although students (61 attendees in a cohort of 212) were very satisfied with the learning sessions (rating scale 1-5, 5 = excellent, mean $\pm$ SEM = 4.58 ± 0.08 , N = 48 respondents) and they had little previous familiarity with the ATS/ERS diagram (2.33 ± 0.16), the students' self-rated understanding at the end of the problem-solving session was not great (3.86 ± 0.11), nor did they think that flow charts would "would help you personally to learn" the material (3.96 ± 0.14). They were confident that with "time to study at home", that they could "now master the ideas behind the ATS/ERS diagram shown at the end" (4.42 ± 0.10). We conclude that optional problem-solving sessions are not particularly attractive to students, but those who do attend believe that their future learning of complex concepts like LFTs will be improved.

C20

Developing authentic assessment by using a design sprint methodology

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The University of Leeds is undergoing an institution wide curriculum redefined process ¹, aiming to transform the curriculum and align with four strategic objectives built around: Partnership, Transformation, Belonging and Sustainability. For the undergraduate programmes in the School of Biomedical Sciences which include, Human Physiology, Physical Activity and Health, Biomedical Sciences, Neuroscience, Pharmacology and Sport and Exercise Sciences, a new form of synoptic assessment is in the design stage, due to be implemented in September 2023.

Since the Covid-19 pandemic, many essay based assessments have moved solely online in time-limited assessments set during an exam period. With the rise of artificial intelligence resources available such as Chat GTP, a greater spotlight is being shone on these “essay style” assessments in terms of their sustainability, but also questioning again how authentic these assessments are with relation to employability skills. Instead should we be encouraging student to understand and critique this rapid development of new technology? An evidence-based report is proposed to replace these previous essay style assessments. This would form one mode of assessment in the new synoptic assessment portfolio planned for these programmes, assessing work-ready competencies.

A design sprint methodology was used to help shape and design the structure of both the new formative and summative evidence-based report assessment. This design sprint was facilitated through experts in people design and involved conducting several 1-1 interviews with students, alumni, staff and external experts in the field of assessment prior to the design sprint.

The design sprint focused on generating ideas, prototypes and receiving feedback. Ideas were generated individually from both staff and students present and then collated to review where multiple ideas overlapped. A choice of formative assessments were proposed with a focus on encouraging students to link their understanding and knowledge through mind maps or an infographic report plan which would be accompanied by a short example paragraph to allow feedback on the students writing style. For the linked summative assessment, students could use both their formative assessment plan and feedback to generate a final evidence-based report. To help ensure this assessment would be ready for Sep 2023 a timeline was created at the end of the design sprint. This set out goals for testing prototypes, listing key stakeholders who should be involved and went.

The co-creation design strategy and intense sprint produced multiple prototypes for assessment, including this evidence-based report, which have the four strategic objectives embedded right from the start. Key stakeholders were engaged from initial design conception, which should help for a smooth rollout of this new style of assessment. Working with student on co-creation of prototypes and testing multiple designs with students and staff should help iron out initial problems and creating innovative new assessment approaches. Synoptic style assessment in the first year is new for both staff and students, but with sufficient scaffolding through well designed formative tasks, this style of assessment should help kick-start student's integrated understanding of the content by the end of year 1

1. <https://www.leeds.ac.uk/curriculum-redefined>

C21

The impact of colour in learning environments on neurodivergent learners: how can we create accessible learning spaces for autistic students?

Lauri Ovaska, Derek Scott

undefined

Neurodiversity refers to the natural variation in brain structure and function, and can be used to define atypical development, such as autism, as neurodivergence rather than a disorder. Autism covers a wide range of various traits, including sensory issues, which may include hyper- or hyposensitivity to sensory stimuli. The inability to cope with sensory issues may lead to increased distress, agitation, and social withdrawal. In the context of learning, sensory issues are also often associated with poorer learning outcomes. Whilst institutions often consider physical accessibility when designing learning spaces, the needs of neurodivergent learners may be less prominent. To address such sensory issues, environmental factors, such as the interior design of learning spaces, must be considered.

To investigate the impact of colour in learning environments on autistic students, a systematic literature review including 7 different studies was conducted. Subsequently, the findings of the literature review were compiled into a set of autism-friendly colour design guidelines and a design criteria checklist, which was used to audit the study spaces on Aberdeen University Campuses (n=15). It was noted that autistic students are a highly heterogenous group, and no single guideline would necessarily apply to all autistic students.

A neutral colour palette seemed to be most appropriate for autism-friendly learning environments, as this helps to create a calming, low-stimulation environment. However, when used alone, neutral colours may feel unwelcoming and be associated with negative experiences. Autistic students seem to prefer cool, low-saturation colours associated with nature that help to create a calming atmosphere. On the contrary, warm, and saturated colours are often associated with negative experiences and may cause agitation. Pattern and colour contrast should also be avoided, as these may also cause visual distraction. However, high saturation colours and colour contrast may be used as tools to aid with navigation or guide attention. Furthermore, to provide means for self-regulation, colour should be used to create designated sensory-rich spaces that autistic students may explore to feel more engaged as well as low-stimulus escape spaces that autistic students may retreat to and thus prevent overstimulation. Colour should also be considered in transition zones between low-stimulus and high-stimulus areas to allow recalibration of the senses, which helps autistic students to adjust to the upcoming sensory environment.

The audit yielded a mean score for learning spaces of 4.30 ± 1.75 (max score = 10, n = 15). It was noted that several study spaces utilised the official University colours, which may not create spaces that are conducive to learning for autistic students. The new Science Teaching Hub was deemed the most autism-friendly learning environment on campus. This may be because the sensory needs of neurodivergent students were considered during its design process.

As autistic students are such a heterogenous group, it is important to provide flexibility in the learning environment by offering areas with different sensory qualities. Moreover, future

research should focus on including autistic individuals in the discussion to ensure their needs are met when ensuring accessibility in learning and work environments.

C22

Mind Maps and Core Concepts can help nurses manage complexity in physiology learning

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Physiology is often the subject that healthcare students on professional programmes, such as nursing, midwifery or therapies, love to hate. The essential basis for safe professional practice is in-depth knowledge of anatomy, physiology, and consequences of physiological disturbance(s) across the Lifespan, yet the evidence shows that pre- and post-registration students alike face considerable difficulty in understanding biosciences and applying theory to practice.

Providing learning activities that support active learning in physiology can therefore be a challenge, given the wide educational background of students and the limited contact time available for educators within the packed professional curriculum. It is difficult to get to know the students well enough for meaningful exchanges about their specific challenges or strategies for learning who may be in very large lecture cohorts.

The Johari Window is a simple tool for illustrating and improving self-awareness and mutual understanding. Although most often used for team development, it has the potential to stimulate students' thinking about their perceptions of, and emotional responses to, learning (Cassidy, 2014; Lowes, 2020). At the start of several modules at different levels, an adapted grid was used and 356 nursing students expressed their perceptions about their previous learning of physiology. The four quadrants covered topics they (students) liked, topics they disliked, aspects of learning that seemed easy and "tricky bits". Responses were analysed using word cloud technology. Students consistently enjoyed the ability to apply their knowledge in practice settings but found the complexity of physiological interactions and relationships difficult.

In response to these findings, a mind mapping activity was utilised to enable students to create a visual display (Vanides, 2005; Safar et al, 2014) that examined the principles and dynamic nature of homeostasis. Each group then selected examples of homeostatic disturbances to deepen their exploration and relate examples of core concepts in physiology to patient care. Since students were drawn from a range of different clinical areas, the discussion could sometimes become quite wide-ranging as they shared their experience.

Learning about core concepts in physiology (Michael & McFarland, 2020) seems to have the potential to create the keystone for a solid and meaningful lifelong learning opportunities that fill the gap between theory and practice learning. Encouraging this open-ended approach to learning physiology served to form a framework for developing learning activities which helped to unpack students' misconceptions, partial understanding and confusion. By exploring core concepts through discussion with other students, the mind mapping activities appeared to help students to verify their understanding and ability to integrate new learning in physiology with their experience in professional settings.

Cassidy, TM (2014) Opening the window to lifelong learning: Applying the Johari Window framework in engineering communication curriculum 2014 IEEE International Professional Communication Conference (IPCC) Professional Communication Conference (IPCC), 2014 IEEE International. :1-4 Oct, 2014 Michael, J., Mcfarland, J. (2020) Another look at the core concepts of physiology: Revisions and resources. Adv. Physiol. Educ., 44, 752-762 Safar, A., Jafer, Y.J., Iqadiri, M.A (2014) Mind maps as facilitative tools in science education College Student Journal ; Winter 2014, Vol. 48(4) p629, 19p Vanides, J., Yin, Y., Tomita, M., & Ruiz-Primo, M. A. (2005). Using concept maps in the science classroom. Science Scope, 28(8), 27-31.

C23

Education about the physiology of death and dying in the training of healthcare practitioners – do we need to do more?

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undefined

We have previously reported work which examined the inclusion of the physiology of death within the medical curriculum (Brown *et al.*, 2022). Our previous focus had mainly examined educational recommendations and resources for doctors, but we were keen to expand this work to other medical and allied healthcare professionals. We aimed to explore whether training recommendations and education on this important but often ignored topic was better for some professions. We hoped that we could identify best practice and use this to make recommendations for other professions when learning about this topic.

A literature review was conducted of 59 educational and professional guidance documents for 14 allied health professions and other conventional medical professionals. Further analysis of the selected terms/keywords within 45 academic textbooks for three advanced clinical practitioner types was undertaken, as well as 15 general, non-profession-aimed medical science textbooks.

Results showed that paramedics had the highest frequency of selected terms, with 'death' being the most common term across both reviews and 'apoptosis' being the most frequent term within the general medical science textbook analysis. More scientific and technical terms, such as 'brain stem death', were found more frequently in the textbooks than in the guidance documents. Nurses had a more holistic approach to care and dying, as reflected in their selected terms, such as 'palliative' and 'end of life' being more common. Physician associates scored the lowest compared to advanced nurse practitioners and paramedic practitioners, likely due to their newness as a profession and use of selected terms that focused more on cellular processes, rather than death or dying of the actual person.

The study concludes that paramedics may have the most significant understanding of the physiology of death, followed by doctors, if the recommendations made in their clinical guidance documents are taught fully and effectively. Paramedics are also very likely to be exposed to a broader range of patient deaths and trauma given the nature of their clinical role. It was also clear that, despite the recommendations regarding a full understanding of lifespan physiology for many healthcare professions, there is a lack of specific learning material available for educators and learners to use when trying to expand their understanding. Developing educational material or learning activities on such a sensitive topic may also be challenging for specific professions (e.g. midwives) where such experiences may potentially be very distressing and hard to simulate. It may be that curriculum guidance documents should be reviewed in light of the recent experiences of some healthcare professionals (e.g. physiotherapists) who dealt more closely and frequently with dying patients during the pandemic than was previously typical.

We conclude that there are elements of good practice relating to death and the dying process in materials for healthcare professionals such as paramedics. Lessons could be learned from their education and resources to enhance the training of other clinical staff. These findings have

implications for the enhancement of healthcare education and highlight the need for more open and honest discussions about death.

Brown, K., Scott, D. A., Ginesi, L. (2022). We need to talk about death. *Europhysiology 2022*, pp. e13875. [ONLINE] DOI: <https://doi.org/10.1111/apha.13875>

C24

Dietary fatty acids increase intestinal L cell numbers independent of the development of obesity

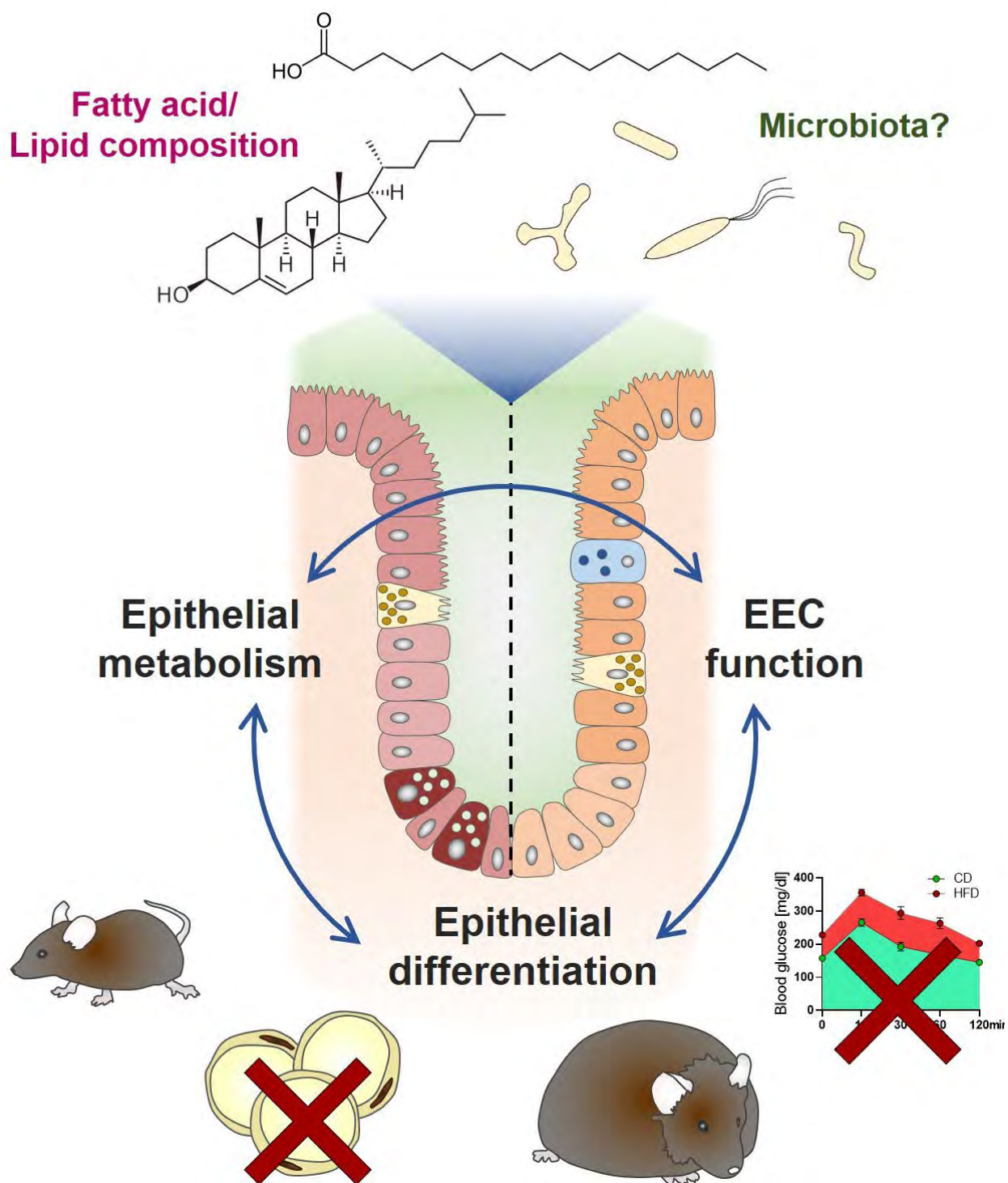
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Enteroendocrine cells (EECs), produce various hormones to coordinate optimal absorptive conditions following food intake, ensuring efficient postprandial assimilation of nutrients. EEC are equipped with sensors for the detection of luminal nutrients including free fatty acids, short-chain and long-chain fatty acids. Dietary habits, obesity and microbiome composition are associated with alterations in EEC function and numbers. In particular, glucagon-like peptide 1 (GLP-1)-producing L-cells seem to be affected. Ileal versus colonic GLP-1 is suggested to confer location-specific functions, stimulating insulin release and reducing gastrointestinal motility, respectively.

To scrutinize the effect of dietary fat and obesity on L-cell density in the intestinal epithelium, we quantified GLP-1⁺ cells in the ileum and colon of mice. BL/6J mice were fed high-fat diets (HFD) based on different fat sources (palm oil (P), lard (L)) (n= 6 – 8 in all feeding groups). Effects of the P-HFD were characterized over time (1, 4, 12 weeks) and for 48 and 60 kJ% of fat. Additionally, using mouse strains with different susceptibilities to diet-induced obesity (DIO), AKR/J (high), BL/6J mice (intermediate), and SWR/J (low/none), we investigated P-HFD 48-mediated effects independent of obesity. L cells per open crypt were quantified in at least 3 non-consecutive tissue sections based on immunohistochemical stainings for Glp-1. All samples analyzed in this study for EEC/ EC numbers were obtained from our tissue biobank and generated in the context of a previously published study (*doi: 10.1002/mnfr.201400840*). Thus, in accordance with 3R principles, no mice were sacrificed for the purpose of this study. In accordance with previous reports indicating enhanced L-cell differentiation induced by dietary lipids, P-HFD increased GLP-1⁺ cell numbers in the ileum and colon of BL/6J mice. In the colon, the effect was already significant after one week (1.98 fold increase (x) over control diet, $p < 0.0001$, t-test), was enhanced after 4 weeks (2.54x, $p < 0.0001$) but stayed stable afterwards (2.43x, $p < 0.0001$), without additional impact of fat content (48 versus 60 kJ%). In contrast, 4 weeks on L-HFD caused only a borderline significant increase in ileal GLP-1⁺ cells (1.27x, $p < 0.0149$), but no significant changes in the colon. Corroborating the hypothesis that dietary fat directly impacts EEC differentiation and not obesity *per se*, the increase in colonic GLP-1⁺ cells was similar in all mouse strains, independent of weight gain. In line with a role of colonic GLP-1 in controlling intestinal transit time, numbers of GLP-1⁺ cells did not correlate with basal blood glucose levels or AUC in SWR/J mice (Spearman correlation). The main difference in composition between P-HFD and L-HFD is the content of palmitic acid and cholesterol (exclusively present in L-HFD). GLP-1⁺ cell numbers remained unaltered in L-HFD fed mice, thus palmitic acid or its metabolites might directly foster EEC differentiation. In contrast, the effect of HFDs on metabolic parameters does not seem to affect intestinal L cell numbers.

This study highlights potential of precise nutritional interventions in the context of metabolic diseases and underlines the necessity for careful interpretation of data from DIO models due to distinct effects of the fat source.



C25

Pten knockout in preosteoblasts leads to changes in bone turnover and strength

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Bone development and remodeling are controlled by the phosphoinositide-3-kinase (PI3K) signaling pathway. We investigated the effects of downregulation of phosphatase and tensin homolog (Pten), a negative regulator of PI3K signaling, in osteoprogenitor cells. Using a mouse model with Pten deficiency in preosteoblasts, we aimed to identify mechanisms that are involved in the regulation of bone turnover and are linked to bone disorders.

Animal experiments were approved by local authorities (Regierungspräsidium Leipzig, Germany (TVV30/19 and TVV32/17). Bone marrow stem cells (BMSCs) were isolated from inducible conditional Pten knockout (Pten cKO) mice with a deletion of Pten exon 5 in *Osterix/Sp7* expressing osteoprogenitor cells (1). Femora, tibiae and BMSCs from Pten cKO and Cre negative control mice were compared. Expression of osteogenic markers, Pten protein and AKT phosphorylation was determined. Bone phenotyping was performed by μ CT and 3-point bending test. Number of osteoclasts and osteoblasts was determined by tartrate resistant acid phosphatase immunohistochemistry. Proliferation of BMSCs was measured by counting nuclei and Ki-67-stained cells. In vitro adipogenic and osteogenic differentiation was determined by Nile Red and alkaline phosphatase staining, respectively, as well as detecting gene expression changes in adipogenic and osteogenic markers. Bone turnover was assessed by ELISA detecting procollagen type 1 amino-terminal propeptide (P1NP) and C-terminal telopeptide (CTX). Measurements were converted to log fold changes $\pm$ SD and normalized to the mean of control animal values. Significant differences were determined by one-sample t-test ($p < 0.05$).

BMSCs from Pten cKO mice were functionally different from control BMSCs. Osteogenic marker Runt-related transcription factor 2 (*Runx2*) was increased 5.4fold in BMSCs from Pten cKO mice, while Pten protein was lowered to 0.7 ± 0.1 fold (normalized to α -Tubulin, $p = 0.003$) and AKT(S473) phosphorylation was increased to 11.2 ± 1.1 fold (normalized to total AKT, $p = 0.03$) compared to control BMSCs ($n = 3$ per group). We detected a higher trabecular bone volume/total volume (males: 1.8 ± 0.3 fold, $p = 0.07$) and higher trabecular bone mineral density (males: 1.7 ± 0.2 fold, $p = 0.08$) in Pten cKO bones of both sexes, while cortical thickness was also increased (males: 1.2 ± 0.06 fold, $p = 0.04$, $n = 6$ per group). Biomechanical analysis revealed a significantly higher maximum force (3.7 ± 0.6 fold, $p = 0.0003$) and increased elastic modulus (2.8 ± 0.5 fold, $p = 0.009$, $n = 6$ per group) of Pten cKO femora. Pten cKO bones from male mice

had a higher number of osteoblasts per bone perimeter (1.9 ± 0.3 fold, $p=0.004$, $n=4$ controls, $n=6$ Pten cKO). Bone turnover markers P1NP and CTX were significantly increased both in Pten cKO male and female mice. Increased proliferation of isolated Pten cKO BMSCs was detected (males: $p=0.0125$, $n=4$ per group). Osteogenic differentiation capacity was significantly enhanced in BMSCs from both male and female Pten cKO mice as shown by Alkaline phosphatase staining and higher expression of *Alkaline phosphatase* ($n=7$, $p=0.016$), transcription factor *Osterix* ($n=7$, $p=0.03$) and *Runx2* ($n=8$ controls, $n=7$ Pten cKO, $p=0.047$) (Figure 1), while adipogenic differentiation was not altered.

Pten knockout in osteoprogenitor cells increases stability and elasticity of mouse long bones and leads to increased proliferation and osteogenic differentiation of bone marrow stromal cells in vitro.

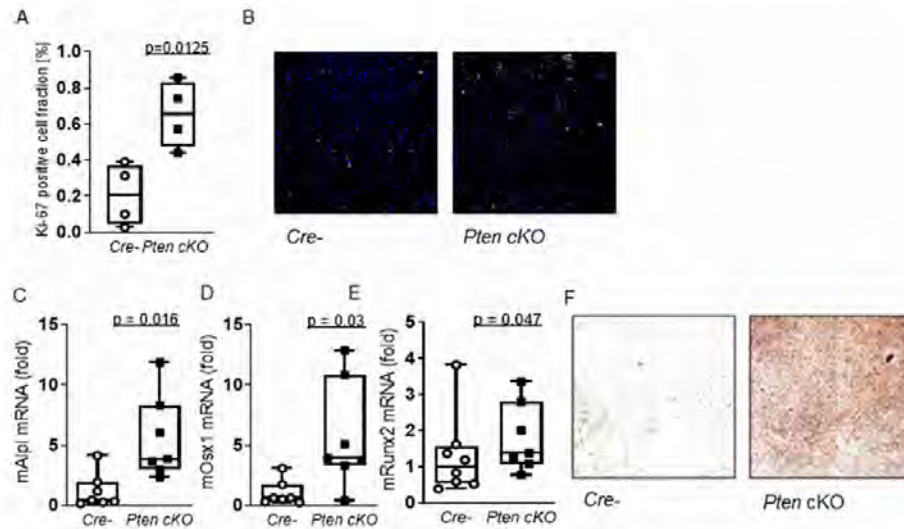


Figure 1: Pten cKO enhances proliferation and capacity for osteogenesis in bone marrow stromal cells (BMSCs).

(A) Proliferation marker Ki-67 immunofluorescence staining (Ki67/Hoechst= proliferative fraction in %) in control and Pten cKO BMSCs at day 1 of proliferation: *Pten* cKO cells show an increased fraction of Ki-67 positive cells to 3.13 ± 0.5 fold ($n=4$, $p=0.0125$). (B) Representative images of Ki-67 (Red) and Hoechst (blue) staining of control (left) and Pten cKO (right) BMSCs (100x magnification). Control or Pten cKO BMSCs were differentiated for 14 days in osteogenic differentiation medium. Osteoblast mineralization was assessed by alkaline phosphatase activity staining. Expression of bone markers C) *Alkaline phosphatase* ($p=0.016$), D) *Osterix* ($p=0.03$) and E) *Runx2* ($p=0.047$), determined using qPCR, was increased at mRNA level in *Pten* cKO BMSCs. Data are presented as fold change normalized to mean of control values $\pm$ SD. F) Representative images of $n=4$ independent experiments (40x magnification)

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C26

The carotid body is involved in GLP-1 effects on glucose homeostasis

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Introduction: GLP-1 is an incretin released by the gut in response to food consumption. Binding to the GLP-1 receptor, (GLP-1R) increases insulin and decreases glucagon secretion by the pancreas, promoting nutrient storage and usage [1]. GLP-1 also acts in the brain to promote satiety. As such, GLP-1R agonists are used in type 2 diabetes (T2D) and obesity to promote glycemic control and decrease weight [2]. The carotid bodies (CBs), peripheral chemoreceptors classically defined as O₂ sensors, have also been pointed to be metabolic sensors involved in energy and glucose homeostasis [3]. Herein, we investigated the role of the CBs on the GLP-1 effects on glucose homeostasis.

Material & Methods: Wistar rats were submitted to 10 weeks of 60% lipid-rich diet (HF) or to a standard diet (NC). After, half of the groups were submitted to carotid sinus nerve (CSN) resection, to abolish CB contribution to GLP-1 effects on metabolism, or to a sham surgery. At a terminal experiment a bolus of liraglutide, a GLP-1R agonist (200 µg/Kg), was administrated in the external carotid artery and glycemia measured for 1h. Insulin, C-peptide, and glucagon in plasma samples were evaluated by a multiplex analysis at 0, 15, 30 and 60min post liraglutide administration. Experiments followed the 2010/63/EU European Union Directive and were approved by NMS Ethics Committee and Portuguese Authority for Animal Health. Differences between data were calculated using One-Way ANOVA and considered significantly different with p-values <0.05.

Results: Liraglutide decreased blood glucose levels by 15.4% and 28.2% in NC and HF animals, effects exacerbated by CSN resection in NC (p<0.05) but not in HF animals. HF diet also increased the time to liraglutide reach a maximal effect on blood glucose (p<0.05) vs NC animals, and impaired the counterregulatory responses to hypoglycemia, effects abolished by CSN resection. Insulin levels increased by 121.6% and 87.2% in response to liraglutide in NC and HF animals, respectively an effect prevented by CSN denervation, with no significative alterations in c-peptide levels between groups. Glucagon levels increased by 44.8% in response to liraglutide administration in NC animals, an effect attenuated in HF diet-animals (20.5% increase). CSN resection in both NC and HF diet animals prevented the counter-regulatory increase in glucagon levels promoted by a decrease in glycemia induced by liraglutide.

Conclusions: CSN resection improves liraglutide effects on blood glucose and insulin levels and on the impaired-HF diet counterregulatory mechanisms to hypoglycemia. CSN resection exacerbates HF-induced impairment of liraglutide positive effects on glucagon secretion, suggesting that CBs modulation of hypoglycemia counterregulatory mechanisms occurs by other mechanism different from glucagon secretion. These results suggest that targeting GLP-1 action on glucose homeostasis involves the contribution of the CB.

C27

The effect of two diabetes interventions on body composition and muscle function outcomes

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Introduction

Type 2 diabetes (T2D) is characterised by chronic hyperglycaemia resulting from insulin resistance and pancreatic beta cell failure. Very-low calorie diets (VLCD) are recommended for management of T2D as they improve both of these defects^{1,2}. However, the deleterious consequences of loss of lean body mass (LBM) with VLCD is becoming of increasing concern³. Similarly to VLCD, new anti-diabetic therapies, such as Glucagon-like peptide-1 receptor agonists (GLP1RA), promote weight loss and glycaemic improvements in T2D, and our recent data demonstrated that exogenous GLP-1 infusions enhanced postprandial muscle protein synthesis⁴. Investigating interactions between anti-diabetic interventions, such as GLP1RA, and VLCD, with body composition is timely, especially since individuals with T2D are vulnerable to accelerated age-related declines in muscle mass. We therefore investigated the effect of the GLP1RA Semaglutide, VLCD or a combination of the two therapies, upon body composition and muscle function outcomes.

Methods

Nineteen people with T2D and BMI >27 kg.m⁻² were allocated to receive either 800 kilocalorie/day VLCD (n=7), once weekly Semaglutide titrated up to 1mg (SEM, n=7), or both in combination (COMB, n=5) for 12-weeks. Dual-energy X-ray absorptiometry scanning was performed at baseline and 12-weeks, along with hand grip strength (HGS) and knee-extensor maximal voluntary contraction (MVC) for assessment of strength. Data were analysed with GraphPad Prism 9.5.0 (La Jolla, USA),

Results

Body weight reduced in all groups (VLCD -14.0kg p<0.0001, SEM -6.4kg p<0.01, COMB -14.9kg, p<0.0001), as did fat mass (VLCD -9.43kg p<0.0001, SEM -3.87kg p<0.01, COMB -10.54kg p<0.0001). Reductions in weight and fat mass were significantly lesser in SEM than in VLCD and COMB (p<0.01 for both). LBM significantly reduced in the VLCD (-3.64kg, p<0.01) and COMB (-4.14kg, p<0.01) groups, with no significant change in the SEM group. There was no significant difference in LBM reductions across the groups. HGS and MVC showed no significant change with any intervention.

Conclusion

Reductions in fat mass occurred with all interventions and were significantly greater with VLCD and the combination of SEM and VLCD compared to SEM alone. Whilst LBM reduced with VLCD and the combination, it did not reduce significantly with SEM, which in the context of

weight loss was suggestive of muscle mass preservation perhaps owing to lower overall weight loss. There was no benefit to combining SEM with VLCD, where similar reductions in LBM occurred to the VLCD group. These interim results suggest that Semaglutide may preserve muscle mass during weight loss, however, whether there is a benefit to combining this drug with VLCD requires further investigation. Despite these observed changes in LBM, there were no significant decrements in our measures of arm and leg strength, perhaps indicating an improvement in muscle quality owing to weight loss.

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C28

Maternal obesity impacts the antioxidant response and adipogenic commitment of neonatal mesenchymal stem cells

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Introduction: Maternal obesity is a risk factor for the development of childhood obesity. The mother's redox state could affect the developing fetus and program the adipocyte's metabolism. We hypothesize that the offspring's mesenchymal stem cells (MSCs), which are adipocytes precursors, have a higher adipogenic commitment through FOXO1 activation and oxidative stress.

Objective: To characterize and study the expression of antioxidant enzymes, together with FOXO1 activation in Wharton jelly- MSCs (WJ-MSC) from the offspring of women with obesity, compared to those from normal-weight women.

Methods: Umbilical cords were obtained from UMCG maternity ward with patients' consent (#2019.175). WJ-MSCs were isolated from newborns of normal-weight women (NW-MSC; Body Mass Index 18.5-24.5 kg/m²) and women with obesity (OB-MSC; Body Mass Index > 30 kg/m²) through the explant method. WJ-MSCs were characterized by surface markers, lineage commitment, clonogenic capacity (CFU-F) and cell growth rate. Basal and H₂O₂-challenged gene expression for superoxide dismutase 1/2 (SOD1/2), glutathione peroxidase (GPx1) and catalase (CAT) were quantified (qRT-PCR). FOXO1 protein expression was quantified in WJ-MSCs during induced-adipogenic commitment for 5 days (DMEM, insulin, IBMX, dexamethasone). Data was analyzed with Mann-Whitney test (mean ± SEM, n=7).

Results: Primary cultures of WJ-MSCs are a reliable source of MSC as shown by immunophenotype and differentiation assays. The OB-MSC presented a lower CFU-F and higher cell population doubling time, compared to NW-MSCs. OB-MSC presented a basal decrease in SOD1/2 and GPX gene expression (p<0.05), compared to NW-MSCs. The OB-MSC presented no response to H₂O₂ SOD2 gene expression, while NW-MSC responded with higher gene expression (p<0.05). OB-MSCs showed a trend to higher levels of FOXO1 protein (p=0.0571).

Conclusion: The OB-MSCs showed decreased antioxidant status, which may result in oxidative distress, compared to NW-MSC. Future studies should thus look at oxidative stress markers. During adipogenic commitment, OB-MSCs presented higher FOXO1 expression, which is a mediator for both adipogenesis and oxidative stress, suggesting that FOXO1 may be involved in the decreased antioxidant enzymes. If this altered redox regulatory activity affects the adipocyte's metabolic function requires further studies.

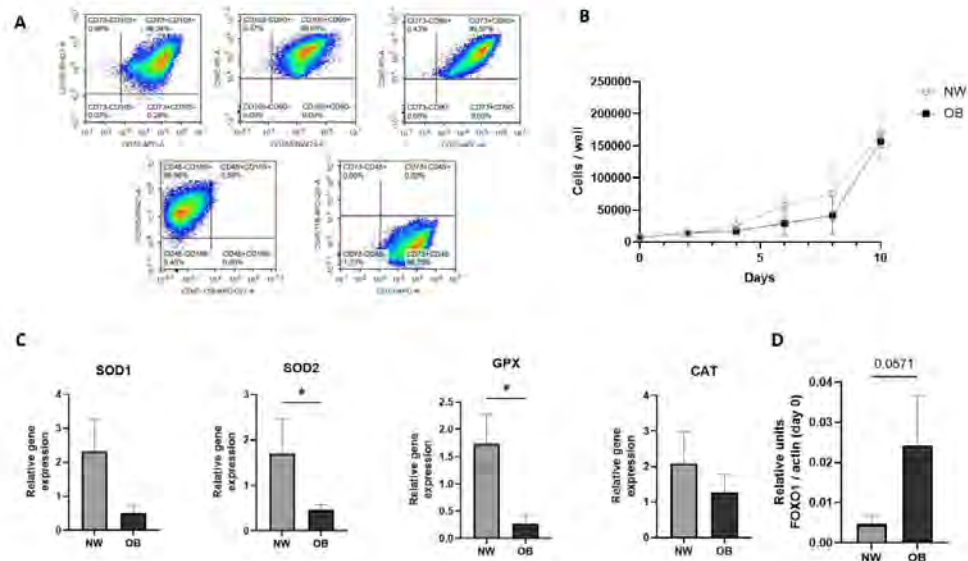


Figure 1. Summary figure. **A.** Immunophenotyping of WJ-MSCs: Cells were positive for CD73, CD90, CD105, and negative for CD45 and CD11b. (N=3). **B.** Cell growth rates: WJ-MSCs were seeded in passage 3 and counted in duplicates every 48 hours. **C.** Basal antioxidant gene expression: WJ-MSCs were seeded and gene expression of SOD1/2, GPX and CAT was measured for NW (N=9) and OB (n=7). **D.** FOXO1 protein expression during adipogenesis: Adipogenesis was induced in WJMSCs from NW and OB for 0-5 days. FOXO1 protein expression was evaluated every 24 hours for NW (n=4) and OB (n=4). Mann-Whitney test (mean + SEM).

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C29

Exposure of bronchial epithelial cells to hyperglycaemia alters the airway surface liquid proteome

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The fluid lining the lumen of the airways (airway surface liquid, ASL) is critical for defence against inhaled pathogens. Cystic fibrosis disease (CF) exhibits compromised ASL defence properties. The development of CF related diabetes (CFRD) which affects 40-50% of adults with CF is associated with a further decline in lung function and increased exacerbations. While the effect of CF on the proteins of the ASL has been well studied, the effect of hyperglycaemia on the ASL proteome in non-CF and CF bronchial epithelial cells remains comparatively underexplored.

We exposed Calu3, non-CF bronchial epithelial cells (NHBE) and CF (CFBE) cultured at air-liquid interface to normoglycemia and hyperglycaemia for 24 hours. We then carried out proteomic profiling on the ASL produced by these cells using tandem mass spectrometry.

We found that NHBE and CFBE ASL shared more proteins than Calu3 ASL. In both NHBE and CFBE, exposure to hyperglycaemia compared to normoglycaemia increased Mucin 5B abundance and pathways associated with peptidyltransferase activity ($p < 0.05$, $n = 4$ respectively). Several proteins involved in immune response to pathogens were decreased ($n = 4$). In NHBE ASL, hyperglycaemia altered proteins involved in metabolism and glycolysis (GLUD1, ATP5B), indicating metabolic dysfunction and cellular stress. In CFBE, ASL proteins associated with decreased immune responses (SPON2, HIST1H4A), deregulated oxidative stress response (PARK7) and altered intracellular trafficking (MVB12A) were changed with exposure to hyperglycaemia ($n = 4$). There were also more unique sequences with AGE adducts in CFBE compared to NHBE ASL ($p < 0.05$, $n = 4$). These data indicate that exposure to hyperglycaemia compromises innate immune activity of the ASL and further promotes cellular stress and inflammation, particularly in CF airways.

C30

Identifying salivary biomarkers for epithelial cell susceptibility to SARS-CoV-2 infection

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BACKGROUND: Our body's first defence against inhaled viruses like SARS-CoV-2 is a protective barrier that lines our respiratory tract called the mucosal-epithelial barrier. This barrier plays a vital role in neutralising the virus before it can infect respiratory epithelial cells. Secretions present in the nasal mucosa are also present in saliva, making it an ideal sample for non-invasive study of this barrier site. In this study, we aimed to identify key proteins found in saliva samples of healthcare workers who had recovered from COVID-19, which could protect against SARS-CoV-2.

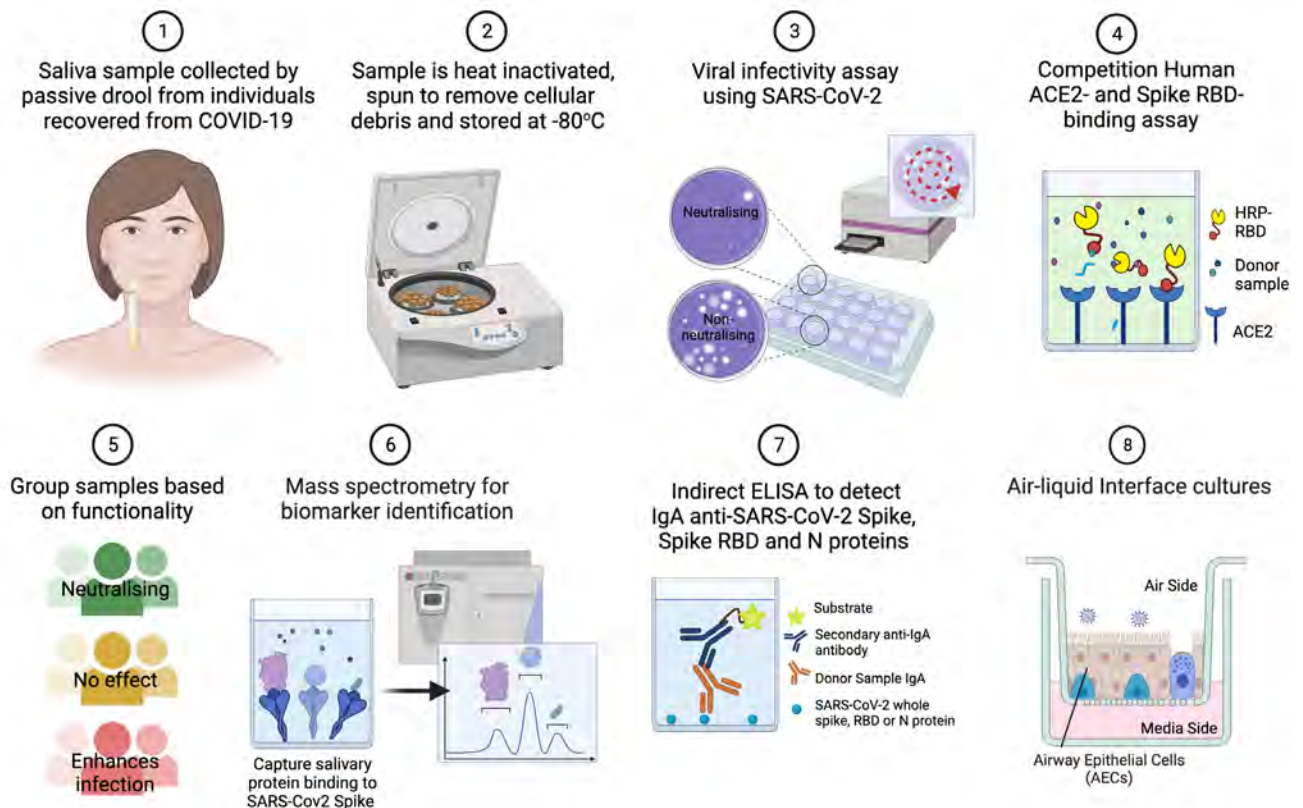
METHODS: We collected 551 saliva samples from consenting staff at Great Ormond Street Children's Hospital who had previously tested positive for SARS-CoV-2 infection, prior to vaccination. Samples were grouped based on their functional ability to protect against infection using an *in vitro* RBD-ACE2 binding and infection assays with VeroE6 epithelial cells. A subset of samples was also tested for viral neutralisation using air-liquid interface (ALI) culture of primary human respiratory cells. The levels of proteins which specifically bind to SARS-CoV-2 spike antigen were measured by baited mass spectrometry and compared between the functional subgroups.

RESULTS: We found that 7.3% (n=29) of the screened saliva samples reduced SARS-CoV-2 infectivity >2-fold (Group A); 89.3% (n=353) had minimal effect on SARS-CoV-2 infectivity (Group B) and 3.3% (n=13) of samples resulted in >2-fold enhancement in SARS-CoV-2 infectivity (Group C). Proteomics analysis of samples from these subgroups (n=10 for each) identified elevated IgA in the most neutralising samples. This was supported by ELISA screening with IgA detected specific for the SARS-CoV-2 antigens Spike in 86% (422 of 488), Nucleocapsid in 85% (418 of 488), and RBD in 83% (377 of 450) of saliva samples. We determined that a salivary concentration of anti-RBD IgA above 500 pg/μg total protein significantly (p=0.035) reduced *in vitro* viral infectivity compared to saliva samples which tested negative for anti-RBD IgA.

Proteomics analysis also revealed elevated vimentin (VIM), antithrombin III, and S100A9 in the samples associated with enhanced SARS-CoV-2 infectivity (Group C). This was supported by further *in vitro* infectivity assays using recombinant VIM, SERPIN and S100A9 protein at concentrations corresponding to the detrimental saliva samples. This demonstrated that vimentin exposure resulted in the largest relative increase in SARS-CoV-2 infection of VeroE6 cells (22.2% increase versus mock). In addition, immunofluorescence staining of SARS-CoV-2 inoculated ALI cultures showed co-localisation of vimentin with SARS-CoV-2 antigen, indicating a role for vimentin in infection of human nasal epithelial cells.

CONCLUSION: Our research suggests that salivary IgA is a useful indicator of recent SARS-CoV-2 infection and higher levels of anti-RBD IgA can help reduce viral infection of epithelial cells. However, other secreted proteins were found to be associated with enhancing *in vitro*

SARS-CoV-2 infectivity. Screening saliva for mucosal biomarkers such as vimentin may be an effective strategy to help identify individuals who are most vulnerable to repeat infection by SARS-CoV-2.



C31

Towards an understanding of proton activated chloride (PAC) channel regulation by GqPCR signalling

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Background: Proton activated chloride (PAC) channels, encoded by the *PACC1* gene, mediate outwardly rectifying chloride currents activated by acidification of the extracellular environment (1, 2). PAC is expressed in a plethora of mammalian cell types, but the physiological mechanisms of PAC modulation are incompletely understood. Recently, phosphatidylinositol-4,5-bisphosphate (PIP₂) was reported to bind and inhibit PAC channel from the extracellular side (3). However, PIP₂ is predominantly an inner-leaflet lipid. Here, we explore whether physiological variations in inner-leaflet PIP₂ levels, such as those associated with activation of G_q-protein coupled receptors (G_qPCRs) may influence PAC activity.

Methods: Whole-cell patch-clamp recordings of PAC currents in human embryonic kidney 293T (HEK293T) cells were used in conjunction with heterologous expression of the acid sensing GqPCR ovarian cancer G-protein coupled receptor 1 (OGR1), the α_1 -adrenergic receptor (α_1 R) or the *Danio rerio* voltage sensitive protein phosphatase (DrVSP). In some experiments, DrVSP with a C302S mutation (DrVSP(C302S)) to abolish phosphatase activity was used as control. Data are given as mean $\pm$ SEM alongside the number of experiments (n). P-values < 0.05 were considered significant.

Results: A chloride current was recorded in HEK293T cells at extracellular pH (pH_e) below 5.5. At pH_e 5 and 7.4, the current was 100.7 $\pm$ 8.6 pA/pF (n=18) and 4.5 $\pm$ 0.5 pA/pF (n=18) when measured at + 95 mV, respectively. The pH_e giving the half-maximal activation (EC₅₀) was 5.2 $\pm$ 0.0 (n=5). HEK293 cells in which the *PACC1* gene was deleted (2) presented negligible currents even at pH_e 5 (4.9 $\pm$ 2.9 pA/pF (n=5)), and reintroduction of *PACC1* cDNA (transcript variant 2) restored PAC currents (838.6 $\pm$ 119.7 pA/pF at pH 5).

The PAC current measured at 5 pH was not affected by heterologous expression of OGR1 or α_1 R (stimulated with phenylephrine (1 mM)), suggesting that the PAC current may not be modulated by the G_qPCR signaling pathway in HEK293T cells. The role of second messengers associated with G_qPCR signalling, Ca²⁺ and PIP₂, was further investigated. PAC current amplitude was not affected when free Ca²⁺ in the intracellular recording solution ([Ca²⁺]_i) was raised from 0 to 1 mM. Inclusion of the PIP₂ scavenger neomycin (1 mM) in the intracellular solution had no effect on PAC current magnitude. In cells transfected with DrVSP the current at a negative potential (-60 mV) did not differ from that measured in cells expressing DrVSP(C302S). However, at a supra-physiological hyperpolarizing potential (+100 mV), the steady-state current was reduced by a factor $\sim$ 1.8 from 152.0 $\pm$ 24.6 pA/pF (n=11) in DrVSP(C302S) to 86.3 $\pm$ 9.1 pA/pF (n=14) with DrVSP.

Conclusions: G_qPCR and PIP₂ signaling did not produce significant modulation of the PAC current in HEK293T in a physiological range of membrane potentials. The data suggest that cellular responses to extracellular acidification that are mediated by OGR1 and PAC may involve different pathways. While further work will be required to establish the crosstalk of pH_e

sensing mechanisms in native cells, the data highlight new aspects of the cellular responses to variations in pH_e homeostasis.

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C32

How ageing airways affect neutrophil migration during early SARS-CoV-2 infection.

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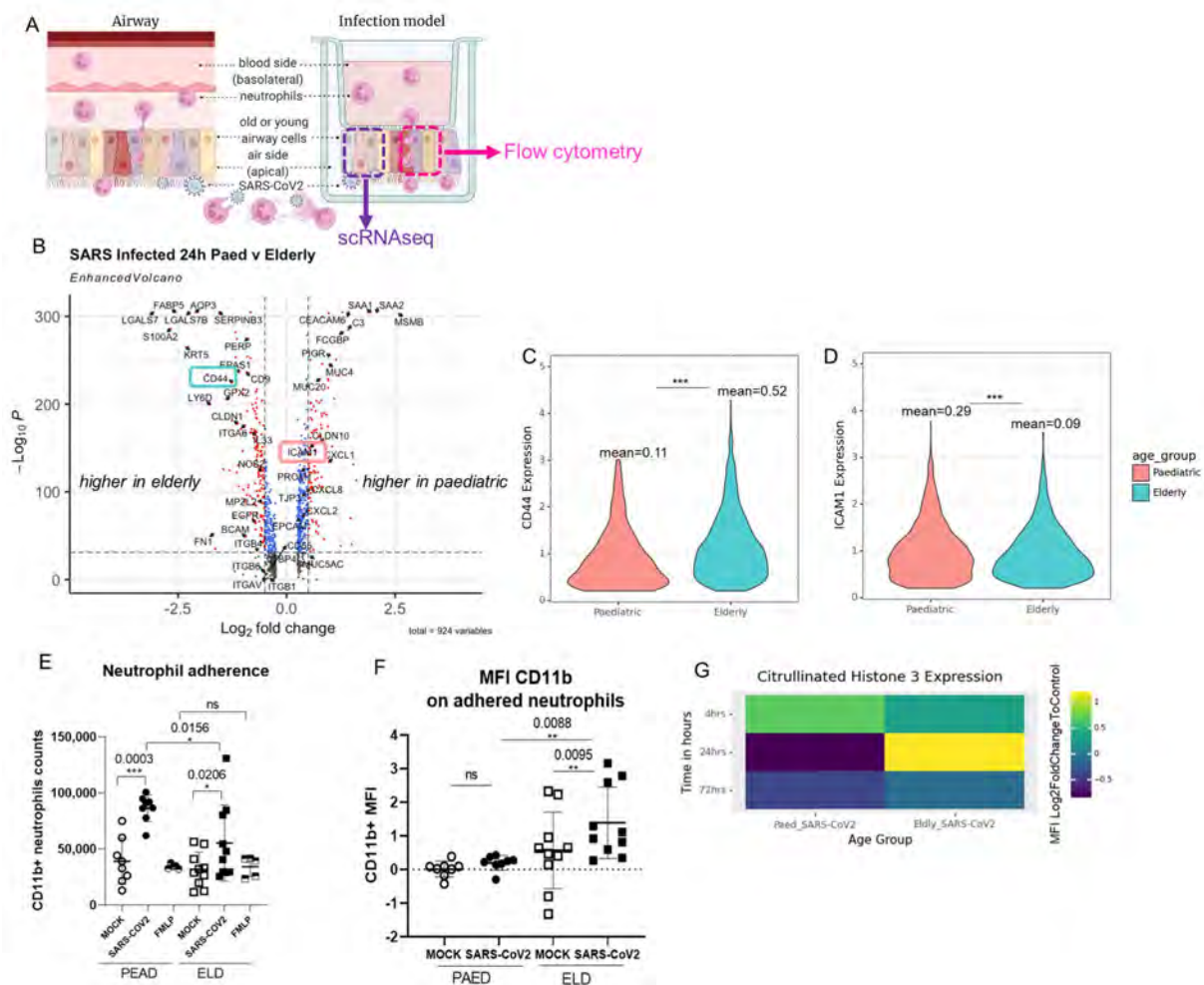
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BACKGROUND: The COVID-19 pandemic caused by the SARS-CoV-2 virus has resulted in over 6.5 million deaths, predominantly in the elderly (Huang *et al.*, 2020). There is little understanding regarding how COVID-19 severity increases with age. Neutrophils are found in large numbers in the airways of the lungs in severe COVID-19 patients (Veras *et al.*, 2020). We aim to understand whether this influx of neutrophils into the airway has a protective or detrimental effect. To do this we investigated the function of neutrophils during SARS-CoV-2 infection and their interaction with the airway epithelium using an experimental infection model of the airway epithelium from children and the elderly (**Figure 1A**).

METHODS: Nasal airway cells obtained from healthy elderly (>70y) and young (<11y) individuals (n=18 total: elderly n=10, paediatric n=8) were differentiated at air-liquid interface as described before (Woodall M *et al.*, 2021). Epithelial cells were then infected with SARS-CoV-2 for 24h. Airway epithelial cells were subsequently analysed by single-cell RNA sequencing (scRNAseq) (**Figure 1A**) to identify differentially expressed genes that could impact neutrophil migration (**Figure 1B**). To test this functionally, human neutrophils were added to the basolateral (blood) side of infected epithelial cells so that they migrate to the apical (air) and infected side of the epithelium, similar to a physiological airway (**Figure 1A**). Neutrophils were then recovered after 1h for flow cytometric analyses (**Figure 1A**).

RESULTS: scRNAseq data showed that CD44; a glycoprotein expressed on the surface of both the airway epithelium and neutrophils; was highly expressed in the elderly airway epithelium 24hrs post SARS-CoV-2 infection (**Figure 1B,C**). Whilst ICAM-1 is more highly expressed in the paediatric epithelium (**Figure 1B,D**). We also found higher numbers of neutrophils adhered to SARS-CoV-2 infected paediatric epithelium compared to SARS-CoV-2 infected elderly epithelium (**Figure 1D**). In addition we found increased activation of neutrophils (CD11b+) (**Figure 1F**) and more Citrullinated Histone 3 positive neutrophils (**Figure 1G**) migrated across the SARS-CoV-2 infected elderly compared to the paediatric epithelium.

CONCLUSION: Our data suggest that neutrophils have a weaker and less stable adhesion to SARS-CoV-2 infected nasal epithelium with increasing age. This may be due to the interaction of LFA-1 on neutrophils and ICAM-1 on the SARS-CoV-2 paediatric airway which is mediated by stronger electrostatic and hydrophobic forces. Whereas the interaction between CD44 on the elderly airway epithelium and neutrophils is facilitated by hyaluronic acid, a polysaccharide of which their binding affinity can vary depending on inflammation. Overall, these findings point to an inflammatory neutrophil phenotype influenced by an elderly epithelium and supports the hypothesis that neutrophils contribute to COVID-19 severity.



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C33

Voltage-gated Na⁺ channel activity in breast cancer cells increases glycolytic rate, which acidifies the tumour microenvironment

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Voltage-gated Na⁺ channels are expressed in many cancer types (1), and in breast cancer Na_v1.5 expression is associated with increased metastasis and poorer survival (2). Na_v1.5 activity has been shown to increase invasion of breast cancer cells by reducing the extracellular pH. This is dependent on Na_v1.5 activity increasing H⁺ extrusion through the Na⁺/H⁺ exchanger NHE1 (3). The mechanism by which Na_v1.5 increases H⁺ release into the tumour microenvironment is unclear. This study investigated whether Na_v1.5 activity increases the rate of glycolysis, since an increased influx of Na⁺ means that more ATP is needed to power Na⁺/K⁺ ATPase to maintain intracellular [Na⁺] at steady state.

In MDA-MB-231 breast cancer cells at an extracellular pH of 6.0 (as can be found in solid tumours), the steady state (“persistent”) current through Na_v1.5 channels at the resting membrane potential of -18.9 mV (4) was $10.3 \pm 2.2\%$ of the maximal transient Na⁺ current, which was -9.19 ± 1.28 pA/pF. For an average cell with capacitance 26.0 ± 2.2 pF, this equated to a persistent inward Na⁺ current of -24.6 ± 3.4 pA.

We showed Na⁺ removal from the cell via Na⁺/K⁺ ATPase relies on glycolysis for ATP in this model, because inhibiting glycolysis with iodoacetate increased intracellular [Na⁺], as measured by the ratiometric indicator SBFI-AM ($n = 6$, $p < 0.01$, one sample t test), whereas inhibiting oxidative phosphorylation with oligomycin did not change the intracellular [Na⁺] ($n = 6$, $p = 0.62$, one sample t test).

Using a Seahorse analyzer, we then showed that Na_v1.5 activity increased glycolysis, measured as the extracellular acidification rate, by 9.8 ± 1.7 mpH units/minute ($n = 3$, $p < 0.0001$, 2-way ANOVA), whereas Na_v1.5 activity did not change oxidative phosphorylation, measured as the oxygen consumption rate ($n = 3$, $p = 0.99$, 2-way ANOVA).

The rate of Na_v1.5-dependent H⁺ production was estimated by assuming that all Na⁺ entering via Na_v1.5 was removed by the Na⁺/K⁺ ATPase, and this was powered by ATP from glycolysis. It was assumed that H⁺ produced via ATP hydrolysis and glycolysis was then removed by NHE1. The estimated rate of pH change in a Seahorse analyzer well was 1.3 mpH units/minute, which was in the same order of magnitude as the measured rate of pH change.

These results indicate that Na⁺ channel activity in cancer cells can increase glycolytic respiration which acidifies the tumour microenvironment, and this may explain how Na_v1.5 increases invasion in breast cancer.

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C34

Transepithelial Fluid and Electrolyte Transport in a Human Choroid Plexus Cell Line in Response to TRPV4 Stimulation

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Introduction: Cerebrospinal fluid (CSF) is produced predominately by the choroid plexus epithelium (CPE). The composition of CSF varies according to physiological, diurnal, and pathophysiological influences. Understanding the transporters that control the production and composition of CSF is of clinical relevance for many diseases. In a genetic rat model of hydrocephalus (*Tmem67*^{-/-}), we have previously shown that antagonists of the transient receptor potential vanilloid 4 (TRPV4) channel, ameliorate the development of excess CSF in the context of hydrocephalus, implicating this channel as a key component of CSF production. The aim of our studies is to use a human CPE cell model, the HIBCPP (human choroid plexus papilloma) to identify the transepithelial electrolyte fluxes that occur in response to TRPV4 stimulation and to determine if the electrolyte flux is accompanied by a measurable fluid movement.

Methods: HIBCPP cultures were grown on Millicell permeable supports. Ussing-style electrophysiology was used to define changes in transepithelial electrogenic fluxes (short circuit current, I_{SC}) and transepithelial permeability (conductance, the inverse of transepithelial resistance) in the presence of TRPV4 agonists and effectors. Parallel cultures were used to measure fluid secretion (net fluid flux from the basolateral media to the apical media) or absorption (the opposite of secretion) 10 minutes after the addition of a TRPV4 agonist.

Results: We previously showed that the HIBCPP cell line has important transporters found in the native epithelium in the correct polarization and forms a moderately tight barrier epithelium consistent with the blood-CSF barrier. TRPV4 agonist stimulation causes a multicomponent change in transepithelial electrolyte flux and a substantial and reversible change in transepithelial permeability as measured by transepithelial conductance. The TRPV4-mediated electrolyte flux appears to be secondary activation of multiple transport proteins stimulated in response to the TRPV4-mediated influx of Ca^{2+} and Na^{+} . The I_{SC} is a complex mixture of movement of both cations and anions causing the I_{SC} to return to baseline within 10 minutes. However, the increased conductance, which remains elevated at 10 minutes, indicates the I_{SC} return to baseline is a function of continuing but opposing transepithelial movements of electrolytes. This flux is accompanied by a statistically significant fluid secretion. The vehicle-treated samples had an increase in fluid secretion of $1.34 \mu\text{L}/\text{cm}^2/10 \text{ min}$ (mean + SEM, $n=5$), a value not statistically different from zero. In response to the TRPV4 agonist, there was a significant ($p=0.0297$) 18.7-fold increase in fluid secretion to $25.11 \mu\text{L}/\text{cm}^2/10 \text{ min}$ ($n=7$). Although *in vivo* the choroid plexus is one of the most secretory epithelia in the body this is, to our knowledge, the only cultured epithelium showing this level of fluid secretion.

Conclusions: The HIBCPP cell line has multiple characteristics of the native choroid plexus epithelium. This line is being used to dissect pathways involved in CSF production and these will be presented. Our results have uncovered unexpected levels of fluid movement in response

to TRPV4 stimulation which can inform the role of opposing electrolyte movements and electroneutral transporters.

C35

Extreme apnea in humans promotes cerebral oxidative-nitrosative stress and structural destabilisation of the neurovascular unit

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Background: Voluntary asphyxia imposed by static apnea represents a unique model to test the functional-structural limits of the neurovascular unit (NVU) in humans exposed to pathological extremes of hypoxaemia and hypercapnia. In the present study, we examined if [1] apnea would be associated with exaggerated cerebral oxidative-nitrosative stress (OXNOS) and structural destabilisation of the NVU and [3] distinguish between hypoxaemia and hypercapnea as the dominant vasoactive stimulus.

Methods: Ten ultra-elite breath-hold divers (6 male/4 female) aged 33 (mean) $\pm$ 9 (SD) years old performed two maximal dry apneas after normoxic hyperventilation (NX: severe hypoxaemia-hypercapnia) and hyperoxic hyperventilation (HX: absence of hypoxaemia while exacerbating hypercapnia) with measurements obtained at eupnea and after apnea. Mean arterial (MAP) and internal jugular venous (IJVP) pressures were recorded directly. The latter served as a validated surrogate of intracranial pressure (ICP). Net transcerebral biomarker exchange was calculated as the product of global cerebral blood flow (gCBF, duplex ultrasound) and radial arterial to internal jugular venous concentration gradients of plasma ascorbate free radical ($A^{\cdot-}$, electron paramagnetic resonance spectroscopy), plasma nitrite (NO_2^- , ozone-based chemiluminescence) and select panel of serum NVU proteins (ELISA, Single Molecule Array). Following confirmation of distribution normality (Shapiro-Wilk *W* tests), data were analysed using a combination of two (*State*: eupnea vs. apnea $\times$ *Site*: arterial vs. venous) and three (*Trial*: NX vs. HX $\times$ *State* $\times$ *Site*) factor repeated measures ANOVAs with post-hoc Bonferroni-corrected paired samples *t*-tests.

Results: Compared to HX, greater increases in MAP ($+61 \pm 9$ vs. $+47 \pm 14$ mmHg, $P = 0.021$) and IJVP ($+12 \pm 2$ vs. $+7 \pm 5$ mmHg, $P = 0.005$) were observed during apnea in NX whereas the increase in gCBF was lower ($+83 \pm 22$ % vs. $+206 \pm 52$ %, $P = <0.001$). Apnea in NX stimulated a greater net cerebral output (venous $>$ arterial) of $A^{\cdot-}$ (-4049 ± 5035 vs. -609 ± 5273 AU/100g/min, $P = 0.042$) and lower uptake (arterial $>$ venous) of NO_2^- (1787 ± 2029 vs. 3806 ± 2334 nM/100g/min, $P = 0.036$), highlighting the key contribution of hypoxaemia to OXNOS. This coincided with a greater net cerebral output of S100B, glial fibrillary acidic protein, ubiquitin carboxy-terminal hydrolase L1, neurofilament light-chain and total tau (all $P < 0.05$).

Conclusions: Collectively, these findings demonstrate that NVU integrity is more impaired during extreme apnea-induced hypoxemic- compared to hyperoxemic-hypercapnic stress highlighting hypoxia as a key stimulus underlying a transient increase in blood-brain barrier permeability and neuro-gliovascular reactivity/damage. Structural changes were linked to the combined elevation in molecular ($\uparrow$ OXNOS) and haemodynamic ($\uparrow$ systemic/intracranial hypertension) stress. Collectively, these novel findings provide a potential mechanism whereby the combined effects of molecular-haemodynamic stress to which the 'apneic brain' is exposed converge at the NVU transiently compromising integrity and function.

C36

Exercise promotes an inflammatory response proportional to the dose of exercise that transiently impairs insulin sensitivity in young healthy males.

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Introduction: Exercise improves glucose disposal and insulin sensitivity of tissues both acutely and chronically but the dose which elicits this response, and the persistence of this effect is yet to be determined.

Aim: To explore the dose-response relationship between exercise and glycaemic control.

Methods: Participants (n=10) (age 23 ± 4 , BMI = 26 ± 5 kg/m²) attended the lab and cycled at 60% $\dot{V}O_2$ max for a time commensurate with expending 0kcal, 175kcal, 350kcal or 700kcal (randomised). Participants were fitted with a continuous glucose monitor (CGM) for the next 72 hours. The evening of these exercise visits, subjects consumed a control meal (772kcal, carbohydrate=66%, fat=18%, and protein=16%). Following a 12 hour overnight fast, subjects undertook an oral glucose tolerance test (OGTT). Bloods were taken from an arterialised dorsal hand vein at baseline (before ingestion of 75g dextrose in 300ml water), and every 30 minutes for two-hours. Indirect calorimetry was taken 20 mins before the test and during the last 20 mins of the OGTT.

Results: The area under the curve (AUC) of glucose during the OGTT was highest for 350kcal of exercise (0kcal = 12.3 ± 1.2 mmol/L * hour; 175kcal = 12.7 ± 2.0 mmol/L * hour; 350kcal = 14.1 ± 2.6 mmol/L * hour; 700kcal = 13.0 ± 1.8 mmol/L * hour, $p = 0.04$). There were no differences in the range or average glucose concentrations recorded from the CGM for 72hours ($p > 0.05$). Insulin AUC concentration was highest after 700kcal of exercise (0kcal = 137.9 ± 41.7 μ UI/mL*hour, 175kcal = 128.1 ± 36.0 μ UI/mL*hour, 350kcal = 142.9 ± 54.7 μ UI/mL*hour, 700kcal = 169.7 ± 37.6 μ UI/mL*hour, $p = 0.03$). FFA concentrations significantly decreased with 700kcal of exercise (0kcal = 5.3 ± 0.4 mmol/L * hour, 175kcal = 4.5 ± 0.8 mmol/L * hour, 350kcal = 4.6 ± 0.9 mmol/L * hour, 700kcal = 4.3 ± 0.8 mmol/L * hour, $p = 0.006$), suggesting exercise increased triglyceride synthesis and lipid storage. The respiratory exchange ratio (RER) was significantly greater at the end of the OGTT ($p < 0.001$) but there were no differences between exercise doses ($p > 0.05$). The AUC for GLP-1 (total) was significantly increased in larger doses of exercise (350kcal and 700kcal) (0kcal = 20.9 ± 9.5 pg/mL * hour, 175kcal = 24.9 ± 12.4 pg/mL * hour, 350kcal = 34.0 ± 11.7 pg/mL * hour, 700kcal = 35.3 ± 8.5 pg/mL * hour, $p = 0.02$). This further suggests greater insulin resistance with greater doses of exercise. IL-1 β AUC concentrations were highest after 700kcal of exercise (0kcal = 2.3 ± 1.0 pg/mL * hour, 175kcal = 1.6 ± 1.0 pg/mL * hour, 350kcal = 3.0 ± 1.9 pg/mL * hour, 700kcal = 3.6 ± 1.2 pg/mL * hour, $p = 0.01$) suggesting that inflammation may be causing this increase in insulin resistance.

Conclusion: Collectively, these data suggest that greater exercise doses cause a greater level of inflammation post-exercise which can acutely impair insulin sensitivity. Further investigations are warranted to better understand the inflammatory mechanisms regulating post-exercise changes in glycaemic control.

C37

The effect of female breast surface area on heat-activated sweat gland density and output

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Introduction:

The production and evaporation of sweat from the skin surface is the human body's principal method of heat loss during heat stress. By 2 years of age, our skin contains 2-5 million sweat glands [1]. The number of sweat glands does not appear to change beyond this age. Hence, sweat gland density decreases with skin expansion during physical growth [1, 2].

In contrast to men, female development includes significant morphological changes across specific body parts, such as the breast. Female breast development, and the resulting breast surface area (BrSA), can vary greatly due to genetic factors, body-mass-index and energy intake early in life. However, it is unclear whether sweat gland density further decreases as breasts grow.

Sweat gland density may impact sweat output per gland for a given sweat rate [3]. This has implications for sweat accumulation in sport bras, which in turn affects breast heat balance and comfort during exercise heat stress in women of different breast sizes. This study aimed to investigate breast-size dependent, regional differences in sweat gland density and output during exercise heat stress in women with large differences in BrSA.

Method:

Fifteen healthy females (24±7yr) with large differences in BrSA (from small to x-large, range=147.2-480.5cm²) performed a 50-min submaximal run in a climatic chamber regulated at 33.0±0.8°C and 53.4±2.0% RH. Sweat gland density (SGD; modified iodine technique [4]) and local sweat rates (LSR; absorbent patches [5]) were measured above and below the nipple, and at the bra triangle, during the final 5-min of exercise. Gastrointestinal (core) temperature and metabolic rate were monitored throughout the run. We used linear regression analyses to evaluate the relationship between: a) SGD and BrSA; and b) sweat output per gland (calculated as LSR/SGD) and BrSA. Furthermore, we assessed regional differences in SGD and sweat output per gland amongst the bra triangle, above and below the nipple, with a repeated-measures ANOVA.

Results:

SGD above ($R^2=0.55$, $p<0.01$, Fig. 1A) and below the nipple ($R^2=0.63$, $p<0.01$, Fig. 1B) decreased with increasing BrSA. This effect was not observed at the bra triangle ($R^2=0.12$, $p=0.101$, Fig. 1C). Sweat output per gland above the nipple increased with BrSA ($R^2=0.29$,

$p=0.02$, Fig. 2A). This effect was not observed below the nipple ($R^2=0.13$, $p=0.10$, Fig. 2B) nor at the bra triangle ($R^2=0.04$, $p=0.24$, Fig. 2C).

SGD was lower at both breast sites (above nipple= 35.6 ± 6.0 glands/cm²; below nipple= 31.2 ± 4.8 glands/cm², $p<0.01$) than at the bra triangle (86.8 ± 5.3 glands/cm², Fig. 3). Sweat output per gland above ($343.4\pm39.6\mu\text{g}$, $p<0.01$), but not below ($416.4\pm62.5\mu\text{g}$, $p=0.89$), the nipple was lower than at the bra triangle ($690.6\pm76.0\mu\text{g}$, Fig. 4).

Conclusion:

Our findings indicate that SGD decreases and sweat output per gland increases with larger breasts, and that SGD and output per gland vary greatly across the breast and bra triangle. It therefore appears that, to maintain breast heat balance, individual sweat glands upregulate their activity to accommodate their lower density across larger breasts. Sport bra design may therefore consider the implications of this on sweat accumulation patterns for women of different breast, thus bra sizes.

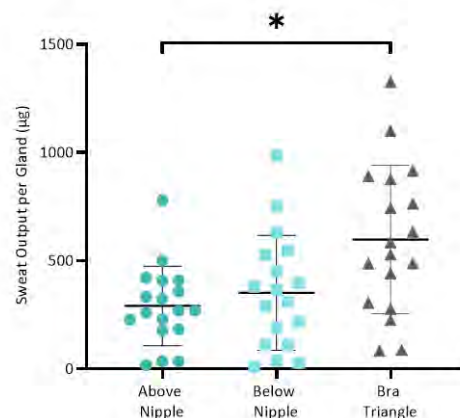


Figure 4. Sweat output per gland regional differences from 3 chest locations. Mean $\pm$ SD. *denotes statistically significant difference in sweat output per gland between test site locations, $p < 0.05$.

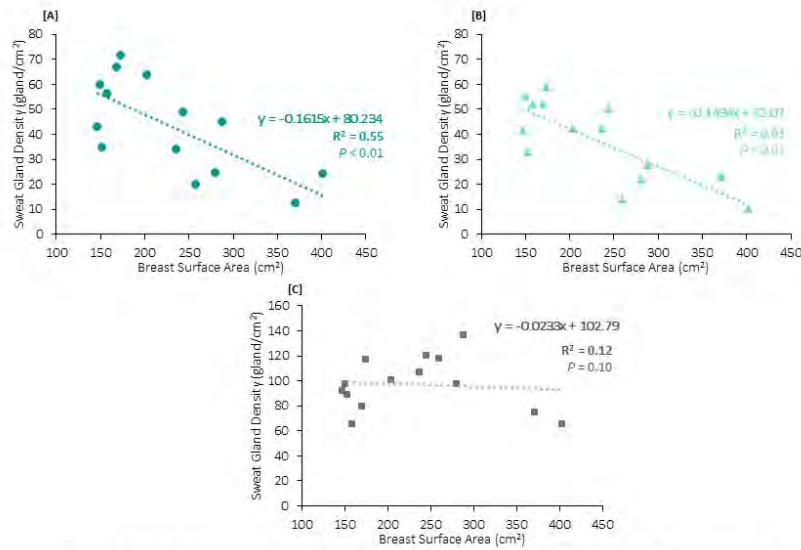


Figure 1. Relationship between breast surface area and sweat gland density at 3 chest locations (n=15). [A.] Above Nipple. [B] Below Nipple. [C] Bra Triangle. Significant negative correlations between breast size and sweat gland density above the nipple and below the nipple ($p < 0.05$).

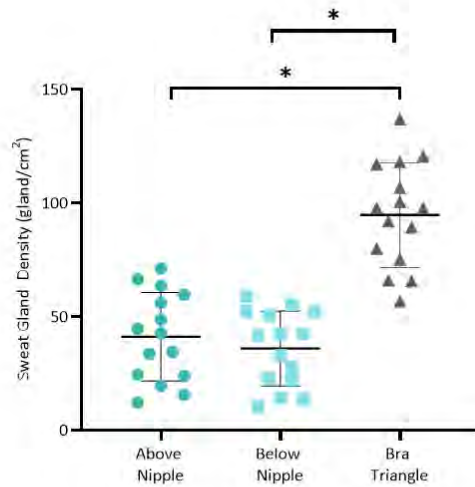


Figure 3. Sweat gland density regional differences from 3 chest locations. Mean $\pm$ SD. *denotes statistically significant difference in total average SGD between test site locations, $p < 0.05$.

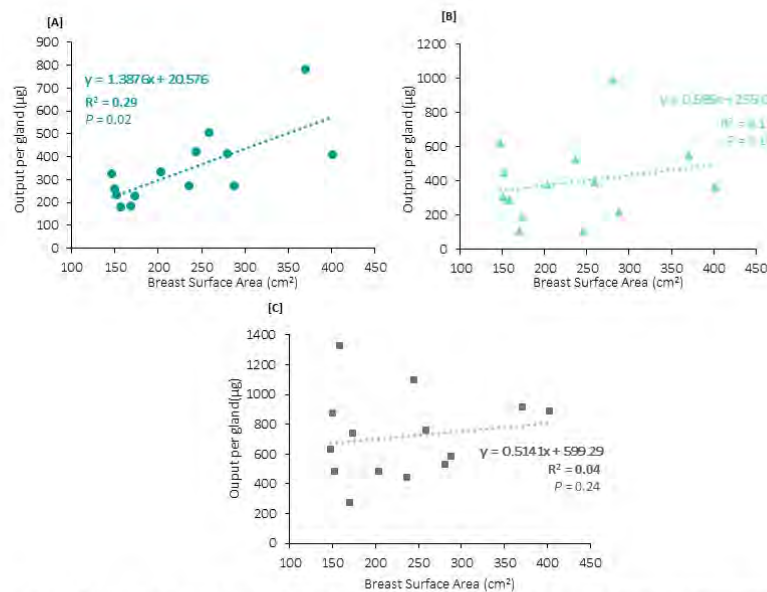


Figure 2. Relationship between breast surface area and sweat output per gland at 3 chest locations (n=15). [A] Above Nipple. [B] Below Nipple. [C] Bra Triangle. Significant positive correlation between breast surface and sweat output per gland above the nipple ($p < 0.05$).

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C38

Morning exercise reduces glycaemia in people with Type 2 Diabetes also being prescribed metformin

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Type 2 Diabetes (T2D) is a significant healthcare challenge. Engaging in physical exercise is beneficial for the treatment of T2D and can improve insulin sensitivity (Gabriel & Zierath, 2017). However, previous evidence suggests that exercise at different times of the day in people with T2D may have opposing outcomes on blood glucose levels throughout the exercise day (Savikj et al., 2019). This may be especially important for those concurrently taking metformin medication (Gabriel & Zierath, 2021). We hypothesise that afternoon/evening moderate intensity exercise is more efficacious than morning exercise at lowering glycaemia in people who are also being prescribed metformin. To test this hypothesis, we conducted a remote crossover exercise intervention using wearable technology. Within this exercise intervention we aimed to monitor adherence and compliance to the exercise protocol. Nine male and nine females with T2D undergoing metformin treatment completed the trial with 2-week baseline recording, six weeks randomly assigned to a morning exercise (7-10am) or afternoon/evening exercise (4-7pm), with a two-week wash-out period. To monitor trial adherence, we assessed step count per day and heart rate (HR). Physical activity was monitored using the Garmin Vivosmart 4 (Garmin Ltd, Olathe, KS, US). Participants were asked to perform 30 minutes of walking at 70% of their estimated max-HR every other day. Glucose levels were measured with continuous glucose monitors FreeStyle Libre 2 sensor (Abbott Diabetes Care Inc, Alameda, CA, US). 24 h hourly mean glucose was estimated. Participants were asked to fill 4-day food diaries during baseline, first and last 2 weeks of each exercise arm. Metformin doses were registered by participants on food diaries. Results are expressed as Mean $\pm$ SEM. Eighteen participants (age 61 ± 2 year) completed the trial with satisfactory adherence. The estimated 70% of max-HR was 111.4 ± 5.5 bpm. During exercise, average HR was 117.2 ± 8.2 bpm and 117.3 ± 11.5 bpm, during morning and evening, respectively ($p > 0.05$). During walking days participants completed an average of 10814 ± 2251 steps and 10373 ± 2183 steps during morning and evening, respectively ($p > 0.05$). During resting days participants walked an average of 6843 ± 2383 steps and 6344 ± 2182 steps during morning and evening, respectively ($p > 0.05$). Thus, no significance change in exercise intensity or compensatory activity was found between the arms of the trial. When analysing the 24 h hourly mean glucose area under the curve (AUC), a significant difference ($p = 0.02$) was found between baseline (210.3 ± 76.68 mmol/L) and morning exercise (180.6 ± 68.37 mmol/L). AUC glucose was significantly lower ($p = 0.01$) in participants taking metformin before breakfast (148 ± 11.05 mmol/L) compared with participants taking metformin after breakfast (220.2 ± 23.58 mmol/L) only when they performed morning exercise. In summary, our data show lower glucose levels after morning moderate intensity exercise in people with T2D also being prescribed metformin. Metformin taken prior to breakfast seems to have a positive effect on AUC glucose levels compared with metformin taken after breakfast when morning exercise is performed. Contrary to our hypothesis and previous findings (Savikj et al., 2019), our data

suggest that the time-of-day effect of exercise on glycemia in people with T2D may be exercise-intensity, and modality-dependent.

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C39

Physically fit adult humans show similar heat tolerance between sexes, even when compared during the luteal phase of the menstrual cycle.

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Background: In adult humans, thermoregulation can differ between sexes for physiological, physical, and psychophysical reasons. Heat tolerance refers to one's capacity for tolerating either heat stress (e.g., work capacity in a hot environment) or heat strain (e.g., core temperature that elicits exhaustion or physiological dysfunction). Both contexts have functional relevance and provide physiological insight. Yet, human heat tolerance data are largely from men, and studies of sex effects are limited to the heat stress context and the follicular phase of the menstrual cycle, i.e., when males and females are most similar physiologically.

Aims: We sought to determine whether heat tolerance differs between men and women, when assessed in the luteal phase and in a heat strain context. We hypothesised that females would have less thermal reserve due to higher baseline core temperature but a similar ceiling.

Methods: Procedures adhered to the ethical approval granted by the Human (Health) Ethics Committee, University of Otago (H20/031). Participants were 9 males and 9 females, pooled from two studies in which equal numbers of males and females cycled at low-moderate intensity in uncompensable heat stress (skin temperature 38-39°C), to volitional exhaustion or rectal temperature of 40°C, whichever occurred first. All participants were habitually physically active, and sexes were of similar fitness (peak aerobic power 54 and 51 mL/min/kg, and 3.6 and 3.7 W/kg) and surface area-to-mass ratio (0.024 and 0.024 m²/kg). Heat stress trials occurred mid-late afternoon, with participants euhydrated and blinded to their core temperature. Previously, each participant had completed an aerobic fitness test and at least one heat familiarisation session involving a 2°C rise in rectal temperature or volitional tolerance. Statistical analyses were unpaired t tests and two-way ANOVA. Results are reported as means and 95% confidence limits (CL), for n=9 vs 9 unless stated otherwise.

Results: Core temperature was not higher at baseline for females than males (37.18 vs 37.06°C; P=0.479; CL: -0.22, 0.45). Neither was thermal reserve smaller (2.07 vs 1.75°C; P=0.271; CL: -0.28, 0.93), nor rate of heating faster (1.25 vs 1.21 °C/h; P=0.841; CL: -0.37, 0.45). Only one participant reached the 40.00°C ethical end point. End tidal CO₂ pressure did not differ at baseline or decrease (5 vs 6 mm Hg) more for females than males during heat stress (Sex: P=0.081; Strain: P=0.001; Sex*Strain: P=0.634). Mean arterial blood pressure and its decrease (2 vs 6 mm Hg) were also similar between sexes (P=0.503; P=0.011; P=0.221). Perfusion of the internal carotid artery also remained similar (-1 vs -9%; P=0.879; P=0.181;

$P=0.294$), as did estimated Intracranial Pressure (1 vs 1 mm Hg, indexed from optic nerve sheath diameter; $P=0.298$; $P=0.073$; $P=0.785$; $n=8$ for both sexes and both variables).

Conclusions: For these fit, fitness-matched females and males, heat tolerance was evidently not lower for females despite the comparison being made during their luteal phase. Cerebral haemodynamics also appeared to be minimally affected. The present results cannot be expected to apply to an unfit population because high fitness is known to lessen menstrual phase effects on sex hormones and thermoregulation.

C40

Influence of ADORA2A and CYP1A2 genotypes on caffeine metabolism in healthy adults

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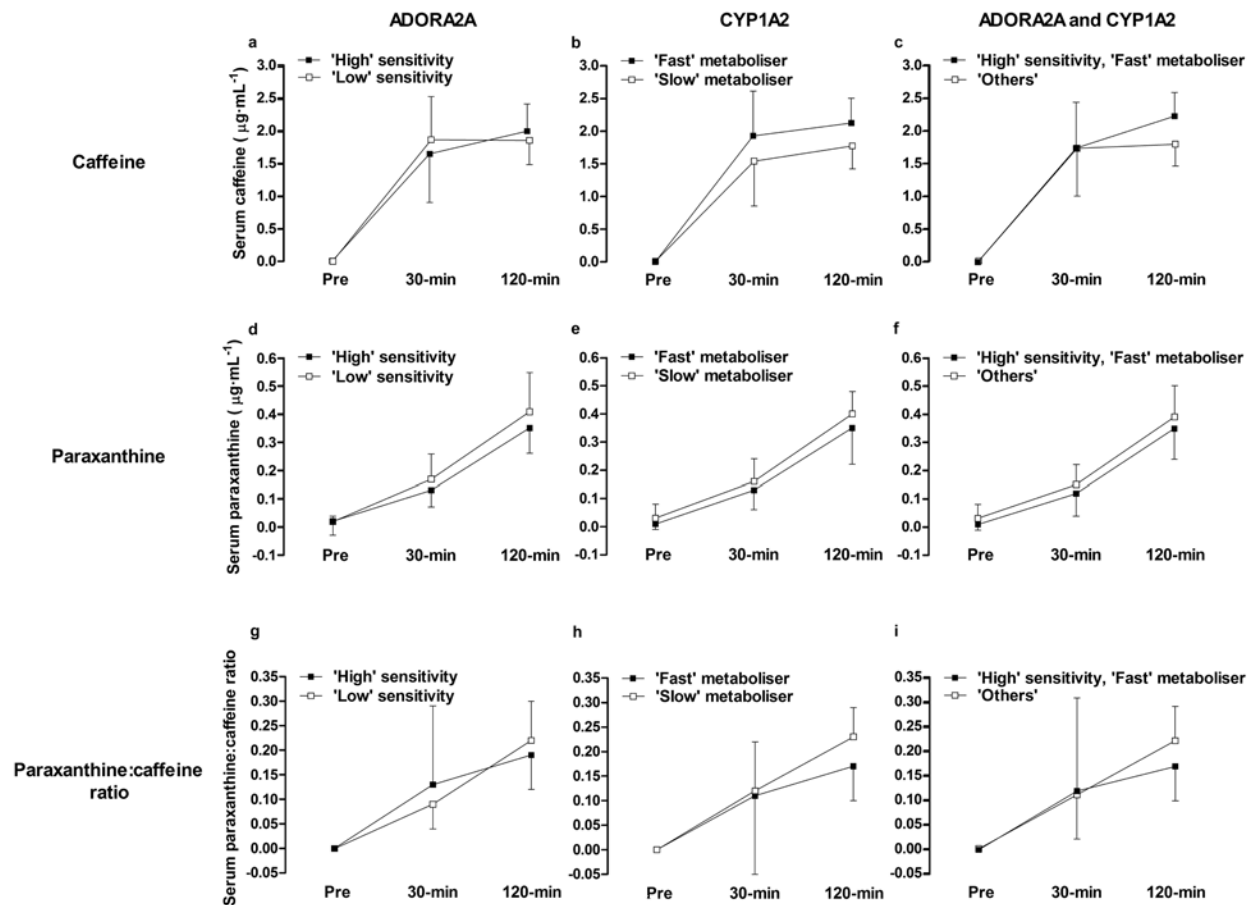
Background: Caffeine, found in coffee, tea, energy drinks, and some foods and supplements, is the most widely consumed psychostimulant in the world. Caffeine pharmacokinetics and pharmacodynamics vary between individuals (Nehlig, 2018), with single-nucleotide polymorphisms (SNPs) in the genes that encode for the adenosine receptor (ADORA2A) and the P450 enzyme (responsible for 95% of the body's caffeine metabolism; CYP1A2) possibly responsible for some inter-individual variability. The purpose of this study was to determine the influence of two commonly occurring ADORA2A and CYP1A2 SNPs on caffeine metabolism.

Methods: Sixteen healthy adults (age 24 ± 5 years; BMI 22.9 ± 2.4 kg·m⁻²; $n = 3$ women) volunteered to participate and consumed a capsule containing 3 mg·kg⁻¹ body mass of caffeine, after an overnight fast and having abstained from caffeine consumption for 48 h. Venous blood samples were collected pre, and 30 and 120-min post caffeine ingestion. Serum caffeine and paraxanthine were measured using high-performance liquid chromatography. Genomic DNA was extracted from whole blood and SNPs in ADORA2A (rs5751876) and CYP1A2 (rs762551) genes were determined by rhAmp assays (Integrated DNA Technologies, USA). Participants were categorised by ADORA2A gene as TT homozygous ('high' sensitivity) or C allele carriers (CT heterozygous or CC homozygous: 'low' sensitivity); and by CYP1A2 gene as AA homozygous ('fast' metabolisers) or C allele carriers (AC or CC: 'slow' metabolisers). Mixed model ANOVAs were used to examine serum caffeine, paraxanthine, and paraxanthine:caffeine ratio.

Results: $n = 10$ participants had 'high' and $n = 6$ 'low' sensitivity ADORA2A genotype; $n = 8$ had 'fast' and $n = 8$ 'slow' metabolism CYP1A2 genotype; and $n = 6$ had both 'high' sensitivity and 'fast' metabolism genotypes (*i.e.*, ADORA2A, TT; CYP1A2, AA). There were no genotype x time interactions for serum caffeine ($P \geq 0.311$), paraxanthine ($P \geq 0.486$), or paraxanthine:caffeine ratio ($P \geq 0.433$; Figure 1). Main effects of time were found for serum caffeine, paraxanthine, and paraxanthine:caffeine ratio ($P < 0.001$). Bonferroni corrected post-hoc t-tests revealed serum caffeine increased from pre-ingestion by 1.73 ± 0.69 and 1.94 ± 0.40 µg·mL⁻¹, at 30 and 120-min post-ingestion ($P < 0.001$). Serum paraxanthine increased from pre-ingestion by 0.12 ± 0.08 and 0.35 ± 0.10 µg·mL⁻¹, at 30 and 120-min post-ingestion ($P < 0.001$). Serum paraxanthine:caffeine ratio increased from pre-ingestion by 0.11 ± 0.13 and 0.20 ± 0.07 , at 30 and 120-min post-ingestion ($P = 0.01$ and $P < 0.001$). There were no main effects of genotype for serum caffeine ($P \geq 0.07$), paraxanthine ($P \geq 0.250$), or paraxanthine:caffeine ratio ($P \geq 0.379$).

Conclusion: Caffeine metabolism 30 and 120-min post caffeine ingestion was not different between healthy adults categorised by ADORA2A or CYP1A2 SNP genotypes. Responses during this period after ingestion may be more influenced by absorption. Additional study is warranted with longer monitoring periods (up to 6 h post-ingestion) to further examine metabolism responses. An influence of ADORA2A and CYP1A2 SNPs on some ergogenic

effects of caffeine have been demonstrated previously (Grgic *et al.*, 2021), but their influence on other physiological effects of caffeine require further examination.



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C41

Hygrosense: mapping skin wetness sensitivity across the body of children, young, and older adults

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Introduction

Perceiving the skin wetness that our body produces (e.g. by sweating) or contacts (e.g. when damp clothing touches our skin), i.e. hygrosensing, is an essential sensory function that supports behavioural thermoregulation [1]. We have previously demonstrated that, in the absence of skin hygroreceptors, young adults perceive skin wetness by integrating multisensory thermal (e.g. cold) and tactile (e.g. stickiness) inputs from skin contact with moisture [2]. Yet, the development and decline of hygrosensation through life remains unclear. This study aimed to investigate differences in wetness sensitivity across the body amongst a large cohort of children, young, and older adults.

Methods

Seventy four participants, including 12 children (4F/8M, mean age: 12±3y; range: 7-15y), 41 younger (21F/20M, mean age: 25±4y; range: 20-34y) and 21 older adults (11F/10M, mean age: 55±6y; range: 45-65y), underwent quantitative sensory testing during which they reported perceived magnitudes (i.e. 100mm Visual Analog Scale from dry to completely wet) of local wetness perceptions arising from the short-duration (i.e. 10s) static application (counter-balanced order) of a cold-wet (i.e. 5°C below local skin temperature), neutral-wet (i.e. equal temperature as local skin temperature), and warm-wet (i.e. 5°C above local skin temperature) handheld temperature-controllable probe (surface: 1.32cm²; water content: 0.8ml) to the centre of the forehead, neck area, and foot dorsum. Perceptual scores from cold-, neutral-, and warm-wet stimulations were analysed for the independent and interactive effects of age (i.e. children vs. younger vs. older adults) and skin site (forehead vs. neck vs. foot) using a 2-way mixed model ANOVA. All methods accorded with ethical legislation.

Results

We found a statistically significant main effect of age on cold-wetness sensing ($p=0.031$). Post-hoc analyses indicated that, irrespective of body region, children were more sensitive than older adults (mean difference: 20.4mm [95%CI: 1.3, 39.5], corresponding to 20% difference; $p=0.033$; Fig. 1A), and as sensitive as younger adults (mean difference: 8.5mm [95%CI: -8.7, 25.8], $p=0.467$). We also found a main effect of skin site on cold-wetness sensing ($p=0.014$). Post-hoc analyses indicated that, irrespective of age, the foot was more sensitive than the neck (mean

difference: 11.1mm [95%CI: 2.2, 20.0], corresponding to 11% difference; $p=0.011$; Fig. 1A). We found no main effect of age on warm ($p=0.842$, Fig. 1B) or neutral wetness perception ($p=0.158$, Fig. 1C), yet we found a main effect of skin site on warm wetness sensing, with the foot being less sensitive than the neck (mean difference: 12.9mm [95%CI: 2.8, 23.0], corresponding to 13% difference; $p=0.008$; Fig. 1A).

Conclusions

We provide novel evidence that children as young as 12 years old present a level of wetness sensitivity that matches young adults in both magnitude and regional patterns (i.e. distal vs. proximal differences between neck and foot). Ageing appears to decrease cold-wetness sensitivity only, which may be underlined by differences in age-induced degeneration of cold-sensing myelinated (A Δ fibers) vs. warm-sensing unmyelinated (C-fibers) thermoreceptors innervating the skin and contributing to wetness sensing [3]. This knowledge may be applied to inform the design of sport garments with sweat management properties matching the wetness sensitivity of different age groups.

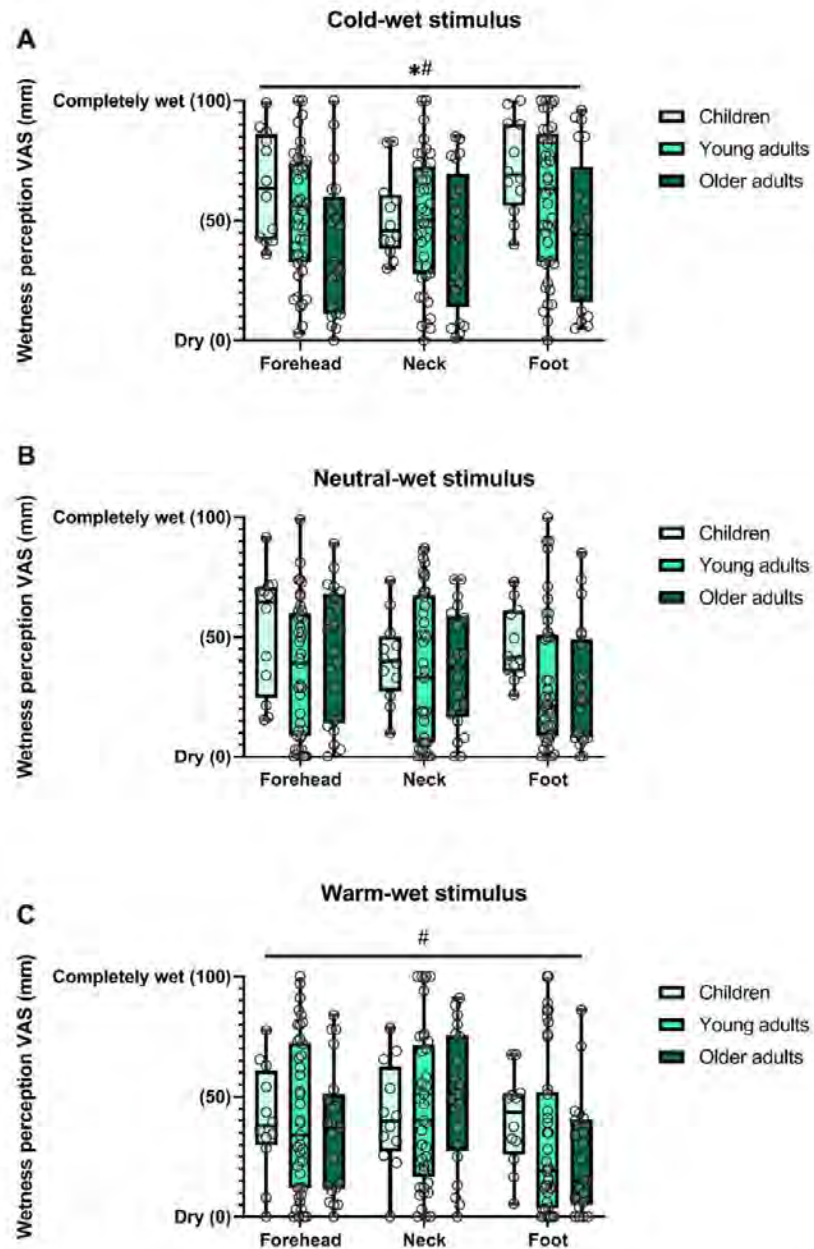


Fig. 1: Regional wetness perception in children, young, and older adults. Cold-wet (A), neutral-wet (B) and warm-wet perceptions are reported (i.e. mean, IQ range, min and max, as well as individual values) for children (N=12), younger (N=41), and older adults (N=21), as a result of stimulation of the forehead, neck, and dorsum of the foot. * denotes a main effect of age, and # denotes a main effect of skin site ($p < 0.05$).

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C42

Complex force control is improved following 6-weeks resistance training in older males independent of motor unit firing variability.

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Resistance exercise training (RET) is widely employed as an effective intervention to increase muscle mass and strength [1]. In older adults, reductions in force control are strongly associated with declines in daily functional tasks such as balance and dexterity [2], but improvements in force control while maintaining steady contractions have been demonstrated in the same population following RET [3]. The motor unit (MU) describes the motor nerve and all the muscle fibres it supplies and decreased MU firing rate (FR) variability has been associated with improved muscle force control [4]. However, the effects of RET on these properties during complex tasks are unknown. This study aimed to determine the effects of 6-weeks RET on strength, force control and MU FR variability of the vastus lateralis (VL).

6 healthy older male volunteers (66±6 yrs) performed 6-weeks supervised unilateral leg extension RET 3 times per week, consisting of 6 sets of 8 repetitions at 75% of 1-repetition maximum (1-RM). At the start and end of the training period strength was assessed by 1-RM and isometric maximum voluntary contraction (MVC). Force control was assessed by a complex force tracking task consisting of 8 oscillations at 25±4% MVC over 30s. The area under the curve (AUC) was calculated after rectifying the difference between the requested and performed force traces. High-density surface electromyography (HD-EMG) of VL was recorded and following decomposition (DEMUSE), MU FR variability was calculated as the coefficient of variation of the inter-spike interval. Paired t-tests were performed to assess differences in strength and force control with multi-level linear regression models assessing differences in FR variability. Significance was assumed when $p < 0.05$.

In the trained leg, 1-RM increased by 25% following 6-weeks RET ($p = 0.001$; 43.8±8.2kg vs 54.9±8.8kg). There was no difference in MVC between baseline and after 6-weeks training ($p = 0.182$; 393±110N vs 437±139N). During the complex phase, AUC representing force variability decreased following RET ($p = 0.037$; 32.0±7.5Ns vs 23.9±7.6Ns). There was no difference in FR variability between baseline and after 6-weeks training ($p = 0.145$; 0.216±0.040% vs 0.233±0.046%).

RET performed for 6-weeks in older males improved muscle strength and complex force control, but these increases were not explained by changes in MU FR variability assessed at similar absolute forces. This suggests an alternative mechanism to MU firing properties is responsible

for force control improvements. RET is an effective intervention for improving muscle strength and function in complex tasks in older people.

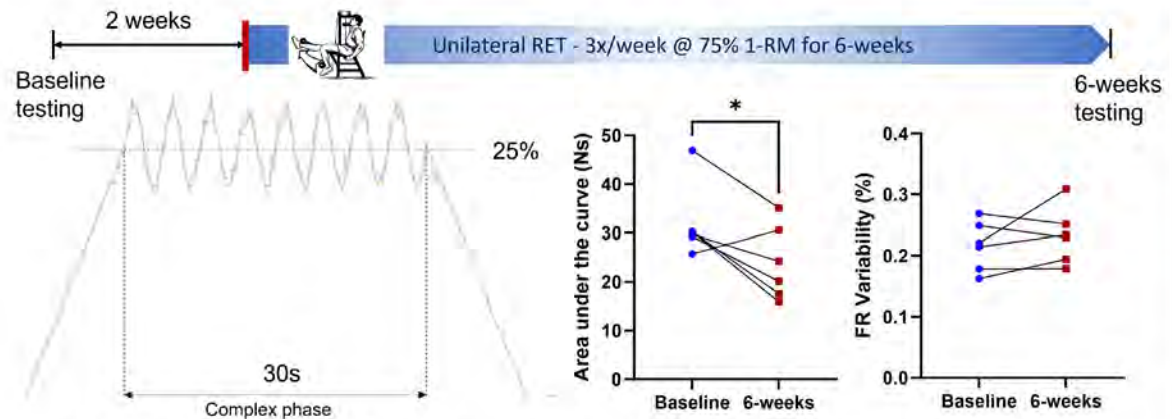


Figure 1. Protocol schematic and example of the complex force tracking task. Force variability (area under the curve) decreased (* $p=0.0372$) but with no change in firing rate (FR) variability following 6-weeks resistance exercise training (RET) at 75% of 1-repetition maximum (1-RM).

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C43

Exploring the impact of short-term unilateral targeted force accuracy training on bilateral muscle function in older adults

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Background: Muscle force output during sustained submaximal isometric contractions fluctuates around an average value, partly due to variations in motor unit firing rates (1). Although 4-weeks targeted force accuracy training (FAT) has been shown to improve muscle force control in younger adults (2), little is known about the impact of these interventions in healthy older adults, or the impact on the untrained limb following unilateral training. Therefore, we investigated whether short-term unilateral FAT could improve muscle function in the trained and untrained limbs of older adults.

Methods: This study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (FMHS 390-1121). After providing written, informed consent to participate, 16 healthy participants (8 male, 8 female. 74±5 years, BMI 26±3 kg/m²) underwent two assessment visits separated by 4-weeks fully-supervised unilateral knee extensor FAT. FAT occurred 3x/wk and consisted of 6 sinusoidal force-tracking contractions at 10, 25 and 40% of maximum voluntary contraction (MVC) in each session. Bilateral knee extensor strength was assessed via MVC and the coefficient of variation of force (force steadiness (FS)) was quantified at 25% MVC. Left and right handgrip strength (HGS) were measured with a handheld dynamometer, and mobility was assessed by the timed up-and-go (TUG). Data were analysed via two-way repeated measures ANOVA (leg/hand x time) and paired Students t-test (TUG). Statistical significance was accepted at $p < 0.05$.

Results: There was no leg x time interaction for MVC ($p = 0.822$), but there was a main effect of time ($p = 0.003$) with MVC increasing in the trained (+15.2%, $p = 0.04$) but not the untrained ($p = 0.09$) limb. There was a significant leg x time interaction ($p = 0.026$) for FS, improving to a greater extent in the trained (+16.2%, $p = 0.0001$) than the untrained (+8.9%, $p = 0.041$) limb. There was no hand x time interaction for HGS ($p = 0.885$), however there was main effect of time

($p=0.001$) with HGS improving in the right (+3.2% $p=0.04$) and left (+3.8%, $p=0.028$) hand. There was a significant improvement in TUG time following the FAT intervention ($p=0.023$).

Conclusion: In older adults, 4-weeks unilateral FAT leads to improved bilateral muscle force control, improved strength of the trained limb and increased bilateral HGS. Importantly, the FAT also improved TUG performance, an important measure of functional ability in older age. These findings demonstrate that low-intensity FAT is able to elicit targeted and cross-education improvements in muscle function. This may inform the development of interventional strategies to improve muscle function in older clinical populations (i.e., as prehabilitation for surgery given its impact in a short time-frame (3)), including in age-associated conditions with unilateral symptom presentation (i.e., stroke) (4).

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C44

The effect of exercise intensity on calcium metabolism

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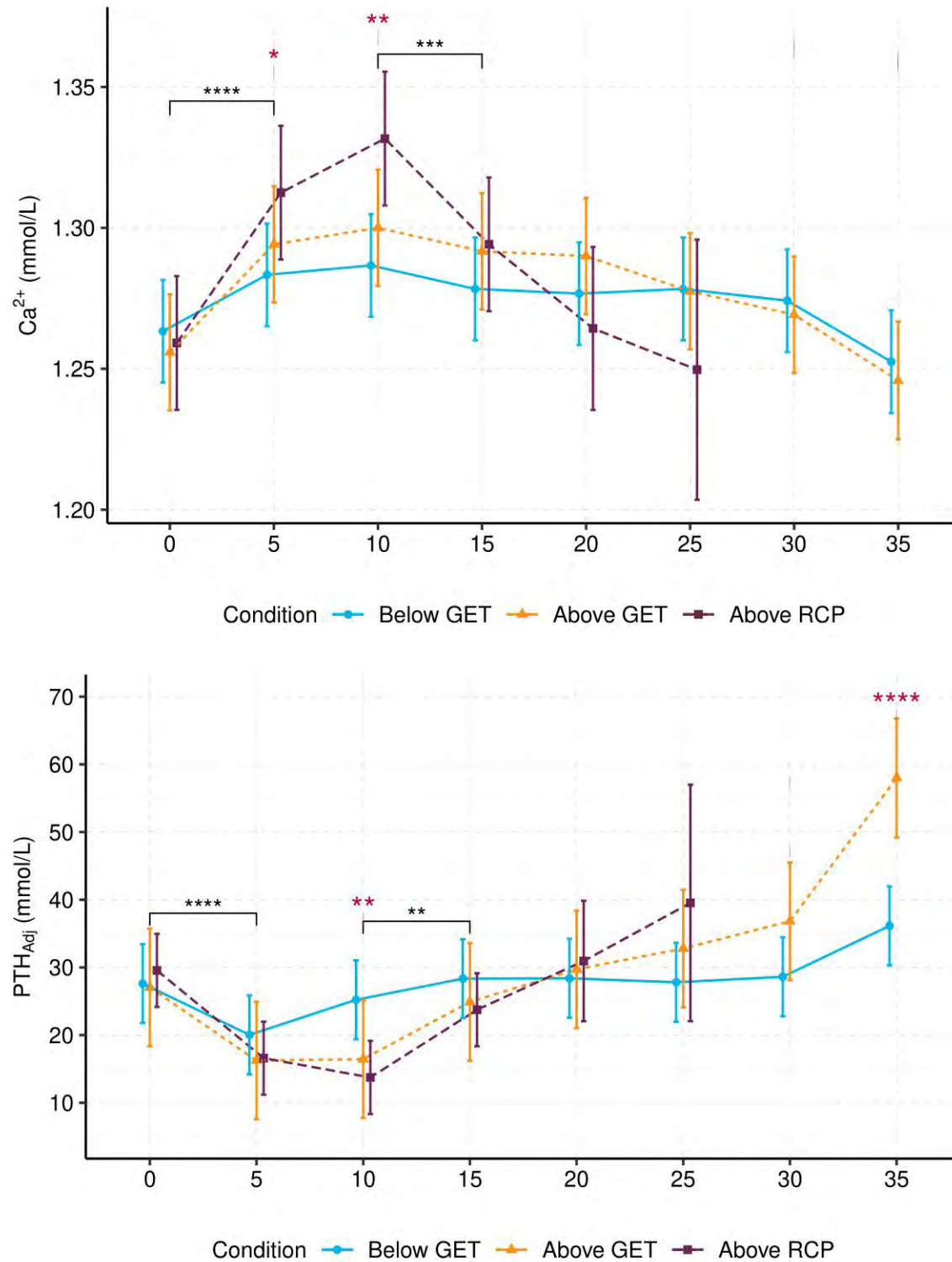
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Introduction: Recent investigations suggest that acute exercise decreases Ca^{2+} , subsequently stimulating parathyroid hormone (PTH) and bone breakdown (Townsend et al., 2016; Kohrt et al., 2018). It has been suggested the exercise-induced decrease of Ca^{2+} may contribute to the low bone mineral density phenomenon observed in many endurance athletes (Duckham et al., 2012; Scofield and Hecht, 2012). The aim of this study was to investigate the effect of exercise intensity, and the associated acid-base changes, on Ca^{2+} and PTH.

Methods: Twelve healthy males ($n = 12$) completed a three-arm, randomised-counterbalanced design experiment. Physiological thresholds and associated workloads were identified from a maximal cardiopulmonary exercise test. Participants completed 30-minutes (or until volitional fatigue) of cycle exercise below gas exchange threshold (GET), above GET, or 10W above the estimated critical power (Above RCP). Blood samples were taken every 5 minutes for 35 minutes, or until volitional fatigue. Ca^{2+} and pH were analysed using an i-STAT point of care device and EG7+ cartridge system. PTH was analysed using ELICA. Data were analysed using linear mixed effects models and omnibus ANOVAs of fixed terms (R Core Team, 2020).

Results: Exercise produced a biphasic intensity-dependent Ca^{2+} response to exercise ($F(12, 189.83) = 3.45$, $p < .001$). Exercise below GET did not alter Ca^{2+} when referenced to baseline ($M_{\text{diff}} = -0.01$ – $-0.02 \text{ mmol}\cdot\text{L}^{-1}$, $SE = 0.01$, $p > 0.05$), but exercise above GET (including above RCP) significantly increased Ca^{2+} , peaking at 10-minutes ($M_{\text{diff}} = 0.04$ – 0.07 , $SE = 0.01$, $p < 0.001$). Plasma-volume adjusted PTH (PTH_{Adj}) was significantly decreased in the initial 10-minutes of exercise above GET/RCP ($M_{\text{diff}} = -15.81$ – -10.61 , $SE = 3.30$, $p < 0.001$). Ca^{2+} decreased throughout the remainder of exercise above GET and RCP, returning to baseline concentrations. PTH_{Adj} mirrored Ca^{2+} 's response: PTH_{Adj} increased from 10 minutes above GET and above RCP, but was only significantly greater than baseline following above GET exercise ($M_{\text{diff}} = 30.93$, $SE = 3.38$, $t(189.33) = 9.14$, $p < 0.001$). Introducing pH as a covariate in the Ca^{2+} model ($b = -0.27$, $SE = 0.06$, $t(189.30) = -4.11$, $p < 0.001$) removed significant interactions at 5 and 10-minutes between exercise below GET and above RCP. However, pH could not account for all Ca^{2+} variation, as the main effect of time ($F(7, 191.77) = 13.85$, $p < 0.001$), and its interaction with condition ($F(12, 190.17) = 4.37$, $p < 0.001$), remained significant. Pooled concentrations of PTH_{Adj} were negatively associated with Ca^{2+} ($R = -0.6$, $p < 0.001$).

Conclusion: These findings suggest GET may act as an intensity threshold for eliciting significant biphasic response in Ca^{2+} and PTH_{Adj} . pH may explain the intensity-dependency of Ca^{2+} during exercise, likely due to the physiochemical competitive binding model (Pedersen, 1972; Fogh-Andersen et al., 1993). However, pH could not account for the entirety of Ca^{2+} 's temporal response, suggesting other exercise-mediated effects may play a role in calcium regulation. It appears Ca^{2+} is important for mediating PTH response to exercise, but that other exercise-induced responses may also influence PTH and subsequent bone metabolism.



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C45

Control of Contraction in Mammalian Skeletal Muscle by the Thick and Thin Filaments

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Muscle contraction is triggered by an increase in intracellular free calcium concentration leading to a structural change in the actin-containing thin filament that allows myosin motors to bind and generate force. The number of motors available for binding to actin is determined by the structure of the myosin-containing thick filaments, so that more motors are available at higher load (Linari *et al.*, 2015; Hill *et al.*, 2021, 2022). Previous studies have focused on the activation of the thick filament at the start of contraction; here we used time-resolved X-ray diffraction to characterise the inactivation of the thick filament when the load is rapidly decreased by imposing rapid shortening during maximal calcium activation.

Small-angle X-ray diffraction patterns were recorded from extensor digitorum longus muscles of the mouse at 27°C using an Eiger 2X-4M detector at the ID02 beamline at the ESRF, Grenoble, France, at a camera length of 3.2m or 2.0m for the low-angle X-ray reflections, and at 31m for the sarcomere reflections. Initial sarcomere length (SL) was set to $2.87 \pm 0.006 \mu\text{m}$ (mean $\pm$ S.E.M) in the resting muscle. Muscles were stimulated continuously for 120ms to produce a fused tetanus, and X-ray data were collected in 2-ms time frames. 60ms after the first stimulus, when SL was $2.67 \pm 0.007 \mu\text{m}$, rapid shortening was imposed for 15ms (SL $2.33 \pm 0.04 \mu\text{m}$), then force redeveloped at fixed muscle length (SL $2.13 \pm 0.008 \mu\text{m}$). Data were collected from approximately 30 tetani in each muscle (n=7). All procedures accorded with current national legislation.

The first order myosin layer line (ML1), associated with the folded helical array of myosin motors in the OFF state of the thick filament, decreased at the start of stimulation but recovered partially towards its resting level during unloaded shortening, indicating partial recovery of the thick filament OFF state. The axial periodicity of the thick filament backbone, signalled by the spacing of the M6 reflection, which increases at the start of stimulation, also partly recovered during unloaded shortening, as did the second actin-based layer line, associated with the azimuthal position of tropomyosin. Thus, both the thin and thick filament are partially inactivated during unloaded shortening, indicating positive coupling between the regulatory states of the two filaments. Unexpectedly, the spacing of the M3 reflection, associated with the axial repeat of myosin motors, which increases during contraction, decreased below the resting level during unloaded shortening. Finally, the sarcomere-based X-ray reflections revealed two distinct phases of relaxation following electrical stimulation: a ~20ms sarcomere-isometric phase followed by ~60ms of chaotic relaxation associated with two distinct sarcomere populations.

These results indicate that activation of both the thick and thin filaments decreases during unloaded shortening at the tetanus plateau. These results are consistent with the mechano-

sensing paradigm in the thick filaments, activation of the thin filaments by myosin motors, and positive coupling between the regulatory states of the thick and thin filaments.

Linari, M. et al. (2015) *Nature*, 528(7581):276-9. Hill, C. et al. (2021) *eLife*, 10:e68211. Hill, C. et al. (2022) *J Physiol*, 600(17):3983-4000.

C46

Disuse induced motor unit adaptation in atrophy resistant and atrophy susceptible muscles

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Disuse atrophy occurs during periods of immobilisation or unloading and is typically characterised by loss of muscle mass and strength. Commonly observed in clinical settings such as bone or joint injury, nerve trauma and bed rest, deleterious effects manifest within as little as 5 days (1). Different muscles express diverging atrophy profiles, with striking differences even within agonist-antagonist muscle pairs such as the atrophy-resistant tibialis anterior (TA) and atrophy-susceptible medial gastrocnemius (MG) (2). While differing reductions in mass across different muscles in response to disuse is relatively well studied, the functional adaptations of motor units (MU) between such muscles are not well understood. The aim of this investigation was to study the adaptation of MU characteristics of the TA and MG as an agonist-antagonist muscle group with respect to their diverging atrophy profiles.

8 young healthy males underwent 15-day unilateral lower limb immobilisation preceded and followed by measurements of muscle cross-sectional area (CSA) using ultrasound and maximal voluntary isometric contractions (MVC) in the immobilised limb. Intramuscular electromyography (iEMG) was used to sample individual MU potentials (MUPs) during isometric contractions at 25% MVC. MUP characteristics were calculated from decomposed iEMG recordings using decomposition-based quantitative electromyography software (DQEMG). CSA and MVC were analysed using repeated-measures 2-way ANOVA. MUP characteristics were analysed using multi-level mixed-effects linear regression. Significance was accepted at $p < 0.05$.

Following immobilisation, MG CSA was reduced ($15.60 \pm 3.20 \text{ cm}^2$ to $13.82 \pm 3.10 \text{ cm}^2$, -11%, $p < 0.001$) while TA MVC remained unchanged ($6.43 \pm 0.93 \text{ cm}^2$ to $6.31 \pm 0.97 \text{ cm}^2$, $p = 0.84$). MVC reduced in both plantar flexion ($2262.50 \pm 86.78 \text{ N}$ to $202.50 \pm 83.58 \text{ N}$, -23%, $p < 0.01$) and dorsiflexion ($202.93 \pm 49.63 \text{ N}$ to $157.58 \pm 34.22 \text{ N}$, -22%, $p < 0.05$). MU firing rate (FR) was significantly reduced in the MG ($\beta = -0.691 \text{ Hz}$, 95% CI: -1.311 to -0.0715, $p < 0.05$) yet remained unchanged in the TA ($\beta = 0.233$, 95% CI: -0.363 to 0.829, $p = 0.44$). MU FR variability significantly increased in the MG ($\beta = 0.0178$, 95% CI: 0.00510 to 0.0305, $p < 0.01$) but was unchanged in the TA ($\beta = -0.00338$, 95% CI: -0.0154 to -0.00866 $p = 0.58$).

As previously reported, the MG reduced in size with immobilisation, while the TA resisted atrophy (3). Despite this divergence in atrophy profiles, reductions in strength were observed in both muscles. Suppression of FR and increased FR variability appear to contribute to functional reductions in the MG only. MU FR is modulated via net synaptic input to spinal motoneurons (4) which may be dysregulated following immobilisation. Impaired neural input to muscle may

explain strength reductions in the absence of size reduction as seen in the TA. Consequently, central neural adaptations as a result of short-term immobilisation warrant further investigation to uncover specific impairments targeting muscle function.

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2. Belavý DL, Miokovic T, Ambrecht G, Richardson CA, Rittweger J & Felsenberg D (2009). Differential atrophy of the lower-limb musculature during prolonged bed-rest. *Eur J Appl Physiol* 107, 489–499. DOI: 10.1007/s00421-009-1136-0.
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4. Heckman CJ, Johnson M, Mottram C & Schuster J (2008). Persistent inward currents in spinal motoneurons and their influence on human motoneuron firing patterns. *Neuroscientist* 14, 264–275. DOI: 10.1177/1073858408314986.

Conclusion: Skeletal muscle from patients following acute trauma injury showed greater expression of atrophy and inflammatory markers. Trauma patient serum exhibited higher circulating pro-inflammatory cytokine concentrations. Primary human myotubes treated with serum from trauma patients showed significant atrophy compared to healthy serum-treated controls. We speculate a mechanism(s) acting via circulating factors may contribute to skeletal muscle pathology following acute trauma.

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Biophysical, thermo-physiological, and perceptual determinants of cool-seeking behaviour during exercise heat-stress in younger and older women

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Introduction

Hot weather and heat extremes have severe detrimental effects on individuals' health, comfort, and productivity [1]. Behavioural thermoregulation represents humans' first line of heat defence due to its greater capacity than energy-demanding autonomic responses (e.g. vasodilation and sweating) [2]. Yet, mechanistic research on the determinants of thermal behaviours and their individual variability with sex and age remain limited [3]. For example, women continue to be largely unrepresented in autonomic and behavioural heat-stress research [4]. This knowledge gap provides barriers to develop interventions (e.g. personalised cooling) and solutions (e.g. body-mapped sport garments) that meet the thermal needs of women across different life stages. This study aimed to evaluate the biophysical, thermo-physiological, and perceptual determinants of cool-seeking behaviour during exercise heat-stress in younger and older women.

Methods

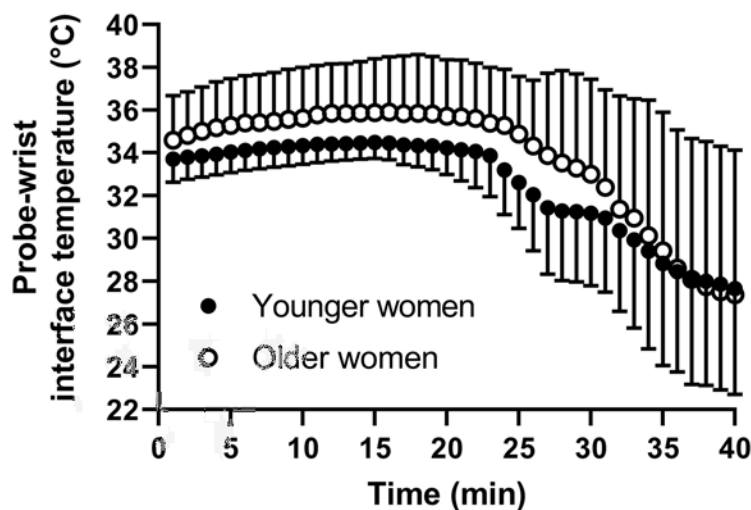
Eleven younger (25±5y; 1.7±0.1m; 63.1±5.2Kg) and 11 older women (53±6y; 1.7±0.1m; 65.4±13.9Kg) performed a 40-min incremental cycling test (workload: 20 to 80W; 20-W increments at 10-min intervals) on a cycle-ergometer in a thermoneutral environment (22 ± 1.7°C; 36±4 RH). Throughout the test, a cooling probe (25cm²) was secured to the participants' wrist, and participants freely adjusted the probe's temperature to offset thermal discomfort arising from exercise heat-stress. We continuously recorded the probe-wrist interface temperature ($T_{\text{interface}}$; micro-thermocouple) to quantify participants' cool-seeking behavior. We also measured participants' rate of metabolic heat production (H_{prod} ; indirect calorimetry), changes in core temperature (T_{core} ; gastrointestinal telemetry), and in mean skin temperature (T_{sk}) and wetness (w ; thermo-hygro- sensors) throughout the exercise. Finally, we quantified participants' cold sensitivity at the wrist via quantitative sensory testing prior to exercise. We compared the onset (time) and amplitude (Δchange) of cooling, and its minimum temperature, between younger and older women, with unpaired t-tests. We modelled the relative contributions of H_{prod} , T_{core} , T_{sk} and w to changes in $T_{\text{interface}}$ for each participant via multiple regression analyses and compared older and younger women with a mixed-model ANOVA. We evaluated the association between wrist cold sensitivity and cooling amplitude via Pearson's correlation.

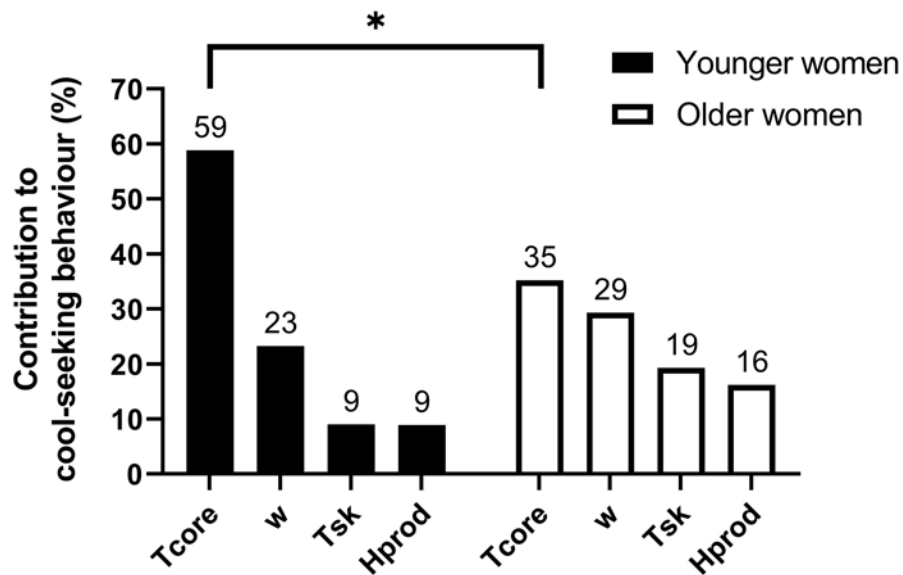
Results

We found no differences in cool-seeking behaviour's onset time (1.4min [95%CI -4.8, 7.7]; $p=0.633$), amplitude (2.2°C [-3.2, 7.7] $p=0.406$) and minimum cooling temperature (-0.3°C [-5.5, 4.9]; $p=0.908$), between younger and older women (Fig. 1). We also found no association between wrist cold sensitivity and cooling amplitude in younger (0.11 ; $p=0.737$) and older women (-0.36 ; $p=0.269$). Multiple regression models indicated that changes in $T_{\text{interface}}$ were primarily described by changes in T_{core} , followed by w , T_{sk} , and H_{prod} ($R^2=0.95\pm0.05$; $p<0.0001$) in both younger and older women (Fig. 2). However, we observed a statistically significant decrease in the relative contribution of T_{core} to changes in $T_{\text{interface}}$ in the older women (-23% [5, 42]; $p=0.006$; Fig. 2).

Conclusions

Younger and older women present similar onset and amplitude of cool-seeking behaviour during exercise heat-stress; however, older women' thermal behaviour appears less reliant on changes in core temperature and more dependent on changes in multiple thermo-physiological (w , T_{sk}) and biophysical (H_{prod}) variables. Predictions of female cool-seeking behaviours based on thermo-physiological and biophysical variables should therefore consider the modulatory effect of ageing.





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Cephalad fluid shifts associated with neuroprotective alterations in cerebral perfusion and haemostasis independent of systemic oxygenation

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Background: Gravity-dependent shifts in central blood volume (CBV) induced by the microgravity of orbital spaceflight pose unique physiological challenges for the astronaut brain. Recent attention has focused on gravity-induced redistribution of fluids toward the head and associated haemostatic consequences associated with altered regional cerebral perfusion (Marshall-Goebel *et al.*, 2019). Changes in posture in terrestrial analogues (stand to head-down tilt) allows for the opportunity to induce a large gravity-dependent shift in CBV to better phenotype underlying mechanisms. Furthermore, the future of human space exploration will require extended extravehicular activities and consequent exposure to low levels of oxygen (hypoxia) that has been associated with blood brain-barrier disruption subject to regional cerebral hyperperfusion and activated coagulation (Bailey *et al.*, 2009; Bailey *et al.*, 2020). The present study aimed to determine to what extent cephalad fluid shifts independently, or in conjunction with inspiratory hypoxia, collectively impact clot microstructure and potential links to altered regional cerebral perfusion.

Methods: Ten healthy males aged 30 (mean) $\pm$ 9 (SD) years old were recruited into a randomised, single-blind, counterbalanced study involving two separate trials separated by 60 min washout. They were examined in two different postural positions (standing head-up and 180° head-down tilt) for 10 min each in normoxia (F_IO₂ = 20.93 %) and hypoxia (F_IO₂ = 12 %). Changes in CBV via thoracic impedance were measured using a tetrapolar high-resolution impedance monitor (THRIM 2994D, UFI, Morro Bay, CA, USA), according to established methods (Bailey *et al.*, 2020). Anterior (internal carotid artery, ICA_Q) and posterior (vertebral artery, VA_Q) blood flow was assessed using duplex ultrasound. Global cerebral blood flow (gCBF) was calculated as (ICA_Q + VA_Q) $\times$ 2. Shear rate (SR) was calculated as 4 $\times$ peak envelope velocity/ arterial diameter. Cerebrovascular conductance index (C_{VCI}) was calculated as Q/mean arterial blood pressure. Cephalic venous blood was obtained without stasis for haemorheological assessment of the fractal dimension (d_f), a novel biomarker of insipient clot microstructure. Following confirmation of distribution normality (Shapiro W Wilks tests), data were analysed using a 2-way (Trial $\times$ Position) repeated measures ANOVA and Bonferonni-corrected paired samples t-tests.

Results: Head-down tilt was generally associated with an increase in thoracic blood volume ($p < 0.001$) and consequent elevation in ICA_{C_{VCI}} ($p = 0.048$) due primarily to an increase in ICA_Q that was not apparent in the posterior circulation (unchanged VA_{Q/C_{VCI}}, Table 1). Thoracic blood volume transfer between head-up and head-down tilt was not compounded by hypoxia

($p=0.870$). Despite global hypoxic cerebral vasodilation (elevated gCBF, $p=0.022$), this did not affect the regional responses to postural tilt. Hypoxia increased d_r ($p=0.035$) with a general and consistent reduction observed during head-down tilt.

Conclusions: These findings collectively demonstrate constrained perfusion to the posterior cerebral circulation and consistent reduction in activated coagulation, reflected by a reduction in incipient clot viscoelastic strength, polymerisation and crosslinking. These changes were independent of systemic oxygenation status and may collectively confer neuroprotective benefits against hyperperfusion-induced structural-functional damage to the neurovascular unit.

Table 1. Cerebral haemodynamics and haematological responses

Inspire:	Normoxia (21% O ₂)			Hypoxia (12% O ₂)		
Position:	Head-up	Head-down	Δ	Head-up	Head-down	Δ
ICA _Q (mL/min)	141 ± 54	182 ± 45	41 ± 69	170 ± 40	206 ± 68	36 ± 75
Position ($P = 0.070$)						
ICA _{SR} (/s)	253 ± 50	238 ± 78	−15 ± 89	311 ± 117	257 ± 71	−54 ± 117
ICA _{CVCI} (mL/min/mmHg)	1.54 ± 0.78	2.00 ± 0.64	0.46 ± 0.78	1.83 ± 0.61	2.32 ± 0.62	0.49 ± 0.86
Position ($P = 0.048$)						
VA _Q (mL/min)	74 ± 17	78 ± 29	4 ± 27	90 ± 30	87 ± 23	−2 ± 34
VA _{SR} (/s)	221 ± 53	227 ± 35	6 ± 46	245 ± 62	237 ± 74	−8 ± 96
VA _{CVCI} (mL/min/mmHg)	0.78 ± 0.21	0.86 ± 0.34	0.07 ± 0.22	0.94 ± 0.32	1.01 ± 0.31	0.06 ± 0.48
gCBF (mL/min)	431 ± 122	520 ± 102	89 ± 150	520 ± 110	586 ± 163	67 ± 150
Inspire ($P = 0.022$)						
g _{SR} (/s)	948 ± 186	930 ± 194	−18 ± 251	1113 ± 336	989 ± 248	−124 ± 386
g _{CVCI} (mL/min/mmHg)	4.66 ± 1.89	5.72 ± 1.57	1.06 ± 1.69	5.55 ± 1.6	6.65 ± 1.6	1.10 ± 2.15
Inspire ($P = 0.025$)						
d_r (AU)	1.94 ± 0.13	1.79 ± 0.09*	−0.49 ± 1.20	1.85 ± 0.11*	1.79 ± 0.08	−0.98 ± 1.00
Inspire ($P = 0.035$); Inspire × Position ($P = 0.029$)						
Thoracic Blood Volume Transfer (mL)			521 ± 289			550 ± 345

*different ($P < 0.05$) compared to Head-up Normoxia; Δ, absolute change from head-up position.

Bailey DM, Bartsch P, Knauth M & Baumgartner RW. (2009). Emerging concepts in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. *Cell Mol Life Sci* 66, 3583-3594. Bailey DM, Laneelle D, Trihan JE, Marchi N, Stacey BS, Tamiya K, Washio T, Tuailon E, Hirtz C, Lehmann S, Ogoh S & Normand H. (2020). Gravitational Transitions Increase Posterior Cerebral Perfusion and Systemic Oxidative-nitrosative Stress: Implications for Neurovascular Unit Integrity. *Neuroscience* 441, 142-160. Marshall-Goebel K, Laurie SS, Alferova IV, Arbeille P, Aunon-Chancellor SM, Ebert DJ, Lee SMC, Macias BR, Martin DS, Pattarini JM, Ploutz-Snyder R, Ribeiro LC, Tarver WJ, Dulchavsky SA, Hargens AR & Stenger MB. (2019). Assessment of Jugular Venous Blood Flow Stasis and Thrombosis During Spaceflight. *JAMA Netw Open* 2, e1915011.

C50

Mechanistic target of rapamycin (mTOR) signaling in aged rats' muscle

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Skeletal muscle aging is associated with increased risk of frailty, morbidity, and mortality and to date has few safe and efficient treatments. Although the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway in younger animals/humans, regulates cell metabolism positively, recent findings suggest mTOR becomes over-active in older age, and that this could be a cause of sarcopenia. In this study, we sought to map mTOR-related signaling as a function of age and fibre type.

This experiment included 24 rat samples collected from: “young” (3-months, N=10), “old” (24-months, N=10), and “very old” (27-months, N=4) rats. All 24 soleus muscles were analyzed, and 8 tibialis muscle (young N=4, old N=4) samples. mTOR related targets were quantified by Western blotting. Data were quantified via densitometry and normalized to Coomassie staining to correct for loading error. Data are shown as mean±SEM, analyzed using Shapiro-Wilk test to test normal distribution, and t-tests or a non-parametric equivalent to compare age. The alpha level of significance was P<0.05.

In soleus muscle, p-mTOR increased with aging; the very old group protein abundance being 2.1-fold higher than the old (P<0.01), and the old being 2.5-fold higher than the young (P<0.01). Downstream, p-rps6 in the very old was 3.1 and 2.4-fold higher than in the young (P<0.001) and old groups (P<0.01), respectively. Another mTOR target, p-4E-BP1, was 1.6-fold higher in the very old than the old (P<0.05). For mTOR upstream targets, p-AKT in the very old group was 2.9 and 2.6-fold greater than the young and the old (both P<0.01), respectively. p-AMPK abundance in the young and very old groups 2.9 and 2.8-fold higher than the old (both P<0.05). The autophagy marker, p-FoxO1a, in the very old group was 1.6-fold greater than the old (P<0.05). In the tibialis muscle, aging did not influence the phosphorylation of the AKT/mTOR/rps6 pathway. Nonetheless, p-AMPK, in young tibialis muscle was 2.5-fold more abundant than in the old group (P<0.01). Finally, p-FoxO1a and p-FoxO3a in the old group were 0.6 and 0.5-fold less abundant than in the young tibialis muscle (P<0.05 and P<0.0001).

Aging alters AKT/mTOR/rps6/FoxO pathway regulation in soleus, perhaps relating to dysregulated proteostasis/autophagy in slow muscle with ageing. Tibialis anterior illustrated altered AMPK/FoxO signaling, suggesting altered autophagy and upstream mTOR signaling. While preliminary in nature, these data support perturbed mTOR signaling with rat muscle ageing which may act in a muscle/fiber type-specific manner.

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Developing a mass spectrometry-based workflow to investigate ubiquitin signalling networks in aged skeletal muscle

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Skeletal muscle mass and function progressively decline with ageing. This decline is known as sarcopenia and is a leading cause of mortality in older individuals. Loss of proteostasis is a common feature of sarcopenia, however the molecular mechanisms involved are poorly understood. Protein ubiquitylation is a key signal for maintaining cellular proteostasis. Therefore, obtaining a comprehensive understanding of protein ubiquitylation events in ageing muscle will provide insights into the molecular mechanisms involved in muscle proteostasis. We have developed a mass spectrometry-based workflow that allows for large-scale analysis of protein ubiquitylation in skeletal muscle. To improve the dynamic range of ubiquitylated protein detection, we included a fractionation process to separate myofibrillar and sarcoplasmic enriched proteins. We employed a cost-effective clean up method called SP4 to remove contaminants and deliver high protein recovery prior to trypsin digest. Finally, we used antibodies to selectively enrich ubiquitylated peptides for mass spectrometry analysis. Human skeletal mixed muscle (n=3) was used to develop this workflow. Label free quantification paired with t-test statistical analysis was used to determine fraction enriched ubiquitylated proteins. We were able to detect 4,591 unique ubiquitylated peptides and 971 unique ubiquitylated proteins. Of these proteins, 710 (73%) were significantly enriched into either muscle fraction. Over 70% of the fraction enriched ubiquitylated proteins were identified in the sarcoplasmic fraction, including heat shock proteins which are important for protein folding. Currently, we are applying this workflow to investigate changes in protein ubiquitylation between young (6 month) and old (22 month) C57BL/6 male mouse gastrocnemius muscle (n=3). Western blot analysis has shown higher abundance of protein ubiquitylation in aged, compared to young muscle. We will run these samples using our mass spectrometry workflow coupled with isobaric labels to obtain a quantitative dataset to determine which proteins undergo altered ubiquitylation in aged muscle. We believe that our new methodology will improve our understanding of the molecular mechanisms contributing to the age-related decline in muscle proteostasis. This information is critical for developing pharmacological interventions aimed at restoring muscle health and achieving healthy ageing. Ethical approval for human samples was obtained through the East Midlands - Derby Research Ethics Committee (18/EM/0004), conformed to the requirements of Research Governance at the University of Birmingham and was conducted in accordance with the Declaration of Helsinki. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

C52

Identification of new human obesity genes from studying a canine obesity model.

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Background: The high heritability of obesity is well established and a plethora of genetic obesity associations in human populations are hard to prioritise for further study. In dogs, an obesity epidemic shares many features with that in people but selective breeding means gene mapping is relatively straightforward. We study pet dogs as a model for human disease using genomics coupled with follow-on molecular, epidemiological and physiological studies. Our overall aim is to understand the mechanistic links between genes and obesity in dogs and humans.

Methods/Results: To identify genetic risk factors for obesity in Labradors we performed a GWAS study using linear mixed models in 241 Labradors. Obesity-associated loci reaching the genomic significance threshold were used to generate genomic risk scores that were predictive of obesity and body weight in an independent set of >250 Labradors and to a lesser extent in a related retriever breed, but not more distantly related breeds. Genomic risk scores provide insight into variable penetrance of the POMC variant and explain why some sub-populations (assistance dogs and chocolate coat colour) have increased obesity risk. The data show genomic risk is in large part mediated via eating behaviour and we demonstrate genomic risk is moderated by environmental exposure to dietary risk factors and exercise.

Fine mapping was performed to focus on candidate obesity genes and variants in the Labrador GWAS. We interrogated human GWAS data from UK BioBank and the GIANT consortium and tested for rare variant enrichment in UK BioBank exomes and the SCOOP cohort of patients with early onset, severe obesity to show several genes identified as having a large effect in dogs are also associated with human obesity; these include SEMA3D, CSNK1A1, CDH8 and CARD11. Another such gene was DENND1B which we show affects endocytosis and trafficking of melanocortin 4 receptors in vitro, providing a novel mechanism underlying the obesity risk.

Conclusion: These data cement the value of dogs as a canine model of complex genetic disease and show how canine studies are of value to improve understanding of both canine and human obesity. We propose several genes as priority candidates for study and propose a new mechanistic link between DENND1B and obesity.

Ethical Statement: The research was carried out in dogs (*Canis familiaris*) kept as pets or assistance dogs. DNA was extracted from oral swabs (saliva). Eating behaviour was assessed using an owner-reported questionnaire (Raffan 2015). Work was approved by the University of Cambridge Dept Veterinary Medicine Ethics and Welfare Review Committee.

Raffan, Eleanor, Stephen Smith, Stephen O’Rahilly and Jane Wardle. “Development, factor structure and application of the Dog Obesity Risk and Appetite (DORA) questionnaire.” *PeerJ* 3 (2015)

C53

Mild hypoxia augments acute temperature sensing in the rat carotid body

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The carotid body (CB) is a sensor of systemic hypoxia, hypercapnia, acidosis and inflammation (Kumar & Bin-Jaliah, 2007). More recently, Gibbons et al (2022) have suggested a role for the CB in thermally-mediated hyperventilation in humans, although previous direct recordings of CB activity with changes in temperature remain equivocal (Alcayaga et al., 1993; Eyzaguirre et al., 1983; McQueen & Eyzaguirre, 1974). Furthermore, the exact mechanism of temperature sensing in the CB remains elusive.

All experiments and procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986. Chemoafferent activity was measured *in vitro* from CBs dissected from adult male Wistar rats (200-300g). CB tissue was surgically removed under non-recovery anaesthesia (isoflurane (3-5%) in O₂ at a flow rate of 1.5L min⁻¹), death via cervical dislocation. CB preparations were superfused with a physiological salt solution equilibrated at 37°C and at a normocapnic, normoxic PO₂ to establish a baseline discharge between 0.25-1.5Hz. The CB was then cooled to 32°C, followed by steady incremental warming to 40°C over approximately 5 minutes (n=24 spikes, 10 animals). This protocol was repeated in hyperoxia (n=6 spikes, 6 animals), mild hypoxia in normocapnia (n=8 spikes, 7 animals) and during the addition of 100 µM 2-APB, a non-selective modulator of TRP channels, in normoxic normocapnia (n=8 spikes, 5 animals). Discharge was recorded at 1°C increments between 32°C and 40°C, results expressed as mean ±SEM and significance (P<0.05) was established by linear regression analysis.

Cooling the superfusate temperature from 37°C to 32°C in normoxia caused a rapid (within seconds) decrease in CB chemoafferent activity (0.84±0.11Hz vs 0.21±0.06Hz) which then increased linearly and significantly to 1.40±0.22Hz at 40°C (mean slope 0.130 Hz.°C⁻¹; r² 0.925; P<0.001). This thermal effect was abolished by hyperoxia (mean slope 0.003 Hz.°C⁻¹; r² 0.098; P>0.40). In mild hypoxia, decreasing the temperature from 37°C to 32°C still led to a rapid attenuation in chemoafferent activity (4.75±0.72Hz vs 0.47±0.12Hz) and subsequent increasing of temperature induced a linear increase in thermal response to 37°C (mean slope 0.766 Hz.°C⁻¹; r² 0.972; P<0.001), the slope of which was increased 2.3 fold between 37°C to 40°C with a discharge of 9.74±1.19Hz at 40°C (mean slope 1.739 Hz.°C⁻¹; r² 0.994). 2-APB greatly blunted the temperature sensing observed in mild hypoxia (mean slope between 32-40°C, 0.109 Hz.°C⁻¹; r² 0.801; P<0.05).

Overall, this data demonstrates that acute temperature sensing in the CB is PO₂ dependent, being abolished by hyperoxia and augmented by mild hypoxia. Thermal sensitivity during mild hypoxia is exaggerated above, rather than below, 37°C, supporting the notion of a primary role for the CB in heat-induced hyperventilation (Gibbons et al., 2022). The augmented response to temperature in mild hypoxia may be regulated by TRP channels but future studies warrant the use of more selective drugs to determine the exact mechanism of acute temperature sensing in the CB.

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C54

Investigating the efficacy of senolytics in wound healing using a human ex-vivo wound model

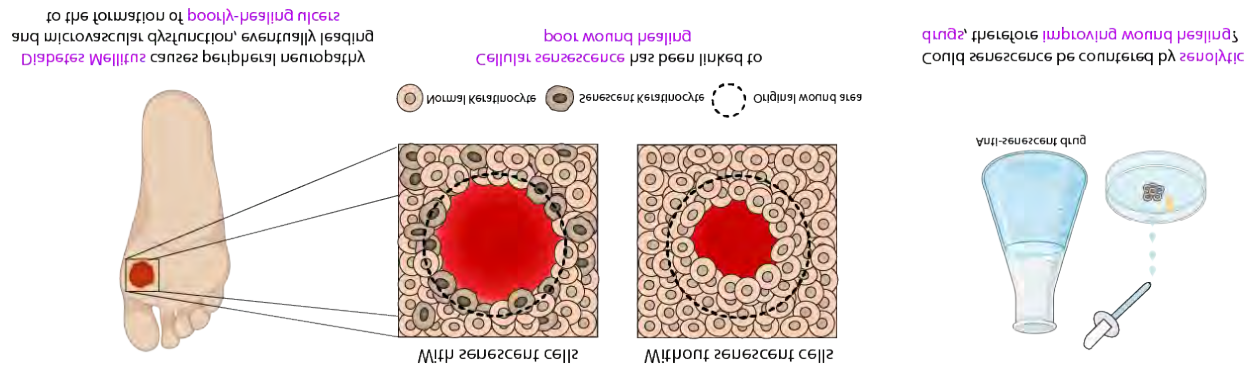
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Diabetes Mellitus (DM) is a lifelong condition characterised by persistent hyperglycaemia due to insulin resistance or impaired insulin production (1). DM causes a wide array of complications, including peripheral neuropathy, which can result in the development of neuropathic ulcers. These ulcers substantially increase infection risk, resulting in further morbidity, and pose significant psychosocial challenges to sufferers of DM (2). Current treatments are inadequate, with healing taking place over months to years, and not at all in some patients (3). Therefore, further understanding of the factors that regulate poor healing is required to develop more adequate therapies. It has been suggested that senescent cells could play a role in wound healing (4). Thus, combating senescence using senescence-targeting (senolytic) drugs may have a beneficial effect on wound healing. We therefore set out to test the healing-promoting effects of two senolytics, metformin and rapamycin, using a human ex-vivo skin wounding model.

Human skin samples were collected post-surgery, with full informed and written patient consent, from Castle Hill Hospital with no other inclusion criteria. Skin was washed and 2mm wounds were created using a biopsy punch. A 6mm biopsy punch was then used to cut out a 6mm explant around each wound, with the 2mm wounds lying centrally within the explants. Metformin and Rapamycin were diluted in dimethyl sulfoxide (DMSO) to a working concentration of 62.5nM and 12.5nM respectively with 2µL of treatment applied topically to wounds. 0.1% DMSO was used as a control treatment. Wounds were incubated in a humidified incubator at 32-37°C and 5% CO₂ for 2 days. Wound explants were fixed and immunofluorescence staining was conducted with a primary Keratin-14 antibody followed by a secondary antibody (AlexaFluor488). Wounds were counter-stained with DAPI and images were acquired using confocal microscopy. Images were analysed using ImageJ. The edge of the wound and non-healed area was drawn around using the freehand tool and the percentage of the original wound area that had healed was calculated for each wound. Significance testing was conducted with ANOVA when there was 3 or more groups, or an unpaired T test for pairwise comparisons.

There was no overall difference found in percentage wound closure when comparing vehicle treatment (DMSO) to Metformin or Rapamycin (n=67; p=0.71). To further investigate, data was stratified into subgroups based on the patient origin of the skin. After stratification, a significant difference was found between the treatment groups for patient 2 (n= 12; p=0.047) but not patients 1 (n=24; p=0.38) and 3 (n=31; p=0.27). Further testing concluded that wound closure with Rapamycin was significantly greater than the control group (p=0.03) in patient 2. The findings suggests that rapamycin has potential efficacy in wound-healing, but that such efficacy is patient-specific. This therefore warrants further investigation and testing of rapamycin on further samples from more patients.



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C55

The exercise metabokine β -aminoisobutyric acid enhances physiological hepatic mitochondrial function and fatty acid β -oxidation

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Background: Exercise provides both a protective and therapeutic approach to target systemic metabolic dysfunction. In part, the health benefits of exercise are mediated by exerkinases as endocrine signals released from skeletal muscle during physical activity. Exerkinases include micro-RNAs, proteins, lipids, and metabolites. The bioactive metabolite endocrine signals have been termed metabokines. β -aminoisobutyric acid (BAIBA) is a non-protein-beta-amino-acid, that functions as a Pgc1 α (a transcriptional co-regulator; peroxisome proliferator-activated receptor- γ co-activator-1 α) and exercise-regulated muscle-derived metabokine. BAIBA modulates crosstalk between skeletal muscle, liver, and fat by inducing white adipocyte browning and hepatic fatty acid β -oxidation (FA β -O). The liver regulates functional processes including homeostasis of systemic lipid and glucose levels through de-novo lipogenesis (DNL), FA β -O, and gluconeogenesis. Liver exhibits a high degree of metabolic flexibility (the ability to adapt to excess or restricted substrate to maintain homeostasis). The signalling and phenotypic effects of BAIBA on hepatic tissue remain poorly characterized. Here the role of BAIBA in regulating beneficial effects on liver metabolism is investigated. We hypothesise that BAIBA can improve hepatic metabolic and mitochondrial function.

Methods: Eight-week-old male C57BL/6J mice (n=20) were fed chow-diet *ad libitum* with/without BAIBA-treatment (100mg/kg/day in drinking water, n=10/group) for 6 weeks under UK Home Office project and personal licences. We investigated BAIBA's effect on 1) hepatic mitochondrial density (citrate synthase {CS} assay) and function (total carnitine palmitoyl transferase {CPT} enzyme activity and O2K-Oxygraph-high-resolution mitochondrial respirometry analysis); 2) expression of genes and proteins associated with FA β -O, mitochondrial function, DNL, and carbohydrate metabolism using RT-qPCR and immunoblotting, respectively. Shapiro-Wilk test for normality, Levene's test for equality of variances, Independent samples t-test for parametric and Mann-Whitney U test (exact-p values) for non-parametric data were used for statistical analysis via IBM SPSS Statistics 26. Significance is considered when p<0.05 with 95% confidence interval.

Results: Hepatic tissue from BAIBA-treated mice was characterized by significantly greater gene expression of *Cpt1a* (carnitine palmitoyl transferase-1a; 66% higher, p=0.015), *Ppara* (peroxisome proliferator-activated receptor-alpha; 97% greater, exact-p=0.035), and a decrease in *Acaca* expression (acetyl-CoA carboxylase-alpha; -61%, p=0.013) with a trend to 44% increase in *Pgc1a*. There was a trend towards lower expression of genes for DNL (as fatty acid synthase and stearoyl-CoA desaturase-1) as well as a trend for enhanced glucose-6-phosphatase and phosphoenolpyruvate carboxykinase-1 representing carbohydrates metabolic activity within the liver of BAIBA-treated mice. In treated group, a significant elevation in hepatic CS activity (28.1% more active, exact-p=0.029) (mitochondrial density) was observed. High-resolution respirometry analysis showed significant functional enhancement of both

mitochondrial content (mean-difference $\pm$ SEM=140.1 $\pm$ 38.66, $p=0.007$; $n=5$ /group) and complex-IV respiration (exact- $p=0.008$; $n=5$ /group), and FA β -O (mean-difference $\pm$ SEM=7.446 $\pm$ 2.676, $p=0.024$; $n=5$ /group) in hepatic tissues from BAIBA-treated mice compared to non-treated controls. Total CPT activity was also higher in the livers of BAIBA treated mice (41.8% greater, $p=0.0124$; $n=10$ -control Vs 9-treated) compared to controls. Western blotting showed significantly higher expression of the metabolic proteins; *Cpt1a* (by 5.04-fold, exact- $p=0.019$) and *Ppara* (by 2.2-fold, $p=0.048$) in the livers of the BAIBA-treated group compared to controls.

Conclusion: BAIBA treatment simulates exercise-like beneficial metabolic effects on liver tissue through enhancing hepatic FA β -O, mitochondrial respiration and function as well as decreasing hepatic DNL.

C56

RNA-Sequencing analysis of skeletal muscle in a loss-of-function model of a novel candidate obesity gene

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Obesity increases the risk for diabetes and cardiovascular disease. Genetic predisposition exacerbates environmental drivers of obesity such as energy dense diets and sedentary lifestyle. We have exploited divergently selected Fat (23% fat as bodyweight) and Lean (4% fat as bodyweight) lines of mice originating from a common base population to identify genes underlying divergent adiposity. A stratified approach using quantitative trait loci (QTL; heritable genetic intervals segregating with adiposity in Fat x Lean F2 populations), metabolic tissue transcriptomics and comparative cross-species bioinformatics identified candidate obesity and leanness genes in adipose tissue (Morton *et al.*, 2011, 2016). Using a similar approach, we have identified novel muscle-expressed genes that segregate with adiposity. A specific phospholipase A2 isoform (we name here PlaX), positioned in the Found in obesity (Fob)-1 QTL, exhibited ~5-fold elevated mRNA levels in the skeletal muscle of Fat mice compared to Lean mice. PlaX activity has been previously linked to the regulation of intracellular membrane vesicle trafficking and generation of lipid signalling mediators (Cervera *et al.*, 2021). Overexpression of PlaX in C2C12 myotubes impaired cellular energetics, glucose transport and increased levels of the active form of AMP-activated protein kinase (AMPK). This led us to hypothesise that skeletal muscle PlaX-overexpression may drive obesity by compromising myocyte energetics and nutrient utilisation. To test this hypothesis, we have generated global PlaX knockout transgenic mice. Our aim in this project was to characterise the skeletal muscle role of PlaX. To achieve this, we performed RNA-sequencing on samples taken from the extensor digitorum longus (EDL) muscle on the Illumina NextSeq 2000 platform. [Differential gene expression analysis and functional enrichment analysis was carried out on samples from five wild type \(WT\) mice and five gene knock-out mice \(KO\), which lack a functional PlaX gene.](#) [Reactome](#) pathway and Gene Ontology ([GO](#)) enrichment analyses were performed using the differentially expressed genes identified at the adjusted statistical threshold (FDR-adjusted (adj.) $p < 0.05$). A threshold of $\text{adj.} p < 0.05$ was used to define significant differentially expressed genes. 339 genes were differentially expressed between KO and WT ($\text{adj.} p < 0.05$). As expected, RNA-sequencing revealed PlaX as the most significantly downregulated gene with a -1.82 fold change ($\text{adj.} p = 2.92 \times 10^{-5}$) in KO vs WT. Among the most significantly differentially expressed genes were Per3, which was downregulated in KO vs WT (-1.5 fold change, $\text{adj.} p = 0.012$), while Pdk4 was upregulated (2.2 fold change, $\text{adj.} p = 0.022$). Both Per3 (Azevedo *et al.*, 2021) and Pdk4 (Jeon *et al.*, 2021) are metabolism-linked genes that have been associated with obesity.

These differentially expressed genes indicate a metabolic response to PlaX loss-of-function in skeletal muscle and support the role of PlaX as a candidate obesity target. To further characterise the role of PlaX in skeletal muscle, we have overexpressed PlaX in L6 skeletal muscle cell line using lentiviral transduction and are currently performing RT-qPCR to measure expression of targeted genes from our RNA-sequencing data. In summary, our genetic strategy

has identified a novel potential skeletal muscle driver of obesity that could be a tractable target for therapeutic development.

Azevedo PG de, Miranda LR, Nicolau ES, Alves RB, Bicalho MAC, Couto PP, Ramos AV, Souza RP de, Longhi R, Friedman E, Marco L De & Bastos-Rodrigues L (2021). Genetic association of the PERIOD3 (PER3) Clock gene with extreme obesity. *Obes Res Clin Pract* 15, 334–338. Cervera IP, Gabriel BM, Aldiss P & Morton NM (2021). The phospholipase A2 family's role in metabolic diseases: Focus on skeletal muscle. *Physiol Rep*; DOI: 10.14814/PHY2.14662. Jeon JH, Thoudam T, Choi EJ, Kim MJ, Harris RA & Lee IK (2021). Loss of metabolic flexibility as a result of overexpression of pyruvate dehydrogenase kinases in muscle, liver and the immune system: Therapeutic targets in metabolic diseases. *J Diabetes Investig* 12, 21–31. Morton NM et al. (2016). Genetic identification of thiosulfate sulfurtransferase as an adipocyte-expressed anti-diabetic target in mice selected for leanness. *Nat Med* 22, 771–779. Morton NM, Nelson YB, Michailidou Z, Di Rollo EM, Ramage L, Hadoke PW, Seckl JR, Bunger L, Horvat S, Kenyon CJ & Dunbar DR (2011). A stratified transcriptomics analysis of polygenic fat and lean mouse adipose tissues identifies novel candidate obesity genes. *PLoS One* 6, e23944.

C57

Lrg1 is a brown adipose tissue thermogenesis and white adipose tissue fatty acid oxidation regulating adipokine

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Background: White adipose tissue (WAT) stores excess energy and acts as an endocrine organ releasing hormone-like factors known as adipokines that regulate whole-body energy balance. Brown adipose tissue (BAT) regulates energy expenditure through futile cycling of the electron transport chain (ETC) by the action of uncoupling protein 1 (UCP1) to generate heat. BAT's heat producing capacity may be a novel approach to treating obesity. Adult humans possess metabolically active BAT, but obesity reduces BAT's thermogenic activity and mass causing BAT to resemble WAT in a process termed "whitening". Leucine-rich- α 2-glycoprotein 1 (*Lrg1*) is an adipose-associated secretory protein and potential adipokine, with plasma levels positively correlated to body mass index in humans. However, the role of LRG1 in the regulation of BAT and sWAT energy metabolism and function has not yet been identified.

Hypothesis: LRG1 may function to inhibit BAT thermogenesis and energy expenditure and drive whitening of BAT. We proposed that inhibiting *Lrg1* may drive BAT thermogenesis with anti-obesity and anti-diabetic effects and thereby identify LRG1 as a therapeutic target.

Methods: Live animal experiments were performed in accordance with Animals (Scientific Procedures) Act 1986. Male *Lrg1* global knockout (KO) mice or wild type (WT) controls were fed standard chow (STD) or high fat diet (HFD) for 10 weeks (n=10). At 18 weeks of age, systemic and BAT metabolic characteristics were determined by whole body indirect calorimetry, high-resolution tissue respirometry, quantitative PCR, histology and immunoblots. Within WAT, fatty acid β -oxidation was assessed using functional assays. Statistical significance was assessed using one-way ANOVA with Dunnett's multiple comparison test or two-way ANOVA with Sidak's multiple comparisons test.

Results: BAT from HFD-fed WT mice exhibit a decrease in mitochondrial respiration through complex 1 of the ETC, demonstrating that loss of LRG1 protects HFD-induced dysfunction in BAT (Fig. 1). Functional loss of *Lrg1* induces a systemic shift towards preferential lipid oxidation in *Lrg1* KO mice with both HFD and STD-fed mice having a lower respiratory exchange ratio (RER) than WT controls (Fig. 2). *Lrg1* KO mice fed HFD have greater BAT wet weight WT mice. H&E histology confirmed that the heavier BAT in *Lrg1* KO mice was not due to whitening of BAT (Fig. 3). *Lrg1* null mice exhibit a molecular phenotype indicative of thermogenic futile cycling, which was observed through increased *Ucp1* gene and protein expression in the *Lrg1* KO mice (Fig. 4).

However, *Lrg1* KO does not protect against weight gain in a mouse model of diet-induced obesity (Fig. 5). *Lrg1* null mice have reduced thermogenic gene expression in white adipose tissue. *Lrg1* null mice have impaired fatty acid β -oxidation in subcutaneous adipose tissue,

demonstrated through a reduction in cellular lipid uptake, reduced *Cpt1a* gene expression, and reduced CPT1a activity.

Conclusion: Loss of *Lrg1* increased BAT UCP1 expression, increased mitochondrial respiration and decreased BAT whitening in models of obesity, indicating that loss of LRG1 increases BAT thermogenesis and protects against obesity-induced dysfunction. However, increased BAT thermogenesis did not lead to reduced weight gain due to decreased fatty acid β -oxidation within sWAT of *Lrg1* null mice.

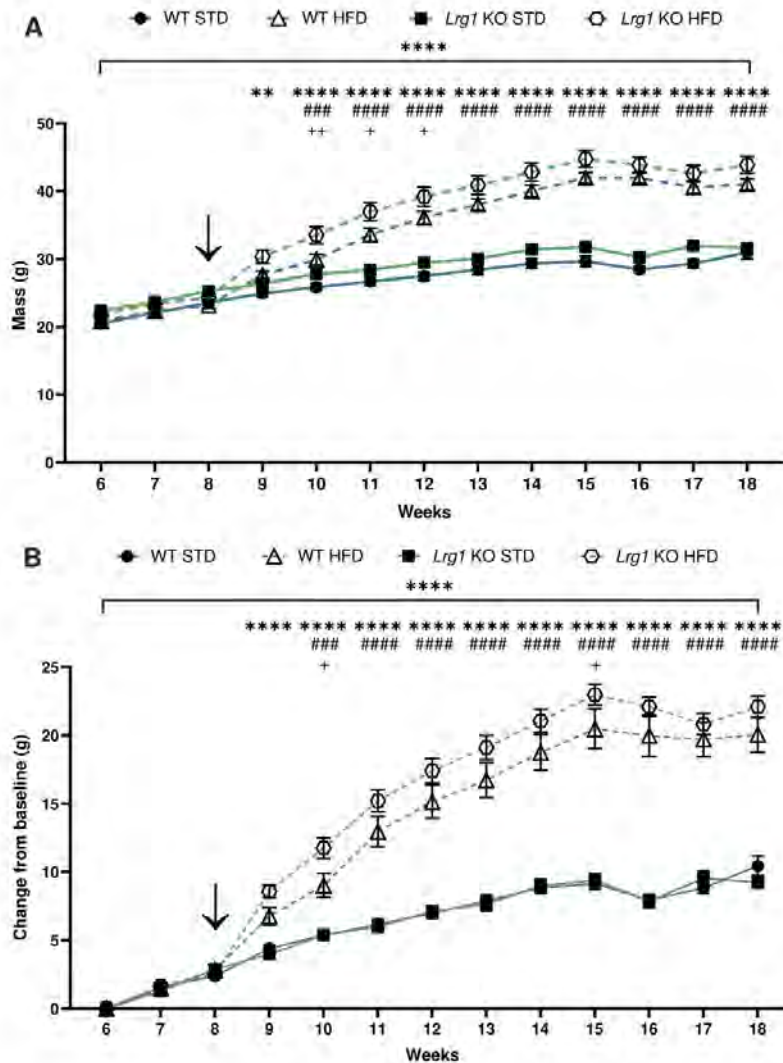


Figure 5 Effect of *Lrg1* KO on body weight of male mice fed standard chow (STD) or high fat diet (HFD) for 10 weeks. Wildtype (WT; blue) and *Lrg1* knockout (KO; green) mice were switched from STD to HFD at 8 weeks of age. Black arrow indicates the beginning of HFD feeding. **A** Raw body weights and **B** change in body weights from baseline at 6 weeks. Analysed using three-way ANOVA with Tukey's multiple comparison test for each time point. Significant effect of time of time indicated by line. Significance between comparisons of *Lrg1* KO mice fed STD compared to HFD are indicated with *, WT mice fed STD compared to HFD are indicated with #, WT mice fed HFD compared to *Lrg1* KO mice are indicated with +, STD WT compared to *Lrg1* KO mice are indicated with -. Data are expressed as mean $\pm$ SEM ($n = 15$).

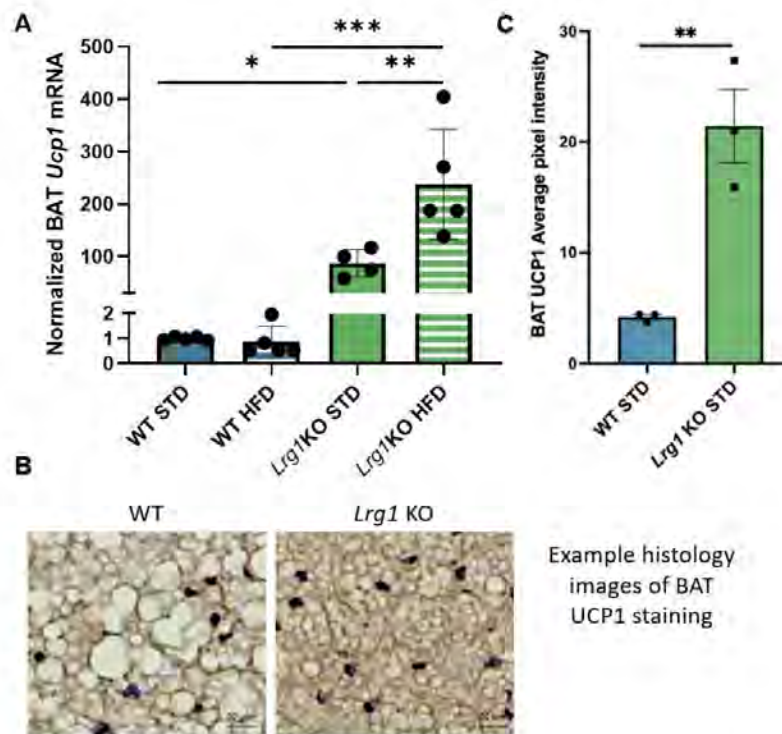


Figure 4 *Lrg1* KO mice have increased *Ucp1* in BAT. **A** Gene expression of *Ucp1* in the BAT of wildtype (WT, blue) and *Lrg1* knockout (KO, green) mice fed either a standard chow (STD) or high fat diet (HFD) (WT n = 5, *Lrg1* KO n = 4). Analysed using two-way ANOVA with Sidak's multiple comparisons test, with significant genotype effect $p \leq 0.0001$. **B** Representative images of *Ucp1* immunostaining used to quantify UCP1 protein content in BAT of WT and *Lrg1* KO mice. **C** Quantification of UCP1 pixel intensity (n = 3). Analysed using an unpaired t-test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Data are expressed as mean $\pm$ SEM with individual data points shown.

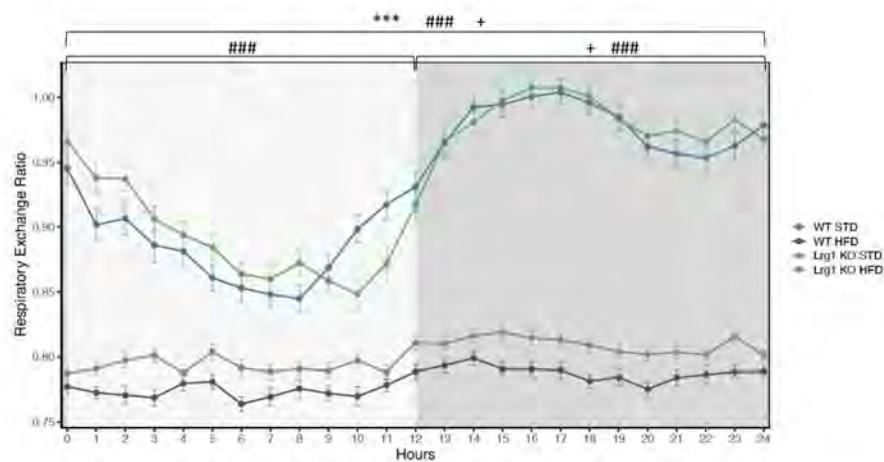


Figure 1 Respiratory Exchange Ratio (RER) *Lrg1* KO and WT male mice fed STD or HFD. RER from wildtype (WT STD: blue, HFD: black) and *Lrg1* Knockout (KO STD green, HFD: grey) mice fed standard chow (STD) or high fat diet (HFD). *Lrg1* KO mice fed STD and HFD mice preferentially oxidize carbohydrates compared with WT control mice fed STD and HFD, respectively. Data were analysed using CalR software. Significance between comparisons of *Lrg1* KO mice fed STD compared to *Lrg1* KO mice fed HFD are indicated with *, WT mice fed STD compared to WT mice fed HFD are indicated with #, WT mice fed HFD compared to *Lrg1* KO mice fed HFD are indicated with +, STD fed WT compared to *Lrg1* KO mice fed STD are indicated with -. Data are expressed as mean $\pm$ SEM (n = 10). * p $\leq$ 0.05, ** p $\leq$ 0.01, *** p $\leq$ 0.001.

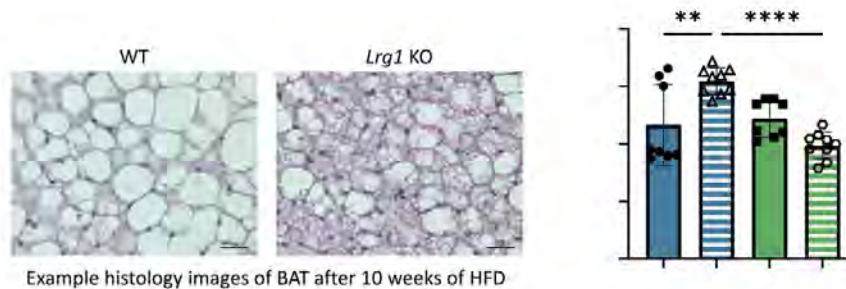


Figure 3 *Lrg1* KO mice fed HFD have decreased lipid accumulation in BAT.

White voids were considered to be lipid vacuoles and quantified. **A** Representative images of H&E staining used to image BAT morphology of HFD fed mice. **B** Percentage area of lipid vacuoles for wild type (WT) and *Lrg1* Knockout (KO) mice fed High Fat Diet (HFD, n = 9) or standard chow (STD, n = 8). Analysed using two-way ANOVA with Sidak's multiple comparisons test, with significant genotype effect $p \leq 0.0001$. ** $p \leq 0.01$, **** $p \leq 0.0001$. Data are expressed as mean $\pm$ SEM with individual data points shown.

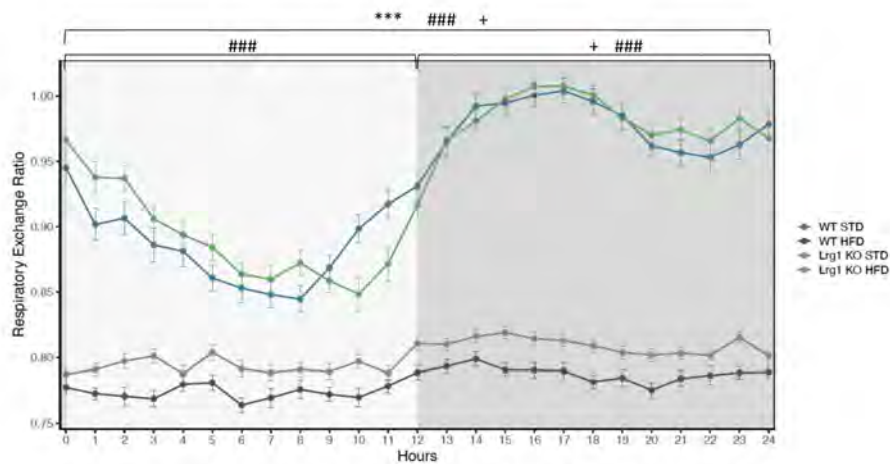


Figure 2 Respiratory Exchange Ratio (RER) *Lrg1* KO and WT male mice fed STD or HFD. RER from wildtype (WT STD: blue, HFD: black) and *Lrg1* Knockout (KO STD green, HFD: grey) mice fed standard chow (STD) or high fat diet (HFD). *Lrg1* KO mice fed STD and HFD mice preferentially oxidize carbohydrates compared with WT control mice fed STD and HFD, respectively. Data were analysed using CalR software. Significance between comparisons of *Lrg1* KO mice fed STD compared to *Lrg1* KO mice fed HFD are indicated with *, WT mice fed STD compared to WT mice fed HFD are indicated with #, WT mice fed HFD compared to *Lrg1* KO mice fed HFD are indicated with +, STD fed WT compared to *Lrg1* KO mice fed STD are indicated with -. Data are expressed as mean $\pm$ SEM ($n = 10$). * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

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Autocrine and paracrine effects of leptin on adipogenesis

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Adipose tissue, which is excessively stored in obesity, not only accumulates lipids, but secretes numerous adipocytokines. One of them is leptin, a peptide hormone produced mainly by white adipose tissue and encoded by the leptin gene (*LEP*). The main role of leptin is energy balance regulation by acting on the hypothalamus. Additionally, leptin contributes to cardiovascular function, immune system activation and regulates the reproductive system through leptin receptor (LEPR) and endocrine mode of action [1,2]. However, the autocrine and paracrine effects of leptin on adipocytes are not completely understood, and the current state of the art provides contradictory data. Nevertheless, it is known that leptin may play an important role in lipid accumulation and metabolism.

Our group identified a somatic variant in the leptin gene (c.250C>A), p.(Gln84Lys) in a spontaneous lipoma. In silico analysis indicated that this variant may result in reduced stability of the protein. Therefore, we aimed to evaluate the effects of leptin knockdown as a model for leptin loss of function and leptin stimulation on adipose progenitor cells – LipPD1 [3].

To access cell viability we used water-soluble tetrazolium salt (WST-1), cell proliferation was examined by fluorescent staining (Hoechst). Next, we studied adipocyte differentiation by staining lipids with Oil Red O (ORO) and fluorescent stain (Nile Red). The effects of leptin knockdown on adipogenesis marker expression was evaluated by real-time PCR. Data was presented as the fold change normalized to controls ($\pm$ SEM), and analyzed using Student's t-test or one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Experiments were repeated independently three times, n=6-8, $p \leq 0.05$ was considered to indicate a statistically significant difference.

We found that leptin knockdown increased cell viability [1.3180($\pm$ 0.0543) fold, $p=0.0001$], and cell number [1.1460($\pm$ 0.0209) fold, $p<0.0001$]. Moreover, leptin knockdown decreased intracellular lipid droplet accumulation – shown after ORO [0.6588($\pm$ 0.0458) fold, $p<0.0001$] and Nile Red staining [0.8254($\pm$ 0.0444) fold, $p=0.0200$]. These changes were associated with reduced expression of adipogenesis markers – proliferator-activated receptor γ (*PPAR γ*) [0.5655($\pm$ 0.2005) fold, $p=0.0500$] and fatty acid synthase (*FASN*) [0.2607($\pm$ 0.0796) fold, $p=0.0007$]. Efficiency of leptin knockdown was confirmed based on reduced leptin expression [0.1700($\pm$ 0.0685) fold, $p=0.0003$]. Next, we showed that treatment with recombinant leptin (10, 100 nM) attenuated viability of adipose progenitors cultured in 10% FCS containing medium [0.7981($\pm$ 0.0462), $p=0.0300$ and 0.8018($\pm$ 0.0419), $p=0.0400$ fold, respectively], but did not

affect adipocyte differentiation [$0.9199(\pm 0.0436)$, $p=0.4247$ and $0.9813(\pm 0.0420)$, $p=0.9922$]. Furthermore, leptin treatment (1 nM) reversed the phenotype observed after leptin knockdown by stimulating adipocyte differentiation [$1.5900(\pm 0.0922)$ fold, $p=0.0084$].

To sum up, our data indicates that leptin knockdown affected adipogenesis by stimulating preadipocyte viability and proliferation, and inhibiting adipocyte differentiation. Leptin treatment attenuated preadipocyte viability without affecting their maturation and reversed the phenotype observed after leptin knockdown by restoring adipocyte differentiation. In the nearest future, we aim to study the effects of the newly discovered leptin variant on adipogenesis.

These findings may contribute to implementing leptin treatment in patients with lipoma, and obese patients with leptin gene variants. Our conclusions may lead to the explanation of potential effects of leptin on adipocyte physiology.

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C59

Unravelling the roles of GEFs and GAPs in Rho GTPase-regulated cytoskeletal, myonuclear, and metabolic dynamics

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Rho GTPases control cytoskeletal dynamics in muscle cells. Cytoskeletal dynamics in turn regulate myonuclear arrangement and GLUT4-mediated glucose uptake. Tight control of Rho GTPase activity is thus crucial to uphold myocellular function. The activity of Rho GTPases is modulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Yet, little is known about the specific GEFs and GAPs responsible for this regulation in skeletal muscle. Additionally, whether cytoskeletal modifications driven by Rho GTPase activity alter myonuclear arrangement and ultimately impair muscle fibre function is unexplored. Thus, we aimed to unravel new mechanisms of control for Rho GTPases in muscle via GEFs and GAPs.

We initially identified >30 GEF and GAP candidates for further study based on published phosphoproteomics data from human muscle biopsies in response to insulin, in insulin-resistant states, and exercise. Muscle-specific knockdown of several GEFs and GAPs, as well as the Rho GTPase CDC42, resulted in reduced motor function measured by climbing ability *in vivo* in *Drosophila* ($P < 0.0001$; one-way ANOVA; $n = 3-17$). We hypothesise that cytoskeletal dynamics drive the compromised physiological function by disrupting myonuclear positioning and thus the localisation of mRNA transcripts, which will be investigated in muscle-specific Rho GTPase knockout mice.

Insight into the role of GEFs and GAPs in GLUT4 dynamics was gained through siRNA-mediated knockdown in mouse adipocytes, which demonstrated up to 35% reductions in insulin-stimulated GLUT4 translocation (two-way ANOVA; $n = 4$ per siRNA knockdown condition). Based on our results in *Drosophila* and mouse adipocytes, and further by cross-species similarity and siRNA knockdown efficiency, we have selected five candidates for detailed analyses in muscle cells. These analyses will include depletion of selected GEFs and GAPs using siRNA in L6-GLUT4myc tagged and C2C12 muscle cells, and characterisation of Rho GTPase activity alongside cytoskeletal and myonuclear dynamics. These findings hold the potential to enhance our understanding of the complex molecular mechanisms underlying skeletal muscle function and homeostasis, ultimately paving the way for novel therapeutic interventions for human muscle-related diseases.

All mouse and *Drosophila* experiments accorded with ethical standards set by current Danish, Finnish, and UK legislation.

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The effect of malnutrition on circulating pre- and post-prandial gastrointestinal hormones.

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Introduction: Malnutrition continues to be a global health challenge with Africa and Asia accounting for the highest prevalence of all forms of malnutrition. Treatment for malnutrition includes the provision of supplemental feeds, which in the case of severe acute malnutrition, involves initial management with F75 feed followed by F100 feed once patients are stable. However, we have been able to show that most children with SAM have severe gut damage which may contribute to malabsorption of nutrients and delay the recovery process. Direct exposure to nutrients results in the release of gastrointestinal peptides with key physiological roles but the effect of malnutrition on hormone production is unknown. We set out to determine the effect of nutrient stimulation on circulating gut hormones in malnourished Zambian children compared to non-malnourished controls.

Method: We measured circulating Glucagon-like peptide 1 & 2, Ghrelin, C-Peptide, Glucagon, Insulin, Leptin, PYY and Secretin pre- and post-prandially in: a) 35 participants with severe acute malnutrition (SAM) admitted to the Lusaka Children's Hospital, b) 19 children admitted to the surgical ward of the University Teaching Hospital for elective surgery and c) 17 children from the community. Blood samples (2mls) were collected into EDTA bottles containing DPP-IV Inhibitor (Merck life sciences) and Pefabloc SC (Sigma). Post-prandial blood samples were collected at least 30 mins after receiving a liquid feed of either F100 (SAM and Community children) or Pediasure. Plasma was analysed using ELISA and the MILLIPLEX Human Metabolic Panel V3. All values are reported as median and interquartile range (IQR). The Wilcoxon matched pairs signed rank test was used to test for a difference in the paired observations and the Kruskal-Wallis ANOVA between groups. Ethical approval to conduct this study was obtained from the University of Zambia biomedical research ethics committee (Reference #: 951-2020 and 2025-2021) and the study was conducted according to the principles of Good Clinical Practice.

Results: There was a significant increase in post-prandial levels of GLP-1, GLP-2, C-peptide, Insulin and PYY across all three groups ($p < 0.05$; Table 1), while there was no significant change in Glucagon, Leptin, and Secretin. Ghrelin only showed a significant difference in the SAM children ($p=0.02$) and not in the other two groups. Of the three groups, the children with SAM had the highest levels of fasted GLP-1 (117pg/ml, IQR 77-162), GLP-2 (5ng/ml, IQR 3.8-6.8) and PYY (360pg/ml, IQR 287-521) while the community children had the highest post-prandial Leptin (454pg/ml, IQR 225-602), and fasted secretin (median 150pg/ml, IQR 43-306) and insulin (872pg/ml, IQR 698-1695). The delta in circulating hormones between groups was only significantly different for insulin ($p < 0.0001$).

Conclusion: Children with SAM have elevated intestinotrophic hormones (GLP-1, GLP-2 and PYY), which may be indicative of an adaptive response to alterations in gastrointestinal structure. However, as GLP-1 and PYY also have glycemic and anorexigenic roles, this elevation may result in decreased appetite and delayed recovery.

Table 1: Table of pre- and post-prandial circulating hormone levels in children

Hormone	Hospitalised children (Elective surgery)		p	Severe acute malnutrition children		p	Community children		p
	Fasted	Fed		Fasted	Fed		Fasted	Fed	
GLP-1 (pg/mL)	48 (33-64)	25 (21-29)	0.0007	117 (77-162)	71 (47-132)	0.0000	56 (37-64)	30 (21-43)	0.0007
GLP-2 (ng/mL)	3.6 (2.9-4.5)	2.7 (0-4.1)	0.03	5 (3.8-6.8)	4.12 (2.5-5.7)	0.0005	2 (0-3)	0 (0-2.8)	0.13
C-peptide (pg/mL)	969 (576-1760)	329 (230-503)	0.0001	1115 (710-1568)	624 (343-834)	0.0001	1270 (846-2015)	413 (312-629)	0.30
Ghrelin (pg/mL)	28(23-32)	28(22-39)	0.61	21 (14-30)	21 (17 -29)	0.02	10 (9.74-15.5)	10.4 (8.8-12.7)	0.10
Glucagon (pg/mL)	56(48-71)	55 (46-65)	0.84	71 (45-93)	64 (38-89)	0.15	38 (27-46)	31 (25-38)	1.66
Insulin (pg/mL)	648 (460-738)	409 (274-492)	0.001	674 (472-985)	617 (433-775)	0.007	872 (698 -1695)	424 (293 -548)	0.0002
Leptin (pg/mL)	-	-		232 (159-554)	276 (221-620)	0.19	393 (288 -572)	454 (225-602)	0.14
PYY (pg/mL)	188 (162-210)	155 (137-184)	0.0016	360 (287-521)	314 (278-414)	0.04	224 (190-429)	229 (128-438)	0.02
Secretin (pg/mL)	49 (33-64)	43 (29-59)	0.45	37 (28-55)	43 (22-52)	0.29	150(43-306)	97 (94-210)	0.28

- Values are expressed as median and interquartile range (IQR). Abbreviations: GLP-1, Glucagon-like peptide 1; GLP-2 Glucagon-like peptide 2; PYY, Peptide Tyrosine Tyrosine

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On the regulation of arterial blood pressure by intracranial baroreceptor mechanism

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Introduction: There is significant evidence for the existence of intracranial baroreceptor mechanism(s) capable of sensing physiological changes in cerebral blood flow (Marina et al., 2020). However, little is known about the sensitivity of intracranial baroreceptors to changes in brain perfusion and their interaction with inputs from the peripheral baroreceptors. The aim of this study was to characterise the cardiovascular (heart rate and systemic arterial blood pressure, ABP) responses to small changes in cerebral perfusion pressure induced by experimental manipulation of intracranial pressure (ICP).

Methods: The experiments were performed in accordance with the UK Animals (Scientific Procedures) Act (1986). Adult Sprague-Dawley rats (250–300 g) were anesthetized with urethane (induction: 1.3 g kg⁻¹, i.p.; maintenance: 10–25 mg kg⁻¹ h⁻¹, i.v.). The femoral artery and vein were cannulated for measurement of ABP and administration of anaesthetic. The trachea was cannulated, and the animal was mechanically ventilated with room air. The left lateral cerebral ventricle was cannulated and connected via a saline-filled mini-catheter to a pressure transducer to record ICP. The right lateral cerebral ventricle was cannulated and connected via a saline-filled mini-catheter to a “water column” to allow controlled manipulation of ICP.

Results: The resting ICP in rats anesthetized with urethane was 6.2 ± 0.7 mmHg (n=8). Following a small craniotomy that reduced ICP to 0, ABP decreased by 21.1 ± 6.0 mmHg (p=0.033; n=6) within 30 minutes of intracranial decompression. Restoring the integrity of the intracranial space increased ABP by 9.1 ± 3.1 mmHg. Increasing ICP by 5, 10, 15 and 20 mmHg (n=8) triggered stereotypical compensatory increases in ABP and heart rate. In response to a 5 mmHg increase in ICP, ABP increased by 18.1 ± 4.1 mmHg (p=0.01) and heart rate increased by 25 ± 11 BPM (p=0.16). In response to a 10 mmHg increase in ICP, ABP increased by 30.0 ± 5.6 mmHg (p=0.003) and heart rate increased by 47 ± 15 BPM (p=0.046). In response to a 15 mmHg increase in ICP, ABP increased by 42.4 ± 6.4 mmHg (p<0.001) and heart rate increased by 70 ± 17 BPM, p=0.016). In response to a 20 mmHg increase in ICP, ABP increased by 49.6 ± 8.3 mmHg (p=0.002) and heart rate increased by 92 ± 17 BPM (p=0.0031). In conditions of complete denervation of arterial baroreceptors (bilateral sino-aortic denervation), ABP responses triggered by increases in ICP were greatly exaggerated.

Conclusion: These data indicate that intracranial baroreceptor mechanism is highly sensitive to changes in cerebral perfusion within the physiological range and suggest that cerebral perfusion pressure is an important determinant of systemic ABP. The data also suggest that activation of intracranial baroreceptor mechanism effectively overrides the inputs from the peripheral baroreceptors.

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C62

Folate intake, nitric oxide bioavailability and cognitive decline in retired rugby union players

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Background:

Repeated concussions in retired rugby union players may increase the risk of cognitive decline [1]. Previously, we have demonstrated that professional rugby union players are characterised by a suppression in the systemic bioavailability of nitric oxide (NO) [1], an established risk factor for Alzheimer's disease that serves as a major cause of disability and dependency in older adults [2]. Evidence suggests that adequate dietary intake, notably folate, protects against cognitive decline and dementia [3] and this may be mediated through a free radical-mediated pathway involving NO. The present study examined if low folate intake and corresponding reduction in systemic NO bioavailability and cerebral perfusion would be associated with mild cognitive impairment in retired rugby union players.

Methods:

Twenty retired rugby union players aged 64 ± 5 years having sustained 3 concussions incurred over 22 years were compared to 21 sex, age-, cardiorespiratory fitness- and education-matched controls with no prior participation in contact sports or concussion history. Fasted venous blood was obtained for the assessment of plasma bioactive NO (reductive ozone-based chemiluminescence), determined as the cumulative concentration of nitrite (NO) and S-nitrosothiols (RSNO). The Montreal Cognitive Assessment (MoCA) was employed to assess cognition and a self-administered validated semi-quantitative food frequency questionnaire (FFQ) was used to estimate typical food intake over the past 12 months. Dietary data were converted into estimated nutrient intakes using a nutritional software package (Q-Builder, Tinuviel Software; Anglesey, UK). Middle cerebral artery blood flow velocity (MCAv) was determined using transcranial doppler ultrasound. Following confirmation of distribution normality (Shapiro Wilks W tests), between-group differences were assessed using independent samples t -tests. Data are expressed as mean $\pm$ standard deviation (SD) and significance established at $P < 0.05$.

Results:

Compared to controls, players were characterised by a lower intake of folate ($327 \pm 81 \mu\text{g}$ vs. $415 \pm 103 \mu\text{g}$, $P = 0.004$), lower basal bioactive NO ($71 \pm 44 \text{ nM/L}$ vs. $86 \pm 35 \text{ nM/L}$, $P = 0.049$), lower MCAv ($45 \pm 9 \text{ cm/s}$ vs. $51 \pm 7 \text{ cm/s}$, $P = 0.004$) and lower MoCA scores (24 ± 3 points vs. 26 ± 2 points, $P = 0.020$), with the latter clinically defined as mild cognitive impairment (MCI).

Conclusions:

No studies have previously investigated nutrient intake and the integrated mechanistic link to cognitive decline in retired rugby union players with an established concussion history. Collectively, these findings demonstrate that retired players are characterized by inadequate folate intake, reduced NO bioavailability and lower cerebral perfusion that likely precede MCI. Folate plays a key role in reducing serum homocysteine concentration, the latter a modifiable risk factor for cognitive decline and dementia [3, 4]. Similarly, folate has been shown to improve vascular NO bioavailability subsequent to a reduction in systemic oxidative-nitrosative stress [5] which may in turn improve both perfusion and cognition [2]. Folate supplementation may confer neuro-prophylactic benefits in retired players with concussion history and attenuate their trajectory towards accelerated brain ageing.

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C63

Suppressed Triose-phosphate isomerase (TPI) activity affects synaptic vesicle release mechanisms and reduces *Drosophila* life span

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Neurodegenerative diseases are associated with redox stress, often linked to aberrant production of reactive species like nitric oxide (NO)¹. NO can compromise TPI function via 3-Nitrotyrosination so enhancing glycation signalling, neuroinflammation, and neurodegeneration. The resulting physiological mechanisms of dysfunction are not well understood. This work uses *Drosophila melanogaster* to identify impacts of altered TPI activity on neuronal physiology, linking aberrant TPI function and redox stress to synaptic dysfunction at the glutamatergic *Drosophila* neuromuscular junction (NMJ).

Drosophila were kept at standard conditions (12hr light-dark-cycle, 25°C). TPI mutant expressing *Drosophila* (wstd¹ and M80T point mutations^{2,3}) were used as a disease model vs w¹¹¹⁸ (wild-type control).

Electrophysiological recordings in two-electrode voltage-clamp were taken from muscle 6/7 in segments A2/3 of third instar larvae fillets in HL-3 buffer (1.5mM Ca²⁺). Evoked and spontaneous excitatory junctional currents (e/sEJCs) were recorded, alongside 60Hz train stimulations and recovery protocols to investigate synaptic depletion and subsequent recovery.

Confocal images of NMJs labelled with HRP and BRP (labelling neuronal tissue and active zone protein bruchpilot (BRP)) were taken on a Zeiss LSM 880 confocal microscope. Longevity was recorded daily.

Data is expressed as mean±SEM (n=no. of muscles). One-way ANOVA was used to test differences, longevity was tested using a Log-rank (Mantel-Cox) test, p<0.05 is significant.

Average sEJC amplitudes were -0.81±0.14nA for w¹¹¹⁸, -0.68±0.08nA for wstd¹, and -0.92±0.05nA for M80T (p>0.05), at a frequency of 1.29 ±0.18Hz for w¹¹¹⁸, 0.83±0.08Hz for wstd¹, and 1.01±0.20Hz for M80T (p>0.05, n=14, 13, 10). Average eEJC amplitudes were -95.9±5.8nA for w¹¹¹⁸, -99.1±3.5nA for wstd¹, and -88.1±8.9nA for M80T (p>0.05, n=13, 13, 10).

Synaptic depletion at 60Hz stimulation (950ms) reduced amplitudes to 41±7% of initial values in wstd¹ and 57±7% in M80T vs 56±4% in w¹¹¹⁸, exponential fits to decaying amplitudes revealed tau values of 148±28ms for wstd¹ and 390±127ms for M80T vs 256±18ms for w¹¹¹⁸ (p<0.05, n=10, 4, 9). Subsequent eEJC recovery times were altered in TPI mutants, tau: 9.2±0.8s for wstd¹ and 8.2±4.4s for M80T, vs 5.8±0.6s for w¹¹¹⁸ (p<0.05, n=10, 2, 9).

Calcium dependency of evoked release (0.25-3mM) was unaltered in wstd¹ larvae compared to w¹¹¹⁸. Confocal imaging studies did not show significant differences in NMJ morphology, bouton counts: 27.6±3.3 in w¹¹¹⁸ and 33.0±3.8 in wstd¹, active zone counts: 262±37 for w¹¹¹⁸ and 224±20 for wstd¹, active zone areas: 134±20pixels in w¹¹¹⁸ and 95±12pixels in wstd¹, and total bouton areas: 751±51pixels in w¹¹¹⁸ and 711±131pixels in wstd¹ (n=7, 10, p>0.05). TPI mutant

flies showed reduced longevity, median values of 40 days in wstd¹ and 42 days in M80T vs 60 days in w¹¹¹⁸ (p<0.05, n=90, 90, 72).

The data suggests that the TPI-mutant phenotype is in part due to altered synaptic vesicle dynamics, possibly associated with vesicle pool organisation or endo/exocytosis, thus expanding our knowledge of TPI involvement in synaptopathology³. Suppressed TPI activity also enhances protein glycation and redox stress, which may potentially be responsible for our findings. Future studies will examine the physiological impact of redox stress, focusing on the link between NO-mediated post-translational modifications and TPI function.

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In early Alzheimer's disease, the voltage-gated calcium channel blocker nimodipine relaxes pericytes, dilates capillaries, reduces capillary blockages, increases cerebral blood flow and decreases brain hypoxia

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We have shown previously that, both in human Alzheimer's disease (AD) and in mice mimicking AD, pericytes constrict capillaries, thus reducing cerebral blood flow, while arteriole and venule diameter is unaffected in the AD mice (Nortley et al., 2019). The decrease of CBF is an early event in AD, and a decrease of brain energy supply is known to upregulate production of amyloid beta, suggesting that maintaining CBF by preventing pericyte constriction might prevent some of the symptoms of AD.

To assess this, we used 2-photon microscopy to image capillaries in vivo (through a cranial window over somatosensory cortex) in wild-type or AD [APP(NL-G-F) knock-in] NG2-dsRed mice in which pericytes fluoresce red. FITC dextran (70 kDa) was administered intravenously to visualise blood flow. Pericyte calcium concentration was assessed using NG2-Cre^{ERT2} mice crossed with floxed GCaMP5g mice, which were administered tamoxifen. After assessing normality of each data distribution, statistical analysis employed (2-tailed) paired or unpaired t-tests or Mann-Whitney tests, as appropriate.

In 4 month old AD mice, a femoral vein infusion of the blood-brain barrier permeable voltage-gated calcium channel blocker nimodipine (220 microg/kg total, over 10 minutes, using a 60 microg/ml solution) decreased the $[Ca^{2+}]_i$ by $16 \pm 4\%$ (mean $\pm$ s.e.m., $p=0.006$) in 14 1st-3rd branch order pericytes, and increased capillary diameter at the pericyte somata by $17 \pm 4\%$ ($p=0.003$). These changes, together with a similar relaxation of arteriolar smooth muscle cells, increased cortical CBF measured by laser Doppler by $50 \pm 6\%$ in 13 AD animals ($p=0.0003$). Blockage of capillaries (probably by circulating neutrophils or other blood cells) occurred in $\sim 1\%$ of capillaries in 13 wild-type mice, but in 13 AD mice near AD plaques $\sim 20\%$ of capillaries were blocked, which was reduced to $\sim 5\%$ by nimodipine ($p<0.0001$ for both differences).

Giving nimodipine in the drinking water for 1.5 months, from 2.5 months of age, to mimic clinical prophylaxis for AD, similarly increased the diameter (at pericytes) of 1st-3rd branch order capillaries from 5.2 ± 0.3 microns ($n=16$ capillaries) to 6.9 ± 0.3 microns ($n=38$, $p=0.004$). In area CA1 of the hippocampus of 9 WT animals, hypoxia (assessed with pimonidazole) was seen on average in 2 neurons and glial cells per image stack, while in 9 AD mice this rose to 10.5 per stack ($p=0.003$), but in 8 AD mice given nimodipine this was reduced to 4.8 per stack ($p=0.03$).

In brain slices made from neurosurgically-derived human cortical tissue, 75 nM amyloid beta constricts capillaries at pericytes (Nortley et al., 2019). Nimodipine (3 microM) reversed this constriction to the extent that the diameter was not significantly different from that in the absence of amyloid beta, suggesting that the actions of nimodipine on mouse pericytes that are described above are also likely to occur for human pericytes.

These data suggest that prophylactic use of agents targeted at pericytes to reduce capillary constriction may preserve CBF and brain oxygenation in the early stages of Alzheimer's disease.

Nortley, R. et al. (2019) Amyloid β oligomers constrict human capillaries in Alzheimer's disease via signaling to pericytes. *Science*. 365, eaav9518.

C65

Thalamic deep brain stimulation relieves hypercapnic induced air hunger.

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Background: We have previously reported a case of a patient with multiple morbidities (COPD and cerebral stroke) who underwent deep brain stimulation (DBS) of multiple sites including the motor thalamus (Green *et al.*, 2019). The COPD related breathlessness was abolished by DBS of the motor thalamus. We therefore hypothesized that DBS of MT relieves experimentally induced hypercapnic air hunger (AH) in patients undergoing DBS of the motor thalamus for movement disorders.

Methods: Ten patients receiving DBS therapy for tremor, who had electrodes implanted bilaterally in the Ventral Intermediate Nucleus of the MT, underwent two 5min steady state hypercapnic AH tests once with DBS in the 'ON' state and once in the 'OFF' state in random order. Patients rated AH on a 10cm visual analogue scale (VAS) every 15s. Test level of hypercapnia was the same for ON and OFF states (mean±sd end-tidal PCO₂ 42±3mmHg). Ventilation was constrained to the same baseline level for ON and OFF states by setting a fixed flow of fresh gas into a 3 litre anaesthetic bag from which patients inspired with a frequency of 12 breaths/min set with a metronome. AH ratings in the last min of each test were averaged and mean levels compared for ON and OFF states..

Results: Nine of ten patients rated less AH with DBS ON (median reduction -12%VAS; range -9 to -52%VAS) shown in figure 1. Only one patient rated more AH in the ON state (increase of 34%VAS). Overall mean±sd steady state AH was 52±28 %VAS for the ON state and 67±27%VAS for the OFF state. This difference was significant (P=0.03; paired t-test) and exceeded minimal clinically important difference for VAS ratings of AH (Ries, 2005).

Conclusion: DBS of the MT significantly relieved experimentally induced air hunger. We suggest that DBS of the MT may directly block the dyspnea signal ascending through the thalamus. The extent of relief suggests that DBS of motor thalamic nuclei may prove to be a viable therapy for intractable dyspnoea in select patients who are worst affected.

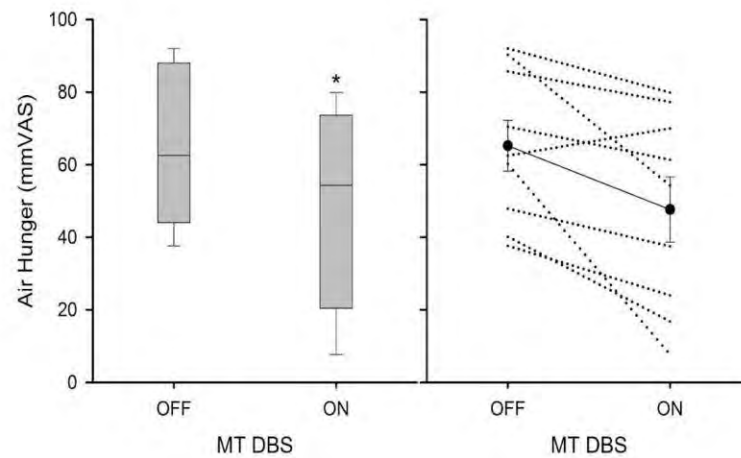


Figure 1 Left panel: Box and whisker plot showing the median (horizontal line), Interquartile range (shaded boxes), and upper/lower extremes (whiskers) for ratings of air hunger (AH) on a 100mm visual analogue scale (VAS) during the steady state of experimentally induced hypercapnia with constrained ventilation with deep brain stimulation (DBS) electrodes in the motor thalamus (MT) DBS switched off (OFF) and switched on (ON) in 9 patients undergoing DBS for treatment of essential tremor. **Right panel:** Individual changes in air hunger ratings between the OFF and ON conditions are shown (dotted lines) along with the Mean $\pm$ SEM change (solid circle). *, $p < 0.05$, two tailed Student's paired t-test

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C66

Sinusoidal electrical stimulation of the dorsolateral prefrontal cortex modulates sympathetic nerve activity to muscle and skin in humans

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Introduction: The dorsolateral prefrontal cortex (dlPFC) is primarily involved in higher-order executive functions, and little is known of its role in control of the autonomic nervous system. Our brain imaging studies have revealed links between the dlPFC and the generation of muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA) in humans (Macefield et al., 2013; Macefield & Henderson, 2016, 2019). We recently showed that sinusoidal electrical stimulation of the dlPFC causes a cyclic modulation of MSNA, heart rate and blood pressure, but had no effect on respiration (Sesa-Ashton et al., 2022). Here we assessed whether stimulation of the dlPFC can also modulate sympathetic outflow to skin.

Methods: Spontaneous bursts of SSNA were recorded from cutaneous fascicles of the right common peroneal nerve via a tungsten microelectrode in 21 healthy participants. Negative-going sympathetic spikes were extracted from the neurogram. Low-frequency sinusoidal stimulation (-2 to 2mA, 0.08 Hz, 100 cycles) was applied to the right dlPFC (EEG electrode site F4, n=21) or left dlPFC (F3, n=12) and the nasion via surface electrodes. The modulation index (peak-trough/peak) was calculated for each stimulation paradigm by constructing cross-correlation histograms between the times of occurrence of the sympathetic spikes and the peak of the sinusoidal stimulus. **Results:** Sinusoidal stimulation of either the right or left dlPFC caused significant cyclic modulation of SSNA (Mann-Whitney, $p < 0.01$), but there was no side-to-side difference. Stimulation also caused cyclic modulation of skin blood flow and sweat release. **Conclusions:** We have shown that sinusoidal stimulation of the dlPFC causes modulation of sympathetic outflow to skin as well as muscle in humans, as directly recorded via intraneural microelectrodes, as well as modulation of their effector-organ responses. This supports an important role for the dlPFC in the control of the sympathetic nervous system, which likely contributes to the ability of mental stress to bring about increases in MSNA, heart rate and blood pressure, and emotions to bring about increases in SSNA, cutaneous vasoconstriction and sweat release.

Macefield VG, James C & Henderson LA (2013) Identification of sites of sympathetic outflow at rest and during emotional arousal: concurrent recordings of sympathetic nerve activity and fMRI of the brain. *Int J Psychophysiol* 89: 451-459 Macefield VG & Henderson LA (2019) Identification of the human sympathetic connectome involved in blood pressure regulation. *NeuroImage* 202: 116119 Sesa-Ashton G, Wong R, McCarthy B, Datta S, Dawood T, Henderson LA & Macefield VG (2022) Stimulation of the dorsolateral prefrontal cortex modulates muscle sympathetic nerve activity and blood pressure in humans. *Cereb Cortex Comm* 3(2): tgac017

PCA001

The Impact of Sestrin2 Deficiency on the Regulation of Endothelial Nitric Oxide Synthase (eNOS)

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Diabetes-related cardiovascular complications are a significant cause of mortality worldwide. Endothelial dysfunction, characterized by decreased nitric oxide (NO) bioavailability, is an early cellular dysfunction linking diabetes to cardiovascular complications. Oxidative stress, caused by excessive reactive oxygen species (ROS) generation and decreased antioxidant capacity, plays a crucial role in diabetes and the onset of endothelial dysfunction. Sestrin2 (Sesn2) is a critical regulator in response to oxidative stress and has a protective role in various studies. Sesn2 levels are decreased in diabetes, which is linked to oxidative stress and endothelial dysfunction. This study aimed to investigate the molecular mechanisms underlying Sesn2 suppression and its contribution to endothelial dysfunction associated with diabetes.

To better understand the molecular mechanisms contributing to endothelial dysfunction in conditions such as diabetes, this study investigated the influence of *Sesn2* deficiency on the expression and activation of endothelial NO synthase (eNOS), the primary source of NO in endothelial cells. EA.hy926 endothelial cells were transfected with specific siRNA duplexes to silence *Sesn2* expression or overexpressed using a pre-designed expression plasmid. The cells were then either challenged or not with thapsigargin, an endoplasmic reticulum (ER) stress activator that triggers an inflammatory response often seen in diabetes. The mRNA expression of eNOS was assessed by qPCR (n=6), and eNOS protein expression and phosphorylation at activatory (Ser1177) and inhibitory (Thr495) sites were evaluated using western blot analysis (n=3-6). Statistical analyses using One-way ANOVA followed by Tukey's multiple comparison post hoc test (normal distribution) or the non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison post hoc test (not normally distributed) were performed, and $P \leq 0.05$ was considered statistically significant.

The silencing of *Sesn2* resulted in a decrease in the phosphorylation of eNOS at the activatory ($38.68 \pm 3.19\%$ of control; $p=0.0002$) and inhibitory ($55.68 \pm 17.35\%$ of control; $p=0.0008$) sites compared to control. This decrease in eNOS phosphorylation was driven by a significant reduction in its protein expression in *Sesn2*-silenced cells compared to controls ($49 \pm 27.03\%$ of control; $p=0.0294$). Similarly, mRNA expression of eNOS was reduced in cells deficient for *Sesn2* ($61.9 \pm 10.4\%$ of control; $p<0.0001$). Interestingly, inhibiting the proteasome with MG132 did not reverse the effects of *Sesn2* silencing on eNOS expression. These findings suggest that Sesn2 plays a critical role in regulating eNOS expression and phosphorylation, likely through a mechanism independent of proteasomal degradation.

This study has provided new insights into the regulatory role of Sesn2 in eNOS expression and highlights the critical role of Sesn2 in endothelial function. The findings suggest that *Sesn2* deficiency leads to decreased eNOS expression and activation, providing a foundation for future research aimed at uncovering the precise mechanisms underlying the interaction between Sesn2 and eNOS. Furthermore, our results suggest that Sesn2 may represent a potential

therapeutic target for treating endothelial dysfunction associated with diabetes and other cardiovascular diseases.

PCA002

NS1643 potentiates T1019PfsX38- and Q1070X-hERG channel variants but exhibits off-target effects in a commonly used cellular model

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The human ether-a-go-go gene (hERG) encodes a voltage-dependent K⁺ ion channel that plays an important role in cardiac repolarisation by terminating the cardiac action potential (AP). Loss of function mutations in hERG cause type-2 long QT syndrome (LQTS-2), which predispose affected individuals to cardiac arrhythmias and sudden death. The development of therapeutic approaches that target the root-cause of LQTS-2 depends upon thorough understanding of the molecular characteristics of disease-causing variants and their responsiveness to novel therapies. T1019PfsX38- and Q1070X-hERG are LQTS-2 causing variants that were reported in Oman and Saudi Arabia (Bhuiyan et al. 2008; Al Senaidi et al. 2014). Previous investigations revealed that these variants exhibit channel gating changes that can affect their activities at the protein level (Bhuiyan et al. 2008; Al Salmani et al. 2022). In this study, we aim to assess the responsiveness of these variants to N,N'-Bis[2-hydroxy-5-(trifluoromethyl)phenyl]urea (NS1643) as a potential investigational drug. Using the whole-cell patch clamp technique as described in Al Salmani et al. (2022), we recorded K⁺ currents from HEK293 cells transiently expressing wild-type- (WT-), T1019PfsX38- or Q1070X-hERG. We used paired and non-paired Student's t-test where appropriate to assess the statistical significance of difference, n is the number of cells. A 400 ms ventricular AP (VAP) clamp revealed that T1019PfsX38- (p = 0.04) but not Q1070X-hERG (p = 0.774) exhibits reduced potassium currents at the repolarisation phase of the AP (75-95 % of the AP duration) when compared with WT-hERG ($I_{(integral)}: WT = 2.63 \pm 0.29$ pA.s/pF (n = 6), T1019PfsX38 = 1.43 ± 0.42 pA.s/pF (n=4), Q1070X = 2.94 ± 1.23 pA.s/pF (n = 4)). The application of NS1643 (10 μ M) to the bath solution increased these $I_{(integral)}$ values by 53%, 80.3% and 78% in cells expressing WT- (p = 0.031, n=6), T1019PfsX38- (p = 0.001, n = 4) and Q1070X-hERG (p = 0.036, n = 4), respectively. To understand these effects further, we measured the voltage and time dependences of channel activation in response to NS1643. NS1643 shifted the half-maximum voltage (V_{mid}) of activation to more negative values (p < 0.01) compared to control but did not increase the maximum amplitudes of currents (control V_{mid} : WT = 2.90 ± 3.25 mV (n = 5), T1019PfsX38 = 6.96 ± 4.94 mV (n = 3), Q1070X = 10.40 ± 2.40 mV (n = 5); NS1643 V_{mid} : WT = -16.70 ± 3.60 mV (n = 5), T1019PfsX38 = -13.57 ± 4.37 mV (n = 3), Q1070X = -15.38 ± 3.69 mV (n = 5)). In addition, NS1643 accelerated the activation process of the three variants at multiple test potentials (p < 0.05, n = 4 – 9). Finally, NS1643 slowed the deactivation process when measured at -40 mV. However, when applied to untransfected HEK293 cells, NS1643 (10 μ M) inhibited endogenous currents that exhibit fast activation and fast inactivation kinetics at potentials ≥ 0 mV (p < 0.01, n = 5). Overall, NS1643 enhances the activities of WT-, T1019PfsX38- and Q1070X-hERG channels but its therapeutic potentials require assessment of possible side-effects.

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PCA003

Effects of Carbon Monoxide on action potentials recorded in iCell2 human induced pluripotent stem cell derived cardiomyocytes

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Exposure to carbon monoxide (CO), a well-known toxin, results in cardiovascular complications, including arrhythmias¹. CO blocks Ca^{2+} , Na^+ , and K^+ currents and can induce early afterdepolarisation arrhythmias (EADs) in rat or guinea pig ventricular myocytes^{2,3}. However, the dominant effect of CO on ionic currents is species dependent and the proarrhythmic mechanism in man is currently uncertain. Here we examined the effect of CO on iCell² human induced pluripotent stem cell derived cardiomyocytes (hiPSC) cardiomyocytes, a model commonly used for human cardiac safety assessment. Whole cell current clamp recordings of action potentials (APs) were assessed using the CO-releasing molecule (CORM-2; 10 μM) or the inactive control (iCORM; 10 μM). Data are presented as mean $\pm$ SEM and the significance level was determined by Student's t-test. Spontaneously beating cardiomyocytes (n=32) exhibited heterogeneous APs. These were grouped into atrial (34.3%), nodal (12.5%), or ventricular shaped APs (53.1%). The CO effect was examined on a ventricular shaped APs triggered with 5-ms depolarizing current injections at 1 Hz pacing rate. AP duration measured at 90% of repolarization ($\text{APD}_{0.9}$) was increased from 307.3 ± 34.1 to 423.4 ± 39.7 ms (n=6, $p < 0.05$). The lengthening of APD_{90} was mirrored by a decrease in the peak of the action potentials from 55.7 ± 7.7 to 46.4 ± 7.4 mV ($p < 0.05$). In some cells, a secondary rising phase, consistent with EADs was apparent. During iCORM perfusion, $\text{APD}_{0.9}$ was stable, with coefficient of variation (CV) of 4.8% and showed no significant change when compared to control conditions (CV= 6.7%). Examining the CO effect on spontaneous APs, exposure to CORM-2 resulted in a progressive prolongation of APs and at longer exposure (6 minutes), cells exhibited a slow depolarisation above baseline, followed by failure to repolarise. In guinea pig myocytes³, CO prolongs the AP due to inhibition of the rapid delayed-rectifier K^+ current (IKr). In iCell² cardiomyocytes, IKr inhibition with E4031 (0.1 μM) prolonged the AP and at higher concentrations (1 μM) induced EADs. These data show that CO has proarrhythmic effects on hiPSC cardiomyocytes and that inhibition of IKr has qualitatively similar effects. Further work is required to establish the direct effects of CO on IKr in iCell2 cardiomyocytes.

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PCA004

Left ventricle remodeling potential of *Phoenix dactylifera* extract in induced diabetic cardiomyopathy in rats

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Introduction: Diabetic cardiomyopathy is characterized by structural and functional alterations in myocardium as persistent hyperglycemia leads towards ventricle hypertrophy and diastolic dysfunction (DD) therefore diabetic people are prone to heart failure. In research based-settings, traditional plant-based extracts prescribed for diabetes have shown promising results.

Objective: Thus, current study aimed to explore ventricle remodeling potential of *Phoenix dactylifera* in induced diabetic cardiomyopathy in rats.

Methodology: For diabetes induction, rats (n=24) were fed with high fat diet and 5% sucrose in drinking water for 2 months with subsequent nicotinamide and streptozotocin administration. Diabetic rats were equally divided into 3 groups: Positive control (PC), standard control (SC; metformin @200mg/kg/bw), treatment group (PD: *Phoenix dactylifera* extract@5mg/kg/bw). Another group negative control (NC; n=8) was fed on normal diet. After 15 days of treatment, rats were decapitated. Body weight, fasting blood glucose (FBG) and ECG, serum glucose, insulin, lipid profile, oxidative stress markers, electrolytes and myocardial enzymes were assessed.

Results: Results (Mean±SEM) showed a significant ($P \leq 0.05$) elevation in FBG, total cholesterol, triglycerides, low-density lipoproteins, total oxidant status, malondialdehyde, CK-MB, LDH, AST and Na^+/K^+ ratio in positive control group, however a significant ($P \leq 0.05$) decrease was observed in body weight, high-density lipoproteins and total antioxidant capacity. On contrary, SC and PD group showed comparable results: antihyperglycemic, antihyperlipidemic, antioxidants and cardioprotective effects were significant ($P \leq 0.05$). ECG showed a significant prolongation in Tend-P and Tend-Q (DD marker) in PC as compared to PD group.

Conclusion: Thus, it is concluded that *Phoenix dactylifera* may have the potential to modulate left ventricle remodeling in induced diabetic cardiomyopathy.

Keywords: Diabetic cardiomyopathy, Hyperinsulinemia, Ventricle remodeling, *Phoenix dactylifera*, Cardio protection.

PCA005

Circulating neuropeptide-Y dynamics during exercise in heart failure

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Introduction:

Around 920000 people in the UK are affected by chronic heart failure (CHF), mainly due to an ageing population⁽¹⁾. High cardiac sympathetic drive and release of the sympathetic co-transmitter neuropeptide-Y (NPY) are significant features of CHF⁽²⁾, and resting venous NPY levels are well known to be associated with morbidity and mortality⁽³⁾, but how the NPY levels change during exercise and how this correlates with functional capacity is unknown.

Method:

We sought to establish the dynamics of circulating NPY levels in heart failure patients and compare these with indices of performance and cardiac function linked to long-term prognosis. Cardiopulmonary exercise testing (CPET) is an established method of quantitative assessment of exercise performance via measurement of ventilation, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production. CPET is applied in the heart failure population where the quality of life and exercise capacity are inextricably linked. Patients at least 6 months post cardiac resynchronisation therapy (CRT) device implantation underwent CPET with venous blood sampling at rest, peak exercise, and recovery as part of the device based synchronized biventricular (SyncAV) study (n=15). Patients' CPET performance measures were compared to the venous serum NPY levels at the rest, peak and recovery. Data is expressed as mean±standard deviation.

Results:

15 heart failure patients (9 males and 6 females, age 70.3±10 years, ejection fraction 29±7 % pre-CRT and 44±7 % post-CRT, $p<0.00001$). NPY levels increased significantly from baseline to peak exercise (40.08±6.90 to 93.46±42.13 pg/ml, $p=0.0004$) and remained elevated during recovery (to 86.84±44.60 pg/ml, $p=0.002$). The peak ($r=0.58$, $p=0.02$), and recovery ($r=0.56$, $p=0.03$) NPY levels as well as the ability to increase NPY from baseline ($r=0.53$, $p=0.04$) significantly correlated with heart rate recovery at 1 minute, but not with peak $\dot{V}O_2$ ($r=0.38$, $p=0.16$).

Conclusion:

In heart failure patients, the ability to increase NPY levels on exertion correlates with heart rate recovery, a known prognostic indicator for mortality.

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PCA006

Laser Doppler flowmetry as a method to assess blood supply to the lower limb joints in children with Legg-Calve-Perthes disease.

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Legg-Calve-Perthes disease is a common pediatric orthopedic pathology of the hip joint caused by impaired blood flow to the femoral head; the disease belongs to the group of osteochondropathies and represents aseptic osteonecrosis of the femoral head, in the severe course of which a functionally significant deformity of the proximal femur is formed. The aim of this study was to assess the degree of blood supply to the lower limb hip joints using laser Doppler flowmetry (LDF) in children with Legg-Calve-Perthes disease, as well as to determine the effectiveness of treatment.[1]

Methods. LDF method, which is non-invasive, was used to measure volumetric blood flow rate and assess the state of microcirculatory bed of the lower extremities. The method is based on probing the tissue with laser radiation and processing of the reflected signal. 20 male patients aged 7-9 years diagnosed with Legg-Calve-Perthes disease of III-IV degree and 20 healthy subjects as a control group were examined using this method on the basis of the pediatric trauma department of the Republican Clinical Hospital. Treatment included prolonged epidural analgesia followed by conservative treatment with drugs that improve blood supply to the joint[2,3]. All investigations and treatment were conducted after written consent of the adolescents' legal representatives. Statistical significance of the results was determined using Student's t test and nonparametric Wilcoxon-Mann-Whitney test.

Results. In the control group, the parameters of blood supply estimation of the hip joint area were the same in both limbs; in the patient group there was a significant decrease in the indices on the affected side. The difference compared to healthy subjects averaged 70% ($p \leq 0,001$). After the course of treatment, the microcirculation indices in the area of the pathological hip joint increased, on average, 5-fold compared with the indices before treatment.[4]

Conclusions. All patients with Legg-Calve-Perthes disease have a significant decrease in microcirculation in the area of the hip joint on the affected side. Prolonged epidural analgesia causes an increase in blood flow and thus has a positive effect on the course of the disease. The obtained results indicate that the method of laser Doppler flowmetry may be useful to confirm the effectiveness of treatment, as well as for early diagnosis of Legg-Calve-Perthes disease in children.

PCA007

Cross-platform validation of hiPSC-derived cardiomyocytes as a better human model for pre-clinical cardiotoxicity studies

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Drug-induced arrhythmia has been a major cause of drug development failure and the market withdrawal of novel compounds. Of particular concern is the block of the ion channel I_{Kr} (hERG) by drugs which can result in torsades de pointes (TdP), a dangerous ventricular fibrillation. Unanticipated toxic effects on contractility such as with the tyrosine kinase inhibitors and anthracyclines are also of increasing concern. Current methods to assess drug-induced toxicity during drug development heavily rely on animal models such as the Langendorff Heart preparation or overexpressed hERG channel cell models. Despite proving effective methods of identifying potential cardiotoxic liabilities during drug development these still create concern over their direct relevance to human physiology and toxicology. Human iPSC-derived cardiomyocytes (hiPSC-CMs) offer the opportunity to screen drugs *in vitro* using a more physiologically relevant model that expresses multiple ion channels and spontaneously contracts.

Axol Bioscience Ltd have shown their human iPSC-derived Ventricular Cardiomyocytes (ax2508) express all significant ventricular cardiomyocyte markers through RNAseq (two replicates) and immunocytochemistry (34 replicates) and have performed extensive electrophysiological characterisation using Multi-Electrode Array (MEA) demonstrating the presence of all key ion channels (min n=4) and a typical ventricular cardiomyocyte-like waveform (10 replicates).

In addition, innoVitro GmbH reproduced the correct contractility responses to isoprenaline, S-Bay K8644, 4-AP and the atrial-specific Carbachol, in ax2508, on their FLEXcyte 96 platform (n=4 per compound and concentration, assessed using the Wilcoxon rank-sum test).

Clyde Biosciences tested the CiPA28 acute cardiotoxic reference compounds on the CelloPTIQ™ platform against Axol's ventricular cardiomyocytes. The cardiomyocytes were grown for 6 days in multiwell format and then transitioned to a serum-free media and loaded with a voltage sensitive dye. Optical measurement of voltage changes allowed the assessment of the effect of each of the 28 compounds (min. n=5), which have varying levels of known TdP risk. hERG block was detected in Axol cardiomyocytes with a range of compounds, as evidenced by action potential duration at 90% repolarisation (APD₉₀) prolongation, APD triangulation and early afterdepolarisations (EADs). For example, clear hERG block was detected at even the lowest conc. of the classic hERG blocker dofetilide (0.3nM) through QT-prolongation (500ms to 550ms) and increased triangulation and the Ca²⁺-channel blocker nifedipine produced a shortened FPD (500ms to 300ms) while the low TdP risk anti-histamine loratidine only had minimal effects. In addition, the predictive power of Axol cardiomyocytes was reinforced when those compounds with multiple ion channel effects and varying risk profiles, such as verapamil, azimilide and ranolazine, modes of action and relative risks were

correctly identified so that verapamil's calcium block correctly counteracted its hERG block, ranolazine increased FPD, by 100ms, but only at intermediate concentrations and azimilide's pro-arrhythmic actions were only apparent at concentrations above 1 μ M. Drug effects were compared to vehicle control within the same well and were assessed using paired t-tests with P-values adjusted for multiple comparisons using the Benjamini-Hochberg procedure.

Therefore, Axol's human iPSC-derived Ventricular Cardiomyocytes can provide a reliable, physiological-relevant model to perform cardiotoxicity studies at scale and within a short time-frame on a range of different platforms.

PCA008

Passive electrical conduction across atrial scar border zone in mouse model of cryoablation

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Introduction

Ablation lines (scar regions created to block abnormal electrical conduction pathways, such as in atrial fibrillation) may become electrically transparent over time, necessitating repeat ablations. Aims/objectives: to explore the suitability of mouse models for studying trans-scar conduction after atrial cryo-ablation.

Method

Ex vivo optical mapping experiments were performed on dissected atrial preparations from cryo-injured, sham operated, and control mice (*Mus musculus*; on days 28 or 56, including male / female animals; n = 6 / condition). Functional whole heart optical mapping experiments were correlated with structural multiphoton imaging data to delineate scar tissue. Degree of tissue fibrosis was assessed in optically cleared hearts (X-Clarity), as well as histological tissue sections. All investigations were performed with ethical approval by the local Institutional Animal Care and Use Committee (Regierungspräsidium Freiburg, G22-047).

Results

Projecting 2D optical mapping data onto 3D multiphoton structural volume stacks of the mouse atrium, we observed passive conduction of excitation waves into the scar tissue, extending beyond the macroscopic boundaries of the border zone in all hearts after cryo-injury. These scar regions were transmural, collagen-rich, and largely devoid of cardiomyocytes. Cryo-lesions were significantly larger than the cryo-probe contact area: measured scar length was 2.73 ± 0.22 mm (compared to 1.5 mm probe length) and scar width was 1.07 ± 0.09 mm (compared to 0.23 mm probe width); lesion area was 2.01 ± 0.14 mm² (compared to the probe area of 0.35 mm²). Values reported as mean $\pm$ SEM, unpaired t test, scar p values = 0.0002 (length), <0.0001 (width) and <0.0001 (area) compared to cryo-probe.

Conclusions

This work supports the idea of a non-myocyte mediated passive conduction of electrical excitation through atrial ablation scars. Electrical coupling of cardiomyocytes and non-myocytes may offer a potential target to steer cardiac electrophysiology post-ablation. This may ultimately reduce the need for re-ablation in patients, constituting a clinically relevant research target.

PCA010

Clinical efficacy and safety of radiofrequency versus cryoballoon ablation for the treatment of paroxysmal atrial fibrillation – a meta-analysis

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Atrial fibrillation, the most prevalent human cardiac arrhythmia, causes unnoticeable to severe symptoms like angina, lethargy, vertigo and dyspnoea. Problematic re-entry circuits perpetually prevent full atrial repolarisation post-atrial systole; preventing optimal atrial filling and ejection, causing ectopic ventricular tachycardia and increasing thromboembolic risk. Paroxysmal atrial fibrillation episodes are isolated or reoccurring and last less than twenty-four hours but gradually increase in frequency and duration until chronic. Effective management slows atrial fibrillation progression. Radiofrequency and cryoballoon ablations are two gold standard interventions for atrial fibrillation where lifestyle and pharmacological intervention fail. Radiofrequency and cryoballoon sever re-entry circuits using heat damage and liquid nitrogen-induced intracellular freezing – respectively. New randomised controlled trials investigating the safety and efficacy of the latest radiofrequency and cryoballoon iterations warrant updated systematic review and meta-analysis. This study aims to elucidate if either radiofrequency or cryoballoon ablation were significantly superior in clinical efficacy or safety.

PubMed and Web of Science databases were searched for relevant randomised controlled trials. Articles with title and abstract terms 'radiofrequency,' 'cryoballoon,' 'ablation,' 'safety,' 'efficacy' or 'paroxysmal atrial fibrillation' were identified via 'AND' and 'OR' Boolean functions. Articles with title and abstract terms 'protocol,' 'reablation,' 'repeat,' 'supplementary,' 'open ablation,' 'combined,' 'economic,' 'financial,' 'comorbidities,' 'animal,' 'systematic review,' 'meta-analysis,' 'child' or 'adolescent' were excluded using the 'NOT' Boolean function. RevMan 5.4 was used for bias assessment, meta-analysis and forest plot representation of included study data.

Eight recent (2011-2021) randomised controlled trials (three single and five multi-centre) with 1950 human patients (1265 male and 685 female; 949 radiofrequency and 1001 cryoballoon) were identified for systematic review and meta-analysis. There was no significant difference in clinical efficacy outcomes for either radiofrequency or cryoballoon ablation: atrial fibrillation reoccurrence (odds ratio [OR] = 1.13, 95% confidence intervals [CI₉₅] = 0.90-1.41, statistical heterogeneity [*I*²] = 0%) and reablation (OR = 0.77, CI₉₅ = 0.35-1.11, *I*² = 0%) rates at 12-months post-ablation or total complication rate (OR = 1.21, CI₉₅ = 0.76-1.92, *I*² = 0%). Radiofrequency and cryoballoon ablation had significantly lower phrenic nerve injury rate (OR = 0.14, CI₉₅ = 0.03-0.62, *I*² = 0%) and shorter total procedure duration (standard mean difference = 0.33, CI₉₅ = 0.19-0.46, *I*² = 0%), respectively.

This report reliably indicates that clinicians and adult patients should not differentiate between radiofrequency and cryoballoon ablation based on clinical efficacy and total complication rate but can reliably discriminate based on phrenic nerve injury rate and total procedure duration. Future high-quality, multicentre, randomised controlled trials with more subgroup classifications (like age, race, gender, paroxysmal versus non-paroxysmal atrial fibrillation) will produce data

that applies to more specific patient groups. More rigorous and transparent reporting of study design and bias risk reduction is required to improve future study validity.

PCA011

The cell-wide web and nuclear envelope invaginations of murine pulmonary arterial myocytes: AMPK triggers contraction by priming and mobilising peripheral and central sarcoplasmic reticulum, and nuclear envelope invagination calcium stores

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Introduction and aims Hypoxia triggers pulmonary artery contraction by mobilising calcium from two distinct compartments of the sarcoplasmic reticulum (SR), one sensitive to block by the sarco/endoplasmic reticulum ATPase (SERCA) inhibitor cyclopiazonic acid (CPA) and the other insensitive [1]. Furthermore, the AMPK-activated protein kinase (AMPK) mediates acute hypoxic pulmonary vasoconstriction in the mouse lung in-vivo [2]. Therefore, we examined the nature of AMPK-induced initiates calcium signalling across the cell-wide web of pulmonary arterial myocytes, a recently discovered network of cytoplasmic nanocourses demarcated by SR nanojunctions [3].

Methods Isolation of pulmonary arterial myocytes and confocal imaging were 18-22 °C; excitation 494 nm; emission 506 nm), ER-tracker (Thermo Fisher) and Draq5 (Thermo Fisher). Confocal images were acquired at 22 °C using a Nikon A1R+ confocal, Galvano scanner and 1.25 n.a. water immersion objective (Nikon).

Results and conclusions ER and Fluo-4 positive nuclear envelope invaginations (NEIs) were thus identified in live cells. Blind and transnuclear NEIs projected deep into the nucleus and were tubular, ~200nm in diameter, and branched. Asynchronous calcium signals were evident at rest within cytoplasmic nanocourses demarcated by NEI and across the cell-wide web beyond the nucleus. Strikingly, when AMPK activators (MK8722 (0.1-1µM; Compound 13, 1-30µM; 991 10µM) [1] were applied extracellularly marked increases in Fluo-4 fluorescence were evoked in NEI subsequent to signal initiation proximal to the plasma membrane by a multi-stage process. For example, extracellular application of 1µM MK8722 reduced the fluorescence intensity of the cytoplasmic calcium indicator Fluo-4 (Phase 1) across all cytoplasmic nanocourses of pulmonary arterial myocytes (fluorescence change in F/F0 = -0.065±0.014; n=6 from n=5 mice). A transient rise (~60s) in fluorescence within peripheral aspects extraperinuclear nanocourses followed (peak change in F/F0 = 0.923±0.126; n=6 from n=5 mice) that propagated inward across the cell-wide web and induced concomitant contraction (Phase 2). A secondary increase in fluorescence within perinuclear but not extraperinuclear nanocourses followed (peak change in F/F0 = 1.315±0.369; n=6 from n=5 mice) which maintained myocyte contraction (Phase 3). In 3 of 6 cells, a further, prolonged increase in Fluo-4 fluorescence occurred in perinuclear nanocourses only (Phase 4; peak change in F/F0 = 0.854±0.18; n=3 from n=3 mice). Intriguingly, MK98722 induced calcium flux into cytoplasmic

nanocourses demarcated by NEI, and these signals were maintained between marked oscillations in calcium flux at proximal perinuclear nanocourses. Consistent with previous proposals [1-3], activation of AMPK, likely triggers contraction of pulmonary arterial myocytes by pre-loading of SR stores and then sequential mobilisation of peripheral SR, central SR and latterly NEI calcium stores, where calcium flux into cytoplasmic nanocourses demarcated by NEI remained elevated between phases of calcium release and contraction across the wider cell.

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PCA012

Estrogen ameliorates cardiac function by reducing GRK2-mediated β_1 AR internalization during acute stress

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Introduction

Stress is an inevitable response to internal and external stimuli that can impair cardiac function, such as stress cardiomyopathy, arrhythmia, and sudden death [1]. Stress response is significantly regulated by the sympatho-adrenomedullary system, resulting in an increased secretion of catecholamines, which activates β -adrenoceptors (β AR) [2]. During stress, GRK2, also known as β -adrenoceptor kinase 1 (β ARK1), predominately translocates to the cell membrane to catalyze the phosphorylation of an already activated β AR, the main receptor that governs the inotropic and chronotropic effects of the heart. Evidence suggests that the female sex hormone estrogen could improve cardiac function during stress by regulating β AR-Gs/Gi signaling pathway [3]. However, the interplay between estrogen and GRK2 is not well understood.

Aim

Here we explored the effects of estrogen on myocardial GRK2 during stress and its effect on GRK2-mediated internalization of β AR.

Methods and results

In vivo and in vitro experiments were performed using female wild-type (WT) and GPER-KO mice, isolated adult mice cardiomyocytes, and hESC-CM.

WT mice were divided into sham-operated and ovariectomy groups. These mice and isolated adult cardiomyocytes were subcutaneously administered with isoproterenol (ISO) and estrogen (E2). The results showed that **(1)** Estrogen enhanced cardiac function by measuring ECG and cardiomyocytes' shortening amplitude, and improved APD (action potential duration), I_{Na} , I_{to} , I_{Ca-L} of hESC-CMs by patch clamp in acute stress. **(2)** Estrogen reduced total GRK2 and membrane GRK2 content in the myocardium during acute stress in mice and hESC-CM by western blot and immunofluorescence. **(3)** By inhibiting or overexpressing GRK2, the results showed that estrogen enhanced the contractile function and electrophysiological indexes of cardiomyocytes via inhibiting GRK2 in acute stress. **(4)** Estrogen reduced GRK2-mediated internalization of β_1 AR in myocardium during acute stress by Immunoprecipitation and immunofluorescence.

WT and GPER -KO mice were also categorized into groups based on the subcutaneous administration of ISO. hESC-CM were subjected to GPER siRNA transinfection, then cells were pre-treated with ISO and estrogen. The results showed that **(1)** GPER attenuated GRK2-induced reduction in cardiac function during acute stress by measuring ECG and cardiomyocytes' shortening amplitude. **(2)** GPER reduced GRK2 content in the myocardium membrane and β_1 AR internalization in acute stress by immunofluorescence.

Statistical analysis

All data analyses were performed with GraphPad Prism 5.01 and presented as means $\pm$ s.e.m. Statistical significance ($P \leq 0.05$) for each variable was estimated by one-way or two-way ANOVA followed by Bonferroni post hoc tests. For animals, $n=6-8$, for cells $n=3-4$.

Conclusion

Exciting new findings from our study demonstrate that estrogen has a powerful protective effect on the heart by reducing the content of GRK2 in the myocardium and preventing the internalization of β_1 AR during acute stress. These findings not only shed light on the complex mechanisms underlying estrogen's beneficial effect on heart function but also highlight GRK2 as a crucial target for treating stress-induced heart disease. By targeting GRK2, we can potentially avoid the side effects of estrogen and develop more effective therapies for women with estrogen deficiencies or elderly patients.

Ethical standards

All animal procedures complied with the guidelines of the Animal Ethics Committee of Xuzhou Medical University (China) (permit number:L2021701001).

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PCA013

Stretch of individual, in situ, Purkinje fibres increases ectopic activations in isolated Sheep left ventricle preparations

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Cardiac mechanical and electrical activity are inter-dependent. The free-running Purkinje fibre network is located upon the ventricular endocardial surface. When the left ventricle (LV) undergoes mechanical stimulation, in the form of acute ventricular dilation, arrhythmogenesis may occur (Hurley et al., 2023). However, it is not yet clear the extent to which the Purkinje fibre network is involved. The aim of this study was to determine whether mechanical stimulation of individual Purkinje fibres leads to the development of ectopic activations.

All work was undertaken in accordance with local ethical regulations and approval from the University of Bordeaux and in accordance with the European Parliament Directive 2010/63/EU. Surgical plane anesthesia was induced in Sheep (n=5; 50-65 kg) with 10 mg/kg sodium pentobarbital and maintained under isoflurane (2% in 100% O₂) prior to euthanasia by an intravenous injection of 2000 mg sodium pentobarbital. Hearts were quickly excised and perfused with cardioplegia and heparin. LV wedges were cannulated at the ostia and coronary-perfused with Tyrode. With the endocardial surface of the heart exposed, LV wedges were electrically stimulated (1.34-1.79 Hz) at the His Bundle by external electrodes. A suture was looped underneath the midpoint of a randomly selected single free-running Purkinje fibre and attached to a force transducer. Thus, raising the force transducer stretched the Purkinje fibre and indicated the timing and level of extending force applied. Endocardial Purkinje fibres were mechanically stimulated by applying a mean extending force of 4.86 ± 0.17 g for 10s, with 10s rest between each stretch. A pseudo-ECG was recorded and the effect of stretch on the number of ectopic excitations was tested by a Wilcoxon signed-rank test.

Stretch provoked single ectopic activations which disrupted the rhythmic patterns of the pseudo-ECG. Ectopic activation occurred a minimum of 1s and maximum of 8.2s upon the initiation of stretch, with no definite distribution pattern through the 10s stretch period.

When individual Purkinje fibres were stretched, 20 ectopics occurred across 7 out of the 21 recordings. When Purkinje fibres were not stretched, a 1.8 fold decrease in ectopic activations was recorded, with only 6 out of the 21 recordings eliciting an ectopic response (mean 0.95 ± 0.33 ectopics when stretched vs. mean 0.52 ± 0.22 ectopics when at rest; $\pm$ SEM; $P < 0.05$; n=21). In addition, when the experimental time period of stretch and rest was considered, ectopic frequency was 81% greater when Purkinje fibres were mechanically stimulated compared to at rest (1.80 ectopics/min upon stretch vs. 0.99 ectopics/min at rest; $P < 0.05$).

The stretch of individual Purkinje fibres led to an increase in the number of ectopic activations in preparations that were already showing ectopic activity. This suggests that Purkinje fibre stretch has the potential to cause electrical destabilisation in compromised tissue. This mode of investigation has the potential to investigate the role of Purkinje fibres as a source for stretch-

induced ectopics or in the maintenance of arrhythmias. However, more detailed electrical mapping of ectopic initiation sites and their conduction is necessary.

Hurley M et al. (2023). Curr. Res. Physiol. 6, 100098

PCA014

End-lysosomal calcium channels work in association of Rab27a to perform vesicular transport

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Introduction: GTPases (e.g. Rab family) received great attention after revealing their role in cell signalling (primarily vesicular transport). Calcium plays a key role in cell signalling in cargo-mediated transport and fusion-release processes of vesicles. Calcium stores within the cells works in association of Rabs proteins to carryout transport and release. But which stores are mainly involved and to what extent is not yet completely understood. Looking at the global picture, endo-lysosomal acidic stores are one of the major calcium stores in cells.

Methods: Our recent investigation on two-pore channels knockout mice suggest two-pore channels deficient mice are not able to release normal levels of hormones and some enzymes. This clearly indicates their direct role in calcium supply for vesicular transport processes. We investigated at both functional and molecular levels to understand mechanisms of calcium recruitment from acidic stores via two pore calcium channels.

Results: Molecular interactome studies of Rab proteins suggest direct relationships between Rab proteins and lysosomal calcium channels. Molecular investigations on GTP/GDP bound Rab27 status suggest two-pore channels interact with Rab proteins and process recruitment of calcium via them from endo-lysosomal stores to complete vesicle transport process. **Conclusion:** This study is of its first kind that suggest lysosomal calcium channels are involved in cell signalling events to make cargo proteins efficient to perform vesicular transport.

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PCA015

Peripheral vascular function in white European and black African descent individuals

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Individuals of black African (BA) descent display a diminished vascular response to various stimuli compared with individuals of white European (WE) descent. Whether the glabrous peripheral microcirculatory responses differ between these racial groups is unknown. We hypothesised that the vascular responses to local heating (LH) and post-occlusive reactive hyperaemia (PORH) would be impaired in the index finger and Great toe pad of BA individuals compared with WE individuals.

Following Loughborough University ethical approval and written informed consent, ten WE (mean [SD] age 25 [4] years, height 1.75 [0.09] m, mass 72.0 [13.0] kg) and eight BA (age 20 [2] years, height 1.72 [0.11] m, mass 81.3 [14.3] kg) participants undertook PORH and LH protocols in 25 °C ambient air. Cutaneous vascular conductance (CVC; flux/mean arterial pressure) was measured at the finger and toe pad with local skin temperature clamped at 33 °C. *PORH protocol*: following a 10 min baseline, finger and toe blood flow was occluded (220 mmHg) for 5 min and then rapidly released. The area of hyperaemia (area under the curve [AUC]) was calculated above the baseline using the trapezoid rule. *LH protocol*: local skin temperature was clamped at 33 °C for 10 min followed by 42 °C for 20 min and then 44 °C for 10 min. Plateau averages at 42 °C and 44 °C were taken from stable 5 min periods. PORH variables were analysed using independent samples t-tests whilst LH was analysed using a mixed model ANOVA.

Table 1. Mean (SD) area under the curve and cutaneous vascular conductance during each protocol for WE and BA groups

		Finger		Toe	
		WE	BA	WE	BA
PORH	AUC	160 (125)	184 (102)	272 (146)	159 (79)
	CVC Peak	4.72 (1.83)	4.12 (1.29)	3.70 (0.96)	2.69 (1.35)
LH	CVC at 42 °C	3.15 (1.13)	2.77 (2.01)	2.11 (0.99)	1.41 (1.07)
	CVC at 44 °C	3.87 (1.26)	3.09 (1.79)	2.50 (1.29)	1.67 (1.16)

Vascular responses of the finger and toe pad were similar in WE and BA participants for each protocol (Table 1, $P > 0.05$), thus the hypothesis is rejected. From the present data, it appears the diminished vascular response previously reported in BA individuals is not present in the glabrous peripheral skin sites.

PCA016

The effect of glucocorticoids on cardiac function in an animal model of metabolic syndrome

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Background: Whether the adverse effects of glucocorticoids on left ventricular (LV) function are exacerbated by the consumption of a high-fructose diet, as a model of metabolic syndrome (MetS), is uncertain. This study aimed to determine whether the presence of MetS exacerbates glucocorticoid-induced changes in LV function. **Methods:** Sprague Dawley rats were divided into control, glucocorticoid (GC), high-fructose (HF), and glucocorticoid+high-fructose (GC+HF) groups. HF and GC+HF rats received 20% fructose solution in drinking water and GC and GC+HF rats received 10mg/kg intraperitoneal injections of methylprednisolone daily for 10 weeks. After 10 weeks, echocardiography was used to assess LV function and cardiac collagen content was determined by picrosirius-red staining. **Results:** Relative wall thickness (RWT) was increased in GC compared to control rats ($p=0.001$). Heart weights and LV weights indexed to body mass and RWT were increased in GC+HF compared to control rats ($p=0.04$, $p=0.009$, and $p=0.03$ respectively). Lateral e' was reduced in GC and GC+HF rats compared to control ($p=0.001$ and $p=0.005$ respectively) and HF ($p<0.0001$ and $p=0.0001$) rats. E/e' was increased in GC and GC+HF rats compared to control ($p<0.0001$ and $p=0.004$ respectively) and HF ($p<0.0001$ and $p=0.02$ respectively) rats. Cardiac collagen content was increased in GC and GC+HF rats compared to control rats ($p=0.001$ and $p<0.0001$ respectively). **Conclusion:** Administration of glucocorticoids caused concentric remodelling and impaired diastolic function by reducing LV relaxation and increasing filling pressures. The administration of glucocorticoids in a model of MetS caused concentric hypertrophy. However, the MetS did not exacerbate the diastolic dysfunction induced by the administration of glucocorticoids.

PCA017

The effect of blood flow restriction on heart rate recovery after submaximal cycling – a preliminary study

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Purpose: Aerobic exercise with blood flow restriction (BFR) is a popular strategy for improving muscle function and aerobic capacity. However, the extent to which BFR affects cardiac autonomic recovery after exercise has not been documented. Therefore, the aim of this study is to investigate the effect of BFR on heart rate recovery (HRR) as a measure of cardiac autonomic activity.

Introduction: BFR involves the use of high cuff pressure to impair blood inflow and venous outflow from the occluded limb. It is commonly used in elite athletes during exercise to simulate oxygen deprivation in the exercising muscles of the occluded limb. Since maintaining the balance between training and recovery is a major goal of training prescription, it would be necessary to know how BFR affects recovery after exercise when such exercises are included in the daily training schedule.

Methods: Eleven healthy athletes (age 26.18 ± 4.23 , body mass index 23.33 ± 1.04) volunteered to perform submaximal cycling under control conditions (noBFR) and with both legs occluded at 80% of the occlusion pressure during exercise (BFR). Exercise sessions were randomly assigned and performed at least 48 hours apart. Each session consisted of 5 minutes of rest, 10 minutes of graded cycling up to 60% of previously determined maximal aerobic capacity, and 15 minutes of passive recovery in a seated position. At the end of the exercise, the leg occlusion was released. During exercise and recovery phase, HR was measured with the Cosmed Quark PFT (Cosmed, Italy). Peak heart rate (HR_{peak}), HRR in 30, 60, and 120 seconds after exercise completion, and HRR relative to HR_{peak} (HRR30%, HRR60% and HRR120%) were determined. Paired t test was applied in SPSS, ver.27 and a confidence level of $p < 0.05$ was chosen (two-tailed test). Cohen's d was determined to quantify significant differences.

Results: HR_{peak} was significantly higher in BFR (164.45 ± 15.03) compared with noBFR (143.45 ± 14.69 , $p < 0.001$, $d = 5.30$), whereas HRR60% was significantly lower in BFR (0.19 ± 0.07) compared with noBFR (0.26 ± 0.06 , $p = 0.013$, $d = 0.98$). There were no significant differences in any other HRR indices.

Conclusions: Our results indicate that cycling with BFR enhances the HR response to aerobic exercise and impairs parasympathetic reactivation after exercise cessation. Increased HR_{peak} at the same load in BFR might be related to increased metabolic stress in the active muscles of the occluded limbs, as described by other authors. HRR60% is associated with parasympathetic reactivation, which seems to be delayed in BFR compared with noBFR according to our results. It has been shown that reoxygenation of occluded muscles is also delayed in BFR (Solsona et. al.2022), shifting the increased metabolic load to the recovery phase. HRR could also be

affected by the increased venous return at the release of leg occlusion after exercise cessation. Further studies are needed to determine the main underlying mechanisms.

Ethical requirements: National Medical Ethics Committee of the Republic of Slovenia (KME RS) has approved this study and has issued a confirmation with KME reference number: **0120-360/2022/3**.

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<https://doi.org/10.3389/fphys.2022.864642>

PCA018

Mechanical stimulation of Piezo1 channels using high throughput automated patch clamp

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Introduction: Piezo1 channels are mechanosensitive ion channels that play a pivotal role in sensing mechanical forces in various cell types. Their dysfunction has been associated with numerous pathophysiological states including generalised lymphatic dysplasia, varicose vein disease, dehydrated hereditary stomatocytosis, and malarial resistance. Given their high physiological relevance, investigating Piezo1 is crucial for the pharmaceutical industry that requires scalable techniques to allow for drug discovery. In this regard, several studies have shown the use of high-throughput automated patch clamp (APC) to explore the function and properties of Piezo1 channels in heterologous expression system as well as primary cells (Rotordam et al. 2018, Parsonage et al., 2022; Karamatic Crew et al., 2023) mainly based on usage of Yoda1, a specific gating modifier of Piezo1 channels (Syeda et al., 2015). However, to our knowledge, a combination of solely mechanical stimulation and high-throughput APC has not yet been available for the study of Piezo1 channels.

Methods: Here we show that optimization of pipetting parameters coupled with the possibility to increase the number of cells per well in the NPC-384 chip of the SyncroPatch 384 lead to Piezo1-mediated currents activated by solely mechanical stimulation (M-Stim). Mouse and human Piezo1 channels expressed in HEK293 cells and untransfected HEK293 cells were tested by M-Stim in the absence and presence of Yoda1. Cells showing stable seal resistance and eliciting inward currents higher than -100 pA that could be inhibited by GdCl₃ (non-specific blocker of mechanosensitive channels) were considered Piezo1 responding cells.

Results: Our results strongly suggest that applying solutions on top of the cells at a fast pipetting speed is crucial for activating Piezo1 channels by M-Stim on the SP384. Moreover, stimulating 4 cells simultaneously in one well of a NPC-384 chip increased the current amplitudes at peak by 2-fold in mPiezo1, from -387.8 pA, 95% CI [297, 511.4] (n=148) to -894.3 pA, 95% CI [757.9, 1043] (n=579) and by 1.5-fold in hPiezo1, from -398.8 pA, 95% CI [332.5, 518] (n=174) to -612.8 pA, 95% CI [544.8, 685.5] (n=346) for hPiezo1. No currents were detected from the untransfected cells. In line with this, the number of responding cells increased significantly from approximately 10% (both mPiezo1 and hPiezo1) to 64.17% ± 3.25% for mPiezo1 and to 52.75% ± 1.60% for hPiezo1 when using 4-hole chips. The number of responding cells was close to 100% when using 4-hole chips in combination with Yoda1.

Conclusions: In this study, M-Stim of Piezo1 channels using a high-throughput planar patch clamp system could be demonstrated. The possibility of comparing and combining mechanical and chemical stimulation in a high-throughput patch clamp assay facilitates the biophysical and pharmacological characterization of Piezo1 channels and thereby provides an important experimental tool for drug discovery.

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PCA019

Influence of nitric oxide and calcium sensitivity on the relaxant action of lauric acid on the corpus cavernosum of male Wistar rats

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Introduction: Lauric acid is the most abundant constituent of coconut oil which is consumed in the tropics. Lauric acid has been reported to have a relaxant action on the corpus cavernosum of the penis, making it a potential cure for erectile dysfunction. However, the mechanisms of action need to be fully established. An erection majorly involves the release of nitric oxide by the endothelium leading to the relaxation of the corpus cavernosum. Also, a reduction in the calcium sensitivity of the smooth muscle cells is an alternative pharmacological target for improving erectile function, as increased calcium sensitivity amplifies contraction.

Aims/Objectives: This study aimed to confirm, by blocking nitric oxide synthesis, if the relaxation of lauric acid involves other pathways aside from the nitric oxide pathway. Also, the inhibitory effect of lauric acid on calcium sensitivity was assessed as a possible alternative relaxation pathway, by evaluating its influence on Ca²⁺- induced contraction.

Method: Ethical approval was obtained from the Ahmadu Bello University Animal Care and Use Committee and the rats were handled in compliance with the World Medical Association Declaration of Helsinki. Corpus cavernosum tissues from five euthanized male Wistar rats were extracted and mounted in an organ bath filled with Krebs solution. Using KCl and Phenylephrine as contractile agents in separate experiments to mimic sympathetic stimulation and depolarization respectively, relaxation responses to cumulative concentrations of lauric acid were evaluated in the control condition and in the presence of N-nitro-L-arginine methyl ester (L-NAME); a nitric oxide synthase inhibitor. In another experiment, to evaluate calcium sensitivity, the tissues were bathed in a Ca²⁺- free physiological solution and pre-incubated with N, N-ethylene glycol tetraacetic acid (EGTA); a calcium chelating agent. Contraction responses to cumulative concentrations of Ca²⁺ in the control condition and in the presence of lauric acid were then evaluated. Results were presented as mean ± S.E.M. Data was analyzed using ANOVA. Values with $p < 0.05$ were considered significant.

Result: For phenylephrine-contracted strips, the percentage relaxation was significantly higher ($p < 0.05$) in the presence of L-NAME compared to the control at 10⁻⁶ M (36.8 ± 1.5 vs 29.8 ± 0.8) and 10⁻⁵ M (43.38 ± 2.2 vs 33.2 ± 2.1). However, for KCl-contracted strips, the percentage relaxation was significantly lower ($p < 0.05$) in the presence of L-NAME compared to the control at the concentrations of 10⁻⁷ M (17.0 ± 2.4 vs 30.6 ± 2.9); 10⁻⁶ M (21.0 ± 3.6 vs 45.4 ± 3.9) and 10⁻⁵ M (24.2 ± 4.0 vs 53.0 ± 4.5) in KCl contracted strips. No significant change was seen in the

contraction response to Ca^{2+} in lauric acid-treated tissues compared to the control at all concentrations.

Conclusion: Relaxation of the corpus cavernosum by lauric acid in phenylephrine-contracted tissues was not solely dependent on the nitric oxide pathway. Lauric acid-induced relaxation was however completely dependent on the nitric oxide pathway in KCl-contracted tissues. Modulation of calcium sensitivity did not account for an alternative pathway for the relaxant action of lauric acid

Keywords: Lauric acid, Corpus cavernosum, Erectile function

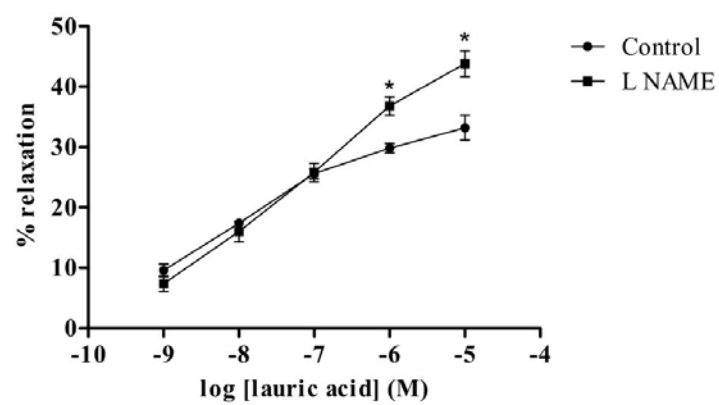


Figure 1: Influence of nitric oxide on the relaxant action of lauric acid in phenylephrine-contracted corpus cavernosum tissues of male Wistar rats

* significant compared to control ($p < 0.05$)

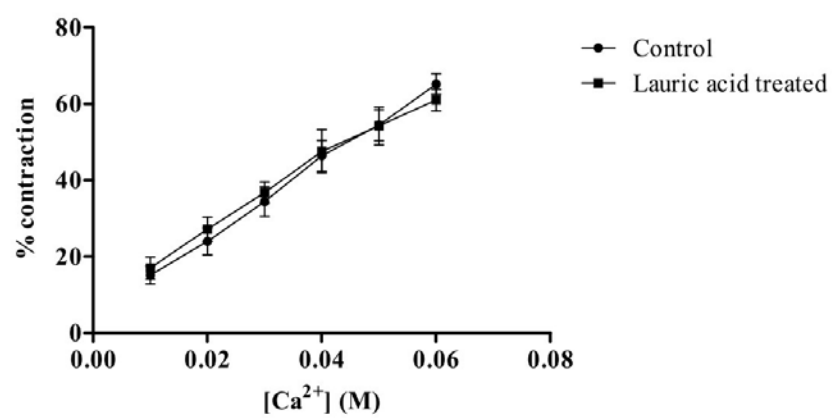


Figure 3: Influence of calcium sensitivity on the relaxant action of lauric acid on corpus cavernosum tissues of male Wistar rats

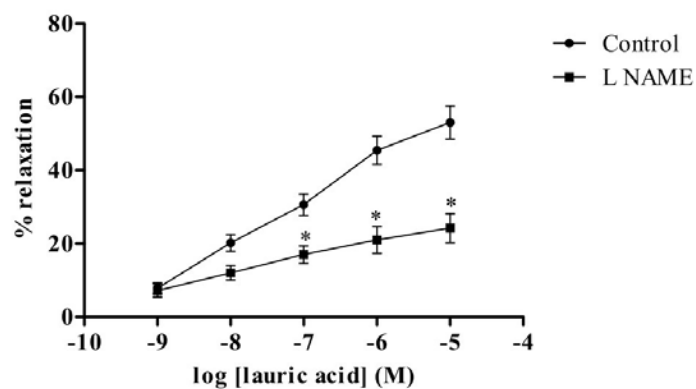


Figure 2: Influence of nitric oxide on the relaxant action of lauric acid in KCl-contracted corpus cavernosum tissues of male Wistar rats

* significant compared to control ($p < 0.05$)

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PCA020

Agreement of carotid-femoral pulse wave velocity and brachial-femoral pulse wave velocity when exploring different path lengths in young healthy individuals

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Carotid-femoral pulse wave velocity (cfPWV) is the gold standard measure of arterial stiffness (Laurent et al, 2006). However, utilising the carotid artery can be challenging due to subcutaneous fat, venous artefacts and breathing affecting the quality of the waveform. Brachial-femoral (bfPWV) has been shown to correlate well with cfPWV (Baier et al, 2018; Keehn et al, 2014) and therefore may be beneficial when measuring central arterial stiffness, whilst avoiding the difficult nature of the carotid artery. Limited research has explored bfPWV and the true value path length, which is imperative when measuring PWV. As such, the aim of this study was to look at the best agreement of bfPWV path length compared with the gold standard cfPWV for both the Vicorder and Ultrasound in a supine and seated posture. Thirty-one young healthy participants (Male: n= 20, Female: n= 11, Age: 24.8 ± 5.2 y, Weight: 74.9 ± 13.6 kg, Height: 1.75 ± 0.8 m, BMI: $24.7 \pm 3.1 \text{ kg/m}^2$) were recruited for this study. Ethical approval was obtained from the institutional human research ethics committee. Participants visited the laboratory on one occasion, Doppler ultrasound measures were taken at the common carotid, brachial and femoral arteries. The Vicorder was used to measure cfPWV and bfPWV, path length measures explored were sternal notch to umbilicus, subtraction (sternal notch to midpoint of femoral cuff minus sternal notch to midpoint of brachial cuff) and midpoint of the brachial cuff to midpoint of the femoral cuff methods. All measures were taken in a supine and seated posture. The Vicorder showed better overall agreement across all path lengths ($\rho = 0.62-0.67$) when measuring bfPWV compared to the ultrasound ($r = 0.44-0.57$) in the supine posture. The subtraction method showed the best agreement in the supine ($\rho = 0.62$) and seated ($\rho = 0.45$) posture when using the Vicorder device, and in the supine ($r = 0.57$) posture when using the ultrasound device. The sternal notch to umbilicus demonstrated the best agreement when using the ultrasound in a seated posture ($r = 0.42$), and when comparing it to the gold standard (cfPWV) measure in supine and seated posture. The findings of this study suggest that bfPWV should be conducted in a supine posture with the subtraction method as the arterial path length. However, sternal notch to umbilicus could also be used if participants are unable to outstretch their arm. The Vicorder device should be used rather than the ultrasound due to the data be collected simultaneously.

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PCA021

The effect of COVID-19 on measures of haemodynamic function: A prospective observational study

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Introduction: Assessment of haemodynamic function provides valuable information regarding cardiovascular performance and can be used to guide diagnosis, treatment, and management of cardiovascular disease. This study investigated the effects of COVID-19 on haemodynamic function in individuals with a history of COVID-19.

Methods: Eighty-four healthy individuals (mean age 60 ± 7 years, 55% were female) with history of confirmed COVID-19 infection; within the previous 18 months and 28 days post-infection, and 40 healthy individuals (mean age 63 ± 7 years, 63% were female) without history of COVID-19 were recruited. Cardiac haemodynamic measurements including heart rate (HR), stroke volume (SV), cardiac output (CO) was determined using echocardiography (Vivid IQ, GE Healthcare, USA) at rest and during peak exercise.

Results: There were no significant differences between COVID-19 and non-COVID-19 groups for body mass index (26.9 ± 4.2 vs 26.5 ± 3.8 kg/m², $p = 0.59$), body surface area (1.8 ± 0.21 vs 1.8 ± 0.22 m², $p = 0.217$), resting systolic and diastolic blood pressures (systolic: 134 ± 17 vs 131 ± 17 mmHg, $p = 0.392$; diastolic: 83 ± 8 vs 81 ± 10 mmHg, $p = 0.156$), and HR, (62 ± 11 vs 60 ± 9 bpm, $p = 0.880$). At rest, participants in the COVID-19 group demonstrated significantly higher SV (77 ± 18 vs 66 ± 17 mL, $p = 0.021$) and CO (4.6 ± 1.1 vs 4.1 ± 0.8 L/min, $p = 0.017$) compared to the non-COVID group. At peak exercise, HR and diastolic blood pressure were significantly higher in the COVID-19 than non-COVID-19 group (HR: 137 ± 23 vs 128 ± 17 bpm, $p = 0.037$; diastolic blood pressure: 93 ± 12 vs 85 ± 12 mmHg, $p = 0.001$). There were no significant differences between the groups in peak exercise systolic blood pressure (198 ± 28 vs 197 ± 27 mmHg, $p = 0.974$), cardiac output (15.1 ± 4.5 vs 16.3 ± 14.0 , $p = 0.487$), or stroke volume (110 ± 31 vs 106 ± 31 , $p = 0.739$).

Conclusion: Individuals with history of COVID-19 may demonstrate increased cardiac work at rest, as demonstrated with higher stroke volume and cardiac output, compared to individuals without history of COVID-19.

PCA022

Evaluation of resting cardiorespiratory parameters in the patients suffering with chronic pain

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Introduction: Various researches have shown correlation between chronic pain and blood pressure changes, thereby involving both sympathetic and parasympathetic systems. Further, the various works done on autonomic functions in chronic pain, still fails to prove that which part of the autonomic nervous system dominates. **Aims:** This study was carried out to understand the effects of chronic pain on resting cardiorespiratory parameters on the patients suffering with chronic pain of severity >3 on visual analogue score (VAS). **Methods:** This study was approved by the Institutional Ethical Committee of Banaras Hindu University. 50 male cases and 50 female cases were selected from the pain clinic, SSL Hospital, Banaras Hindu University, Varanasi, India. 50 male and 50 female age sex matched controls were also selected in this study. The electrocardiogram (ECG), systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and respiratory frequency (RF) were recorded in the cases and controls both. **Results:** Mean SBP/DBP of male cases was found lesser than the male controls ($P > 0.05$). Mean SBP/DBP of female cases was more than the female controls ($p < 0.05$). Mean SBP/DBP of male cases was found lesser than female cases ($P > 0.05$) and the Mean SBP/DBP of male controls was greater than female controls ($P > 0.05$). The HR of male cases and female cases were significantly greater than the male and female controls respectively ($p < 0.05$). The HR of male cases versus female cases and male controls versus female controls were not different ($P > 0.05$). There is no difference in the RF changes in all the groups. **Conclusions:** The data reveal that the sympathetic tone has not changed in substantial amount in the male cases but it is increased in the female cases significantly, this indicates the loss of sympathetic tone in male cases in comparison to the female cases. However, the parasympathetic tone seems to be decreased in both male and female cases.

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and sympathetic nervous activity in females with chronic neck and shoulder pain, BMC Musculoskeletal Disorders, 2012; 13:146-152.

PCA023

Exhausted coronary reserve in growth restricted cohorts: Implications for adult-onset cardiovascular diseases

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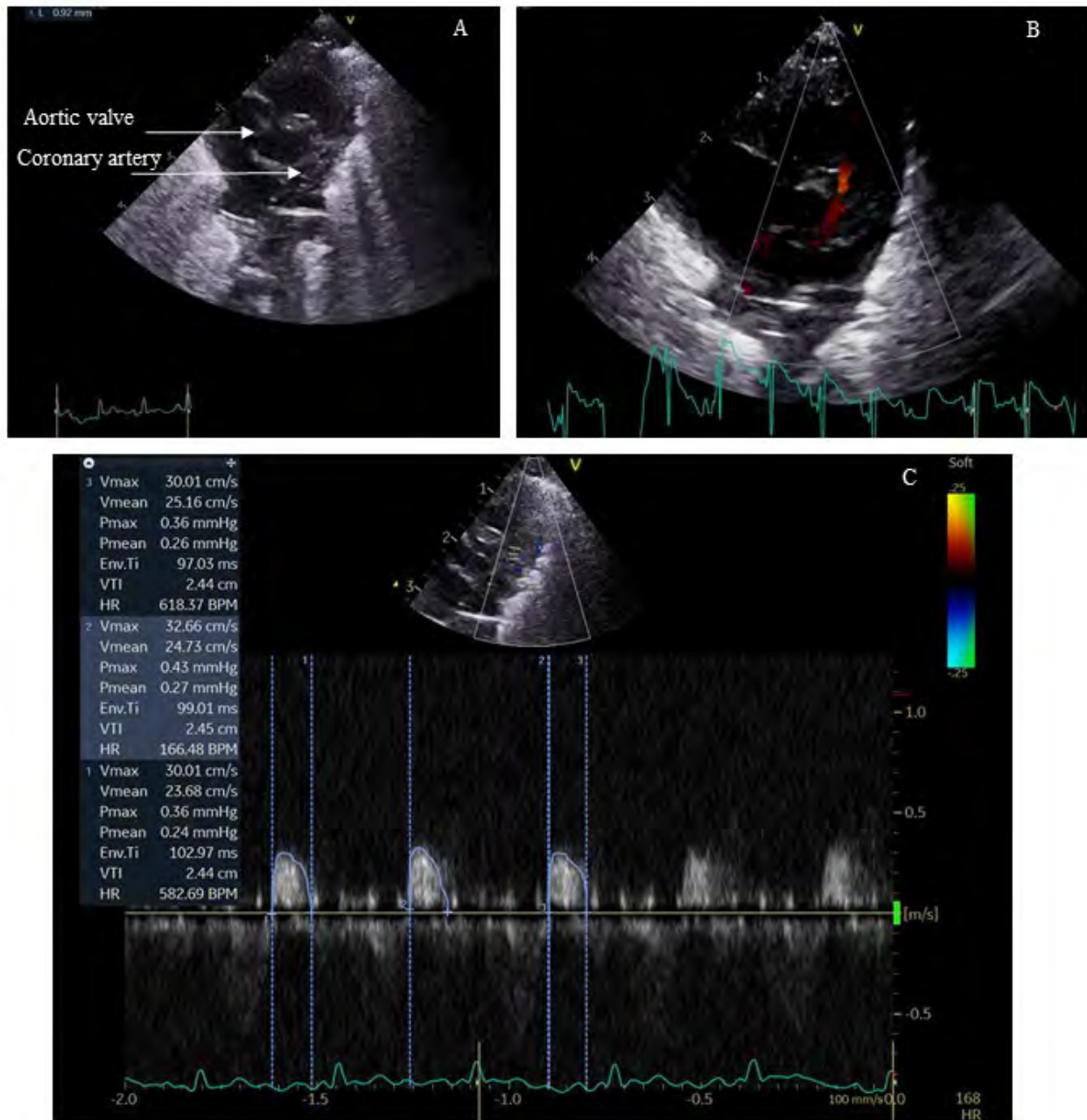
Background: Fetal growth restriction (FGR) resulting from placental insufficiency affects 18-30% pregnancies delivered at <32/40 weeks gestation. Compared to preterm appropriate for gestational age (AGA) human fetuses, FGR fetuses have a significantly earlier visualization of coronary artery blood flow (CABF-‘heart sparing effect’) but impaired cardiac function. Whether this dichotomy persists after birth has not been characterised. Experimental data from lambs has noted increased CABF in the setting of fetal hypoxaemia.

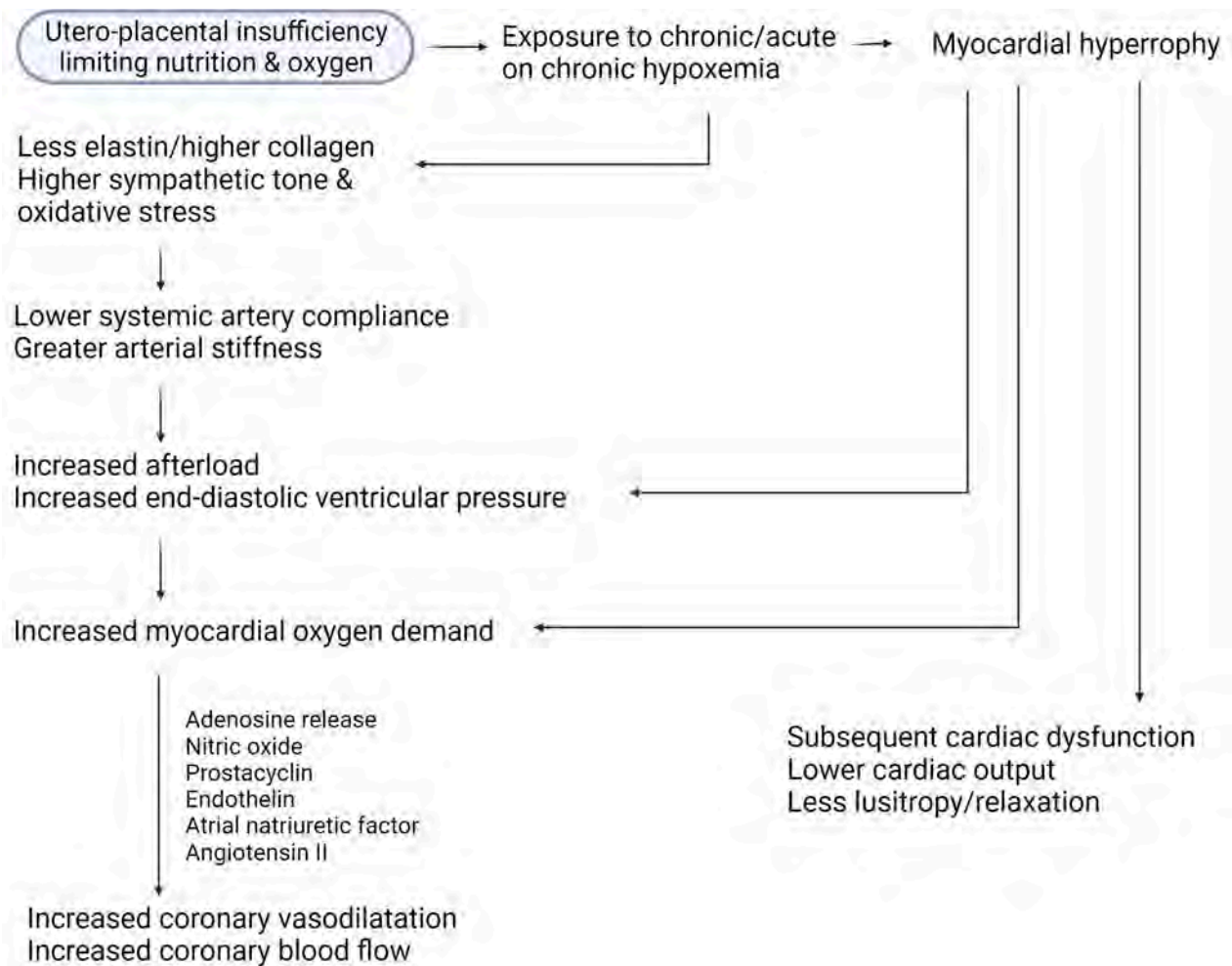
Methods: This echocardiography study compared CABF and cardiac function in preterm FGR infants, against AGA infants during the initial postnatal weeks of life. Cardiac function and CABF were measured with Vivid E95 Advantage Cardiovascular Ultrasound using a 12 Hz probe. Diastolic CABF was measured in the left anterior descending artery (main vessel supplying left ventricle [LV]), using colour Doppler flow analysis. Study was approved by the institution ethics board.

Results: Twenty eight FGR infants were compared with 26 AGA infants (gestation and birthweight, 29.7±1.3 vs 29.9±1 weeks, $P=0.6$ and 918±174 vs 1398±263g, $P<0.001$, respectively). In FGR infants, the LV was dilated and hypertrophied (↑end-diastolic diameter, posterior wall thickness and mass). The end-diastolic diameter in FGR vs AGA infants was 14.6±9.6 vs 13.6±7.3mm, respectively, $p=0.0001$, and the LV mass was 6.4±0.8 vs 5.3±3g, respectively, $p<0.001$. Diastolic and systolic function were impaired (↑ trans-mitral E/A ratio in FGR infants; 0.84±0.05 vs 0.79±0.03, $P=0.0002$) and (mean velocity of circumferential fibre shortening, 1.86±0.32 vs 2.7±0.5 circ/s, $P<0.001$). FGR infants had higher CABF (diastolic velocity time integral, 2.4±0.9 vs 1.65±0.8cm, $P=0.002$). Indexing coronary flow to diastolic and systolic function noted significant differences between the groups (coronary flow: E/A [FGR vs AGA], 2.9±1.1 vs 2.1±1, $P=0.01$ and coronary flow: mVCFc [FGR vs AGA], 1.33±0.5 vs 0.63±0.3, $P<0.001$). Diastolic blood pressure was significantly higher in FGR infants (30±2 vs 25±3 mm Hg, $P<0.001$). Figure 1 summarizes interactions of various hemodynamic forces in the hypoxemic milieu.

Conclusions: We noted postnatal persistence of fetal circulatory adaptation even though detached from *in-utero* hypoxemic state. Greater CABF postnatally may reflect an altered metabolic state, fetal programming, higher prostaglandin levels in FGR, and effects of altered myocardial architecture leading to ↑ O₂ consumption/requirements, possibly a combination of all the above. While coronary perfusion was higher in FGR infants (heart sparing effect), this was not accompanied by improved cardiac function, mimicking fetal age dichotomy as well the dichotomy in the cerebral circulation (increased flow [brain sparing effect] but sub-optimal neurodevelopment). Secondly, FGR cohorts might be unable to further augment CABF as per acute need (already exhausted ‘coronary flow reserve’). The failure of myocardium to relax (combined with intrinsic myocardial changes) and the coronary vascular resistance to drop in the face of increasing demand may heighten the risk of cardiovascular disease, when faced with additional workload such as strenuous exercise, obesity or hypertension. Epidemiologic studies

previously demonstrated $\uparrow$ cardiovascular disease related mortality amongst adults who had low birthweight. Whether assessments of CABF in FGR-born adults provide predictive ability for CVD in middle-older ages, needs prospective study.





PCA024

Quantifying the functional implications of uncertainties in I_{Kr} and I_{Ks} in human atrial cellular electrophysiology

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Cardiovascular disease is a leading cause of morbidity and hospitalisation at a large financial cost to society and an emotional burden to individuals (Amini et al., 2021). Electrical dysfunction, where the normal rhythm of the heart is disrupted, is a major cause of this morbidity. Therefore it is essential that channel kinetics and the interplay between channels is fully understood as to the role they play in regular and diseased states, including atrial fibrillation.

Computational models of the heart are continuing to grow in sophistication and accuracy and have been a valuable tool for understanding the functional implications of ion channel kinetics. There are multiple contemporary computational models that describe human atrial cellular electrophysiology, that all exhibit different action potential (AP) morphologies underlain by fundamental differences in the formulation and relative expression many ion channels. These differences in part reflect inter-cellular and inter-subject heterogeneity but are also related to inter-laboratory variability and how uncertainties have been included in the model. The formulation of two of the major repolarising currents, the rapid and slow delayed rectifier currents (I_{Kr} and I_{Ks} , respectively), is a major uncertainty in these models, due to the significant challenges in measuring the activity of these currents in isolated cellular preparations. Due to the importance of these channel currents for terminal repolarisation, uncertainties in their formulations could have major implications for pro-arrhythmogenic cellular dynamics, such as alternans and early after depolarisations (EADs), and therefore may critically impact the conclusions of modelling studies.

This project aims to quantify the effects of modulating both I_{Kr} and I_{Ks} in six published mathematical models (Courtemanche et al., 1998; Grandi et al., 2011; Chang et al., 2014; Colman et al., 2018). First, the range of values for the conductance of each current that resulted in AP durations (APD) that fit within physiological ranges was explored. Within these ranges, the impact of variability on APD restitution and alternans was investigated, as well as the interaction with variability in the L-type calcium channel (I_{CaL}).

When scaling both I_{Kr} and I_{Ks} there was no clear difference was found in APD_{30} . However, there was a large difference, including the presence of alternans, at APD_{90} in all six models (Figure 1). It was determined that I_{Kr} plays a larger role of the two channels with increased prolongation and reduction. For example, a decrease of 37.59 ms at APD_{90} was found in the original Grandi model compared with I_{Kr} scaled to by a factor of three, but only a decrease of 1.11 ms was found with the respective scaling of I_{Ks} . Inter-model differences were substantial across the range of behaviour studied, and some behaviours (such as EADs or non-repolarising APs) emerged only in a subset of the models.

In the future there is hope that computational models can be used in clinic to personalise medicines towards a patient's cellular profile. However, this work highlights the importance of

accurately capturing I_{Kr} and I_{Ks} in fundamental general models of human atrial electrophysiology before truly patient-specific models can be considered.

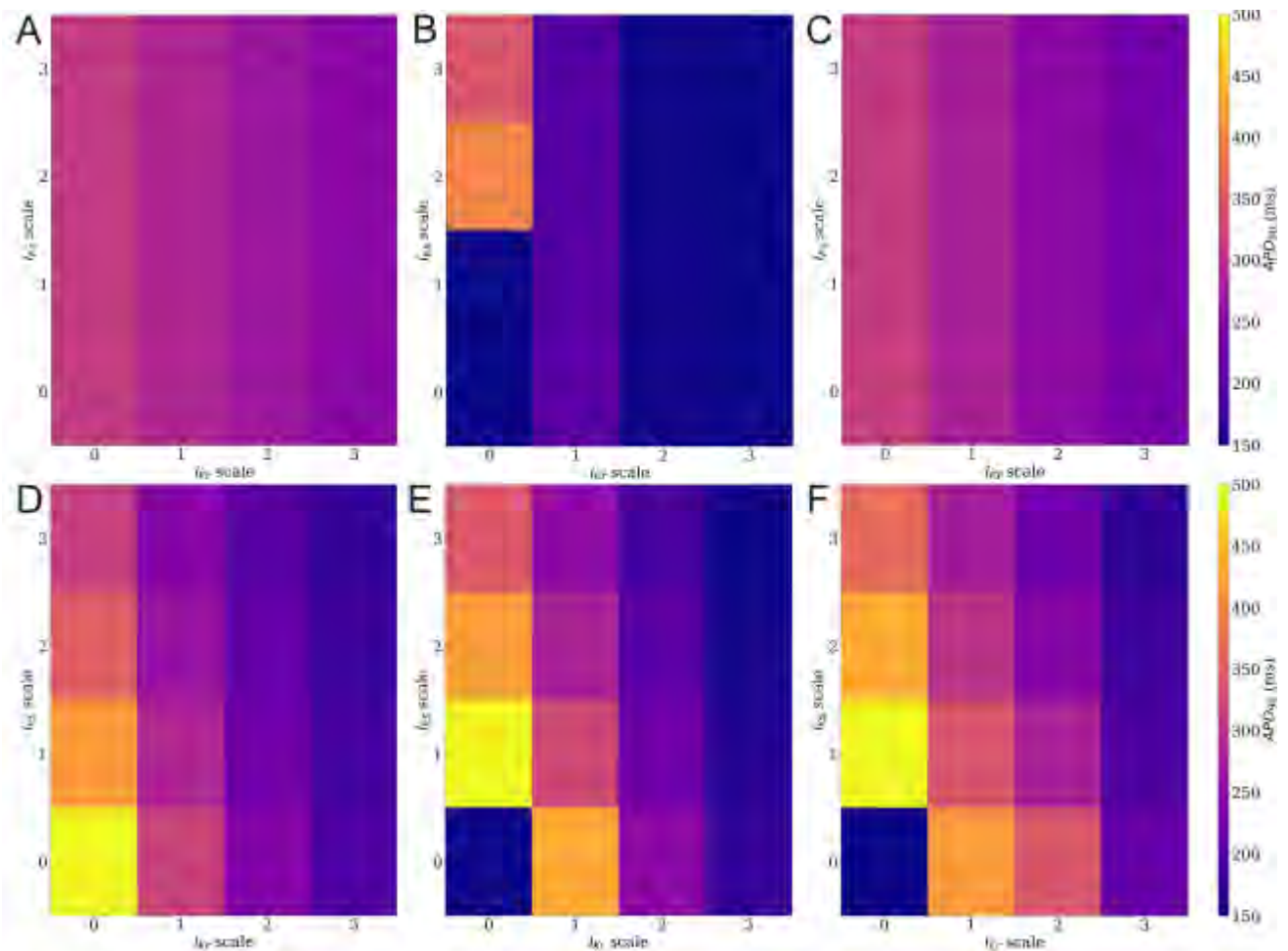


Figure 1: Combination scaling of I_{Kr} and I_{Ks}

APD₉₀ following combination scaling of I_{Kr} and I_{Ks} from 0-3 in the six models. Outputs ranged from alternans at 20.76 to 644.16 ms. Colour scale ranged from 150 to 500 ms with colour extrapolation where out of range (A) hAM_GB (B) hAM_WL_GB (C) hAM_GB_mWL (D) hAM_CRN (E) hAM_WL_CRN (F) hAM_CRN_mWL.

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PCA025

Liraglutide reduced the dark period core body temperature and curtailed cardiac sympathetic activity during the restraint stress.

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Glucagon-like peptide 1 (GLP-1) receptor agonists are proposed as a treatment option in patients with heart failure. However, the recommendation remains controversial because several clinical trials did not effectively improve cardiovascular outcomes in heart failure patients (1). One of the problems not settled is the concern about the positive chronotropic and sympathomimetic effect of GLP-1 receptor agonists (2).

We conducted an experiment with the aim of discovering the potentially hazardous effects of chronic treatment with long-acting GLP-1 receptor agonist liraglutide on the hemodynamics and autonomic nervous system (ANS).

During general anesthesia (120 mg.kg⁻¹ ketamine & 6 mg.kg⁻¹ xylazine i.p.), we implanted 10-month-old male Sprague-Dawley rats (n = 14, randomly assigned to the control or treated groups, n = 7) with telemetric transmitters (HD-S11, Data Sciences, USA). Implants allowed simultaneous monitoring of aortic pressure, ECG, core body temperature, and locomotor activity. After baseline 24-hour (12 h light-dark cycles, dark started at 7.00 a.m.) recording of telemetric signals, we used pharmacological tests and 30 minutes long restraint to estimate ANS activity (3, 4). We applied (i.p.) liraglutide daily, gradually increasing the dose. We started with 0.1 mg/kg of liraglutide for 18 days, continued with 0.3 mg/kg for 55 days, and 1mg/kg for 59 days. We injected (i.p.) saline to control rats. Telemetric signals were recorded weekly for 24 hours. We performed pharmacological and restraint stress ANS tests one month after injecting liraglutide 0.3 mg.kg⁻¹ or 1 mg.kg⁻¹. In addition, we calculated time and frequency domain indices of cardiovascular variability (3, 4). Data were analyzed with the multivariate (Wilks) repeated measure ANOVA. The post hoc Tukey HSD test was used if the interaction between the main effects (treatment & time) was significant.

While body weight remained steady in control rats (645 (SD 80) g), liraglutide-treated rats lost 17 % (p < 0.001) of their body weight at the end of the experiment. We found no significant change in mean arterial pressure, but liraglutide accelerated the heart rate by 10% (p < 0.001) during the light period increasing it from 278 (SD 10) bpm to 305 (SD 14) bpm. We recorded a significant (p < 0.001) reduction in the core body temperature (-0.4 (SD 0.2)°C) associated with liraglutide treatment during the dark period. No pharmacological tests or any heart rate variability or systolic pressure variability indices pointed to possible alterations in autonomic regulation of the hemodynamics. However, during the restraint stress test, liraglutide-treated rats showed a significantly (p = 0.013) lower elevation of mean arterial pressure by 30% and longer pre-ejection time by 28% (p = 0.011) than control rats.

In conclusion, chronic treatment with liraglutide did not affect the mean arterial pressure but accelerated heart rate during the light period. Surprisingly, we found signs of the sympatholytic effect of liraglutide, i.e., reducing the core body temperature during the dark period and prolonging pre-ejection time during the restraint stress.

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PCA026

Evaluating student perception of learning using a virtual reality experience of altitude sickness

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Virtual simulation offers the benefit of putting the user within the learned content; offering interactive lessons where experiential learning improves the rate at which we understand new concepts (So *et al.*, 2019; Angel-Urdinola *et al.*, 2021). As educators, we aim to provide learners with 'real world' scenarios whilst allowing learners to fail in safe and controlled simulated environments. Evidence supporting simulation learning appears in medical and nursing literature, little is known of student perception of simulation learning in basic science, or building confidence in application of threshold concepts beyond laboratory environments associated with basic sciences. It is unknown as to whether simulation learning is effective in promoting confidence in threshold concepts compared with lecture learning.

This study was approved by the Science and Engineering Research Ethics Committee, Manchester Metropolitan University. "Exercise and Environmental Physiology", is a level 5, 30 credit unit as part of BSc (Hons) Human Physiology. Students were administered an anonymous survey after a 2-hour lecture session on altitude physiology and 1-hour Computer automatic virtual environment (CAVE) simulation. Student confidence in identifying the signs and symptoms of altitude sickness and word association of students feelings in identifying signs and symptoms of altitude sickness was assessed. After CAVE simulation, students were surveyed on their perception of the experience and whether it added to the learning experience. Students rated from "Strongly Disagree", "Disagree", "Neither Agree nor Disagree", "Agree" or "Strongly Agree". Word associations were 9 options and/or free text. Differences between student confidence identifying signs and symptoms and word association of perception toward identifying signs and symptoms were assessed using χ^2 goodness of fit (SPSS 28, IBM).

After CAVE simulation, 80% (n=10) "Strongly Agree" and 20% "Agree" with being able to identify signs and symptoms of altitude sickness, compared with 0% "Strongly Agree" and 71% "Agree" after lecture (n=7) ($p<0.001$). Words associated with the lecture activity when compared with CAVE simulation were different (Figure 2; $p<0.001$). 100% "Strongly Agree"/"Agree" they enjoyed CAVE simulation, felt it helped improve knowledge and skills in addition to lecture, was engaging and would recommend to others for applying knowledge to real-world scenarios. 90% "Strongly Agree"/ "Agree" that the CAVE simulation covered what they expected, met learning needs, was appropriate to aid learning, was a high standard, easy to follow, gained new knowledge and learned how to apply knowledge to real-world scenarios. 80% "Strongly Agree"/"Agree" that it exceeded expectations. Some respondents however responded with themes of "Pressure", "Stressed" and neutrally in learning efficacy, suggesting some felt this environment is not conducive to confidence and learning. Despite this, those students did still respond with agreement to gaining/applying knowledge and understanding threshold concepts.

The CAVE environment presents an exciting and innovative way for educators in basic science to expose students to real-world scenarios in a safe, controlled environment and simultaneously meet threshold concepts of learning. Some caution however is advised in creating experiences where all students feel able to participate and to not exceed stress thresholds where learning may no longer take place (Vogel & Schwabe, 2016).



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PCA027

Soapmaking 101: a vehicle for teaching practical and discipline-specific skills

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Background: Experimental practical laboratory classes are used in undergraduate physiology education to enhance learning and develop essential numeracy, analytical and technical skills. However, the COVID-19 pandemic prevented medical sciences students from undertaking a range of standard, in-person physiology labs. Students requested that other ways be found to provide them more practical skills and analytical experience, even when safety measures restricted laboratory work.

Method: To address the need to provide students with practical skills and analytical experience whilst working remotely, we developed a soapmaking practical which was streamed live from the lecturer's kitchen. The remote experiment involved manufacturing soap by a saponification reaction and was later modified for subsequent in-person laboratory classes which were limited by COVID-19 safety measures following the return to labs. We hypothesised that the practical would provide a platform for students to gain relevant practical experience and enhance student attainment whilst learning remotely.

Results: Live streaming allowed students to watch an experiment in action including the collection of data, observing problem solving when errors occurred and maintenance of a lab book. Additionally, it enabled discussion of COVID-19 related physiology, career prospects and pastoral care. Students followed along with the protocol, input data and undertook assessment on the practical using the cloud-based system, Lt, which allowed staff and students to access resources through a variety of devices remotely. Analysis of results showed that students achieved a significantly higher mean grade (2020-2022: $91.97 \pm 1.016\%$, $n = 81$) on the soapmaking practical compared to the respiratory module assessment delivered the following year (2022-2023: $82.16 \pm 1.051\%$, $n = 43$). The mean grade was 9.81% higher (unpaired Student's t-test, $P < 0.0001$) for soapmaking compared to the respiratory practical assessment, and the mean grades for soapmaking did not differ significantly between the 2020-2021 and 2021-2022 (unpaired Student's t-test, $P > 0.05$). 2020-2021 survey data showed that 53% of students agreed or strongly agreed that the soapmaking practical enhanced their learning. Following this feedback minor adjustments were made to the practical and in the 2021-2022 survey 100% of students agreed or strongly agreed that the soapmaking practical enhanced their learning.

Discussion: This novel soapmaking practical provided a platform to improve core skills numeracy, analytical and research skills despite the limitations placed on practical laboratory classes. Our results indicate that the soapmaking practical is an effective way to enhance student understanding of skin physiology and experimental processes. Furthermore, the practical enabled exploration of issues relating to health and safety such as toxicology, allergy and anaphylaxis which are important considerations for industrial manufacturing and helped to prepare medical sciences students for the demands of their future careers.

PCA028

Competition—a method of promoting teaching : Brief introduction of Physiological quiz for Chinese students in medical and health-related majors

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Physiological quiz for Chinese students in medical and health-related majors began in 2015 and are held annually, about in June or July. The quizzes were mainly hosted and undertaken by the Physiology department of Xuzhou Medical University, and were strongly supported by the Organizing Committee of the international physiology quiz, the Education Working Committee of the Chinese Physiological Society and the Jiangsu physiological science society. So far, seven sessions have been successfully held. The first five sessions were held offline, and all the instructors and participating students attended the quizzes at Xuzhou Medical University. The teachers and students of more than 60 universities in Chinese mainland region participated in the offline quizzes. (115 universities participated in the seventh session). Each university sent one or two representative teams, each team consisting of 3-5 students. Due to the pandemic of COVID-19, the sixth and seven sessions were held online. During the student quiz, teachers also specially held a physiology teaching seminar to give suggestions on how to improve students' interest in physiology learning. For example, the theme of last year's seminar was "The Application of Modern Information Technology in Physiology Teaching"; The theme of this year's discussion is "Discussion on Digital Teaching Forms of Physiology".

The Physiology quizzes mainly includes the written test and the oral test. All schools in the first two sessions had taken the written test and oral test. In the following sessions, because there are too many schools and teams, we could only choose the teams which ranked in fronts in the written test to participate in the oral test.

Our physiology quizzes have greatly increased students' interest in learning. The benefit scope of the quizzes is not only the students who finally participate in the national quizzes, because these students are selected by each university from hundreds of students who participate in the quizzes of their own universities. So, in fact, many medical and related students from participating universities participated in the quiz. Therefore, the Chinese physiological quizzes have comprehensively improved the physiological knowledge level of medicine and related majors. The quiz has become a means of improving the teaching level that students find interesting and willing to participate actively.

PCA029

Using design sprints to develop resources for the effective communication of synoptic assessment for year 1 undergraduate students

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New undergraduate curricula for the BSc courses in Human Physiology, Physical Activity & Health, Biomedical Sciences, Neuroscience, Pharmacology and Sports and Exercise Sciences have been developed for the start of September 2023 as part of a new university wide student education strategy. Four strategic objectives are underpinning this process are shown in table 1.

Table 1: Four strategic objectives for student education at the University of Leeds.

Partnership	Engage students as partners in their education, through active, inclusive and research-based approaches to learning.
Transformation	Provide an outstanding education, that is evidence-based, improves students' learning outcomes, and is underpinned and enhanced by sector-leading pedagogies, digital resources and technologies.
Belonging	Foster an engaged and lifelong community of students, staff, alumni and partners.
Sustainability	Embed a sustainable approach to delivering high-quality, research-based education

Reviewing the Leeds curriculum, through focus groups of staff and students highlighted the pressure and stress student were placed under from multiple exams, some of which were not authentic or inclusive. This aligned with comments from recent National student survey results. Staff observed that many students are solely focused on succeeding in module assessments and not appreciating wider applications of knowledge and skills. A synoptic style assessment was proposed across the year 1 programmes, which would include a variety of different authentic assessment methods, designed to integrate students' knowledge and skills learnt across multiple modules. A variety of authentic assessment methods were discussed including evidence information reports, problem solving exercises and reflective essays ,experimental reports and team work outputs (with some individual planning elements), co-created by the students. The design team were concerned around the style of the assessment being very new to first year students and the communication of synoptic assessment to students being challenging.

A novel method of design was implemented to facilitate this process, the design sprint methodology (Grabill J, et al. 2022). This involved 3 phases of activity. The discovery phase

involved two independent researchers holding one-to-one interviews with assessment experts in the field externally, a variety of academic staff, current students and alumni. These 'lived experience' insights were then used in the second phase.

The second phase was ideating, involving a 2 day sprint activity where multiple perspectives were presented to consider and help shape ideas and determine the priority of tasks. This allowed for big ideas to be developed into time limited tasks, to then be prototyped with various stakeholders. The sprint involved design expert facilitators and a range of students and staff over two full days.

The outcomes of the sprint involved producing a visual aid on synoptic assessment co-created by students, having programme level support for students aiding academic belonging, creating clear consistent assignment briefs, marking criteria, marking and feedback guidance for staff, alongside opportunities for formative feedback via various mechanisms (generic, peer and individual). The final phase is to develop the above resources, and test them with staff and students ready for implementation. This methodology including multiple insights has been extremely valuable moving ideas to development of resources to support student education and curriculum design.

Grabill J, Gretter S & Skogsberg E (2022) Design for Change in Higher Education. John Hopkins University Press, Baltimore

PCA030

A staff-student journey towards inclusivity in a physiology-focused degree programme

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The murder of George Floyd, the degree awarding gap between Black and White students currently stands at 18.5%¹ and the School of Medicine's commitment to the British Medical Association charter to prevent and address racial harassment in 2020², collectively highlighted a need to critically appraise our course. The aim of the initiative was to determine whether we were serving our diverse student community with the appropriate content towards building an inclusive course curriculum and cocreating it with them.

The BSc in Medical Physiology and Therapeutics had its first intake over a decade ago and was closely aligned to widening participation initiatives locally. Since its inception, the course has attracted a significant number of Black and minority ethnic students (BME). During the academic years spanning 2017-22, the BME student course cohort comprised over 50% of the total with BME women being the single largest category (30.7 - 40.9%). Building on an EDI induction workshop introduced in academic year 2020-21, a call to join a student-staff BME review group attracted 5 students to support curricular changes to ensure all felt valued and represented in our curriculum.

The student volunteer group, supported by three academic members was convened early 2021. The group met at regular intervals and were tasked to capture, report and advise where gaps in the curriculum (specific modules and subjects) were identified with consideration of BME perspectives. In addition, the group were asked to highlight areas they felt helped to see themselves reflected in the curriculum. Having our student voices and input was pivotal in order to shape our curriculum and address any inequality, considering the overall aim to identify potential areas for change and ensure a more inclusive, equitable experience for all.

A total of 14 modules over the three year programme were reviewed in terms of content in the initial year which has been pivotal to ongoing curricular changes and developments. From our founding student working group, in excess of 20 topics were identified as being of relevance to our BME students, many being beyond skin colour alone. In addition to proactive action and identifying key aspects that need addressing, opinions of how a more inclusive approach to ethnicity could be incorporated into subjects for the future were gained, and representative aspects of our assessment practice were also highlighted. Positive comments received suggested our students felt well represented in the course from initial intake to the programme.

The original working group has ignited our journey towards equality, diversity and inclusivity in our curriculum review and staff-student partnership in working continues. This initial work showcases the early findings of our original students on the BME working group spanning diverse areas of our course.

1) Advance HE (2022) Students' statistical report <https://www.advance-he.ac.uk/knowledge-hub/equality-higher-education-statistical-reports-2022> 2) A charter for medical schools to

prevent and address racial harassment (British Medical Association, 2020)
<https://www.bma.org.uk/advice-and-support/equality-and-diversity-guidance/race-equality-in-medicine/racial-harassment-charter-for-medical-schools>

PCA031

Video/animation as an authentic assessment in physiology and neuroscience teaching – a comparison between first- and second-year students.

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Being able to successfully communicate complex scientific concepts is an important part of learning at higher education level. Poster talks, oral presentations and elevator pitches are well established methods of assessing communication skills. Another method adapted in first year Physiology and Anatomy and second year Neuroanatomy modules was an outreach video/animation group assessments aimed at GCSC and lay patients, respectively.

Here we compare the results when two different cohorts of students in the same UK higher education institutions produced their own video/animation to communicate physiology-related knowledge to different target groups. Our aim was to establish if this assessment strategy could be successfully used with different student groups and instructors and compare student attainment during such assignments. We also report the reflections of lecturers who assessed these video/animation assessments. A comprehensive marking rubric was created based on Peeters *et al.* (2010) and adapted to different modules.

First year Anatomy and Physiology students (n=299) were assigned into 72 groups of 2-6 students. They had ~12 teaching weeks to complete the assignment. Second year Neuroanatomy students were allocated into 7 groups of 6-10 students. These students had 4 teaching weeks to complete the task. Overall, average grades were in the first class range, with first year students achieving ~78% while second year students got on average ~74%.

Both module managers reflected that the students demonstrated creativity, innovation, originality, were able to distil complex concepts into more accessible forms and pitch them at the right level. Adaptation of comprehensive rubric significantly decreased marking time and provided broad feedback to students. The assessment was stimulating and helped students develop their ability to work as a team member. An assessment as such brings out the creativity in students and is easily adjustable to other subjects.

These projects could be undertaken whether a student was studying face-to-face, online or in hybrid mode. Topic choice could make it harder to link the assessment explicitly with intended learning outcomes, and topic complexity could influence the effectiveness of the infographic produced.

We conclude that video/animation assessments are an effective way to encourage group work, ownership, creativity, and investigation by students, which is irrespective of academic stage.

Peeters, M.J., Sahloff, E.G., Stone, G.E., 2010. A Standardized Rubric to Evaluate Student Presentations. *Am J Pharm Educ* 74, 171. <https://doi.org/10.5688/aj7409171>

PCA032

TGR5, GLP-1, and GIP expression in diabetic Wistar rats in response to *Ficus exasperata* Vahl leaf extract

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Introduction

Ficus exasperata has been reported to have several therapeutic potentials. However, there is paucity of reports on its effects on mRNA expression of genes associated with glycemic control in diabetic Wistar rats.

Aim/Objective

This study was designed to investigate the effects of methanol extract of *Ficus exasperata* (MEFE) on mRNA expression of Takeda G-protein coupled receptor 5 (TGR5), Glucagon-like peptide-1 (GLP-1), Glucose dependent insulinotropic polypeptide (GIP) and other glycemic indices in diabetic male Wistar rats.

Methods

Fresh leaves of *Ficus exasperata* were gotten from Ondo State and authenticated at the Department of Botany, University of Ibadan, Nigeria with voucher specimen number (UIH: 240407). It was extracted using methanol and characterized using GCMS analysis. Twenty (20) rats were divided into four (4) groups (n=5) as follows: Group I (Diabetic Untreated), Group II (Normal control), Group III (diabetes + 200 mg/kg MEFE), and Group IV (diabetes + 0.3 units Insulin). Diabetes was induced via single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Treatments were administered daily for 28 days. Thereafter, the rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) for blood collection and tissue harvesting (Beeton, 2007). The blood glucose and insulin concentration were assessed by glucose oxidase and ELISA method while superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and total protein were evaluated using commercially available randox kits. GLP-1, TGR5 and GIP expression were quantified using quantitative polymerase chain reaction (qPCR). *In-silico* docking of MEFE compounds to P2Y1 receptor was done using Cavity-detection guided blind-docking software. Histology of the pancreatic islets were examined using hematoxylin and eosin stains. Data were analyzed using graphpad prism, p<0.05 was statistically significant. All procedures involving the use of animals were performed according to ethics and guidelines for animal care and use for research by University of Medical Sciences Ethical Review Committee and also in compliance with the guidelines provided by the Medical Association Declaration of Helsinki on ethical principles for medical research involving experimental animals [World Medical Association, 2013].

Results

GC-MS result revealed the presence of 23 constituents with kaur-16-ene having the highest binding affinity (-8.2 Kcal) against P2Y1 receptor on islet cells. There was a significant decrease in blood glucose in diabetes+ MEFE (200 mg/kg) compared to DU. However, there was a significant increase in insulin, TGR5, GLP-1, and GIP expression, SOD, catalase and GPx in diabetes+ MEFE (200 mg/kg) compared to DU. The diabetic group + MEFE (200 mg/kg) showed regenerated islet cells compared to DU.

Conclusion

In diabetic rats, the observed results of MEFE suggest its antioxidative and stimulatory effects on genes that effectively controlled blood glucose levels.

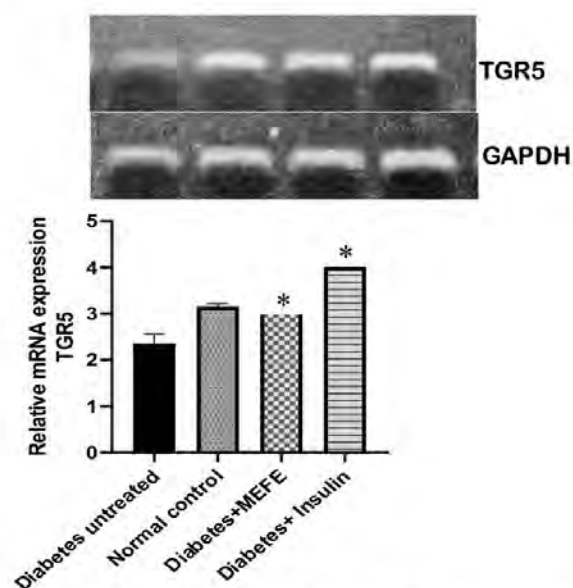


Figure 5: Effects of methanol extract of *Ficus exasperata* on mRNA expression of TGR5 in normal and diabetic treated rats. Result were expressed as mean±SEM n=5, p<0.05 *indicate value significantly different from diabetic untreated.

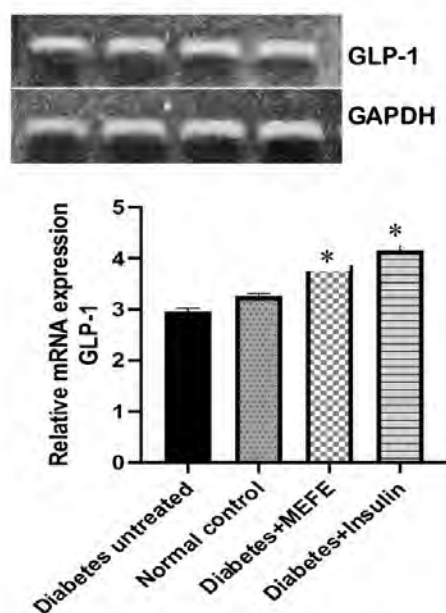


Table 5. Shows the physiochemical and pharmacokinetics of kau-16-ene

Physiochemical properties and pharmacokinetics of kaur-16-ene	
Canonical SMILES	<chem>C=C1CC23CC1CCC3C1(C(CC2)C(C)(C)CCC1)C</chem>
Formula	C ₂₀ H ₃₂
Molecular Weight	272.47 g/mol
Gastrointestinal Absorption	Low
Blood Brain Barrier permeability	No
Pgp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Water solubility	Moderately soluble
Bioavailability score	0.55

Table 6. Effects of methanol extract of *Ficus exasperata* on biochemical indices in normal and diabetic treated rats.

Groups	SOD (U/mL)	Glutathione (U/L)	Catalase (U/mg)	Insulin Conc. (μIU/mL)	Total Protein (g/dL)
I	1.50 ± 0.06	4.51 ± 0.44	1.52 ± 0.14	1.01 ± 0.13	3.12 ± 0.12
II	2.88 ± 0.08	8.32 ± 0.41	2.88 ± 0.25	2.80 ± 0.05	4.00 ± 0.53
III	2.80 ± 0.05*	8.26 ± 0.35*	2.63 ± 0.42*	1.90 ± 0.03*	2.31 ± 0.67
IV	2.92 ± 0.06*	9.34 ± 0.55*	0.86 ± 0.22	2.10 ± 0.15*	4.3 ± 0.56

Result were expressed as mean ± SEM, p < 0.05 * indicate value significantly different from diabetic untreated. SOD (Superoxide dismutase), GPx (Glutathione peroxidase), CAT (Catalase)

RESULTS



Figure 1. *Ficus exasperata* Vahl leaves (White Fig)

Table 1. Shows the primer sequence of target genes

Gene	Primer sequence (5' → 3')	
glucagon-like peptide-1 (GLP-1)	F	TCCCAAAGGAGCTCCACCTG
	R	TTCTCCTCCGTGTCTTGAGGG
Takeda G protein- coupled receptor 5 (TGR5)	F	TGTCACACAACCACTGAG
	R	CAAGCAGGGAGAGGAAACAA
Glucose-dependent insulintropic polypeptide (GIP)	F	TAAGAAGAGCTGTTGGCTCGG
	R	GTCCCCTCTGCATACCTTGG
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F	GCAAGGATACTGAGAGCAAGAG
	R	CATCTCCCTCACAATTCCATCC

Table 2. Effects of methanol extract of *Ficus exasperata* on body weight in normal and diabetic rats.

Groups	IBW (g)	FBW (g)	Weight difference (g)
I	138.00 ± 3.21	117.00 ± 2.90*	21.00
II	126.20 ± 4.40	158.0 ± 6.70	31.80
III	129.00 ± 3.53	129.90 ± 3.00	0.90
IV	141.20±4.15	194.30±7.20*	53.10

Result were expressed as mean±SEM n=5, p<0.05 *indicate value significantly different from initial body weights. IBW (Initial body weight), FBW (Final body weight).

Table 4. shows the binding affinity and percentage concentration of bioconstituents in methanol extract of *Ficus exasperata*

S/N	RT (min)	Compound	Binding affinity (kcal/mol)	Percentage concentration
1	11.550	Cycloisolongifolene, 8,9-dehydro-	-6.9	1.790
2	12.209	Eudesma-4(15),7-dien-1.beta -ol	-7.6	3.878
3	13.714	Neophytadiene	-7.2	1.433
4	14.143	9-Eicosene, (E)	-7.2	1.359
5	14.604	Pentadecanoic acid, 14-methyl-	-6.4	10.080
6	15.009	n-Hexadecanoic acid	-6.2	14.315
7	15.266	Hexadecanoic acid, ethyl ester	-6.7	4.355
8	15.598	7-Tetradecyne	-6.5	2.840
9	15.857	Kaur-16-ene	-8.2	3.335
10	16.217	10,13-Octadecadienoic acid, methyl	-7.6	2.453
11	16.272	9-Octadecenoic acid (Z)-	-7.2	5.799
12	16.327	11-Octadecenoic acid, methyl ester	-6.8	1.621
13	16.382	Oxirane, decyl-	-6.1	1.486
14	16.504	Methyl stearate	-6.7	8.154
15	16.597	Linoelaidic acid	-7.3	3.279
16	16.650	cis-Vaccenic acid	-6.8	8.214
17	16.831	Linoleic acid ethyl ester	-7.0	7.281
18	16.880	Ethyl Oleate	-7.1	6.794
19	17.100	Octadecanoic acid, ethyl ester	-6.4	0.448
20	17.633	Alpha-Farnesene	-7.2	1.597
21	17.872	Hexane, 1-chloro-5-methyl-	-5.0	4.926
22	18.689	Caryophyllene oxide	-7.2	1.855
23	19.217	Bicyclo[5.1.0]octane, 8-methylene-	-6.2	2.709



Fig. 3 (A,B&C). Shows the structural formulae, ADMET properties and *in silico* docking analysis of kaur-16-ene respectively

Table 3. Effects of methanol extract of *Ficus exasperata* on blood glucose concentration after diabetes induction.

Groups	IBG (g)	FBG (mg/dL)	DBG (mg/dL)
I	211.30 ± 4.10	248.50 ± 7.90	37.20
II	73.30 ± 2.90	68.50 ± 2.40	4.80
III	203.20 ± 2.50	120.30 ± 3.40*	82.90
IV	222.20±2.70	65.60±2.70*	156.60

Result were expressed as mean±SEM n=5, p<0.05 *indicate value significantly different from initial body glucose. IBG (Initial blood glucose), FBG (Final blood glucose).

Abundance

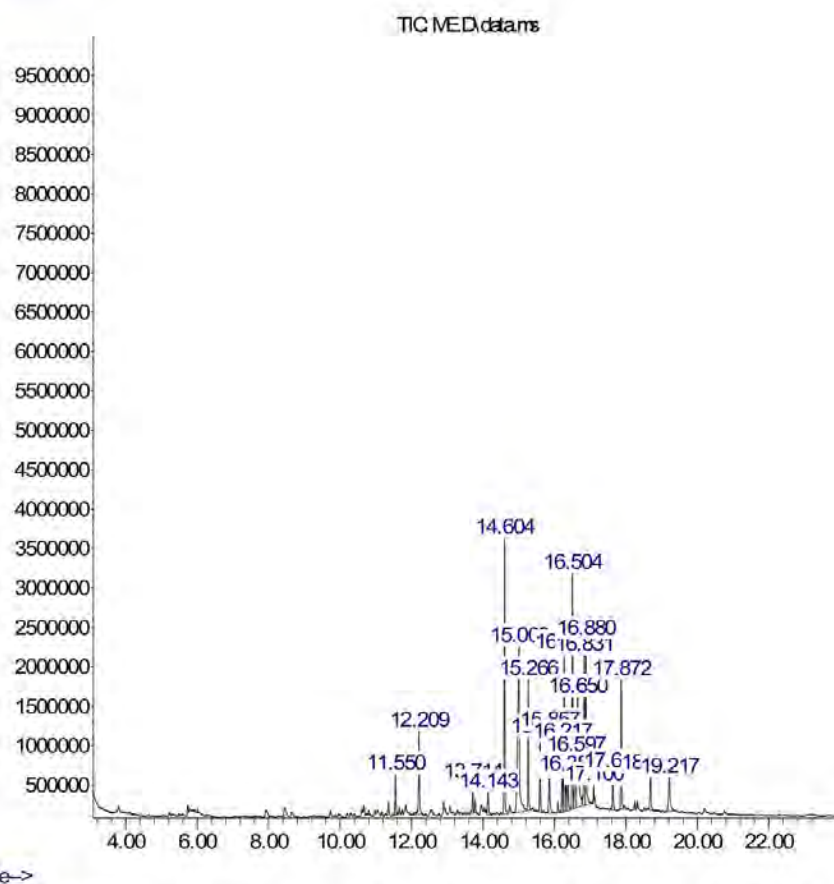


Figure 2. Chromatogram showing the peak of compounds present in methanol extract of *Ficus exasperata*.

Beeton, C., Garcia, A., & Chandy, K. G. (2007). Drawing blood from rats through the saphenous vein and by cardiac puncture. *Journal of visualized experiments : JoVE*, (7), 266. World Medical Association (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191–2194.

PCA033

Reduced maternal progesterone level increases apoptosis in rodent placenta

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Background: Progesterone is a vital hormone that maintains placental and fetal wellbeing during pregnancy. Progesterone has different effects in different tissues, where in some it induces proliferation, while in others it induces apoptosis. Since low levels of progesterone are correlated with smaller placentas and intrauterine growth restriction, we aim to evaluate apoptosis in different zones of the placenta in a model of progesterone withdrawal during pregnancy. **Methodology:** Sprague Dawley rats were used and divided into 3 groups (control, reduced-progesterone and restored-progesterone groups). On day 15 gestation (dg) ovariectomy was performed for reduced and restored progesterone groups, and a subcutaneous mini-pump was implanted to release estradiol (40 ng/hr). Estradiol was also injected twice daily on 17 and 18 dg (s.c. 250 ng in 0.2 ml peanut oil) and on 19 and 20 dg (500 ng in 0.2 ml peanut oil). The reduced progesterone group received approximately one third of the normal progesterone level detected at 22 dg. Progesterone was administered once at 15 dg and twice from 16 until 20 dg (s.c. 0.5 mg in 0.2 ml peanut oil). In the restored progesterone group, progesterone was administered once at 15 dg and twice at 16 dg (s.c. 10 mg in 0.2 ml peanut oil), twice at 17 dg (s.c. 7.5 mg in 0.2 ml peanut oil) and twice at 19-20 dg (s.c. 5 mg in 0.2 ml peanut oil). The control group received the same number of peanut oil injections as the experimental groups. Fetal and placental weights, maternal progesterone levels and gene and protein expressions of placental apoptotic (p53, Bax) and anti-apoptotic (Bcl2) markers were measure at 16, 19 and 21 dg. **Results:** The reduced-progesterone group showed reduction in basal zone and placental weights at 19 dg (18% and 16 %, respectively). Placental efficiency was also reduced by 16% in the reduced-progesterone group at 21 dg. The expression of the apoptotic markers p53 and Bax were significantly increased in both labyrinth and basal zones of the placenta. The anti-apoptotic marker, Bcl2, also showed a significant increase in both placental zones. **Conclusion:** Progesterone is essential for maintaining placental growth and significant reduction in its level results in smaller placentas with increased expression of apoptotic markers leading to pregnancy complications. But you have increases in apoptotic and antiapoptotic markers

	0 dg	15 dg	16 dg	17 dg	18 dg	19 dg	20 dg	21 dg
Reduced and Restored progesterone groups	Detection of sperm in vaginal smear	Ovariectomy Implantation of subcutaneous mini pump with estradiol (40ng/hr)		2X daily s.c injections of estradiol (250 ng/0.2 ml) peanut oil		2X daily s.c injections of estradiol (500 ng/0.2 ml) peanut oil		

	0 dg	15 dg	16 dg	17 dg	18 dg	19 dg	20 dg	21 dg
Progesterone-Reduced Group	1X daily s.c injections of Progesterone (0.5 mg/0.2 ml) peanut oil	1X daily s.c injections of Progesterone (0.5 mg/0.2 ml) peanut oil						
Progesterone-Restored Group	1X daily s.c injections of Progesterone (10 mg/0.2 ml) peanut oil		2X daily s.c injections of Progesterone (10 mg/0.2 ml) peanut oil	2X daily s.c injections of Progesterone (7.5 mg/0.2 ml) peanut oil		2X daily s.c injections of Progesterone (5mg/0.2 ml) peanut oil		
Control	1X daily s.c of 0.2 ml peanut oil	2X daily s.c of 0.2 ml peanut oil						
			Sample Collection			Sample Collection		Sample Collection

Mark, P. J., Smith, J. T., & Waddell, B. J. (2006). Placental and fetal growth retardation following partial progesterone withdrawal in rat pregnancy. *Placenta*, 27(2-3), 208-214.
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<https://doi.org/10.1093/nar/gkt32>

PCA034

Effects of Incubating Temperature Manipulation on Sex Determination in Korat Chickens

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Sex determination in chickens has been the subject of investigation. The goal is to comprehend the processes involved in sex determination and the elements that influence how sex is determined. It was recently reported for the first time that temperature affects sex determination in Australian brush turkey (*Alectura lathami*) (Göth & Booth, 2005). Investigation in quail (Yoshida et al., 1996) and broiler chickens (Collins, 2013; Elmehdawi, 2013) were also reported, but not in Korat chickens. This research aimed to manipulate incubating temperature to determine the male sex in Korat chickens (*Gallus domesticus*). The effect of increasing/decreasing the temperature from the standard temperature throughout the entire range toward gender determination was studied. The eggs used in this research were Korat chicken eggs. Eggs were incubated at 36°C, 37.7°C, and 38°C (100 eggs per experiment, a total of 300 eggs, randomly assigned). The incubation stages were examined by measuring the degree of the translucence of the eggshell. The incubator temperature was controlled at 36°C, 37.7°C, and 38°C, with the humidity, maintained at 55% throughout incubation. Other factors like ventilation were also regulated to prevent confounding effects. Only eggs developed during the first week (7 days) were used for statistical analysis. Sex was examined twice between days 1 and 21. The first was to investigate the newborn's vent sexing (Tran et al., 2010), and the second by observing sexual dimorphism. Blood was collected from day-old hatched chicks, then extracted DNA using a kit and tested for W chromosome by Polymerase Chain Reaction (PCR) method to confirm sex chromosomes. Testes were randomly collected from 5-week-old chickens that had changed sex. Histology was examined by hematoxylin and eosin staining and then studied by microscopy. The procedures of the experiments were approved in accordance with the advice of the Institutional Animal Care and Use Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand. The increase in temperature over the period (38.0°C) had a higher percentage of males than females (52.5% versus 47.5%), according to the research findings. $P > 0.05$ indicates that there was no statistically significant difference. It was discovered that hatched male chicks with high temperatures across the range (38.0°C), as confirmed by the presence of the testicles by histological examination, W chromosomes (female chromosomes), were detected, accounting for 9.7%, when blood from day-old hatched chicks was taken for DNA extraction and W chromosome identification using PCR method. Thus, the incubation temperature, especially the high temperature throughout the period (38.0°C), can change the sex of the chicks from female to male. It is the first scientifically proven in broilers that incubating temperature manipulation can result in sex change, and the hatching rate is not different from regular hatching. Therefore, controlling the temperature during incubation to determine the sex of Korat chickens to males can generate profits for farmers by setting the temperature at the incubator so that the eggs hatch more males than females. It is also a non-invasive method that does not negatively affect consumers; farmers can do it themselves.

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PCA035

Systematic discovery of solute carrier (SLC)-lipid interactions using *in silico* methods

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Background and Aims: Solute carrier (SLC) proteins are secondary, tertiary, and facilitative transporters making up a significant portion of cellular membrane proteins responsible for transmembrane transport of solutes [1]. Due to their pivotal roles in controlling cellular homeostasis, SLCs have been linked to a large variety of diseases and are increasingly seen as having a yet unexploited therapeutic potential [2]. Despite their membrane localization, little is known about how lipid bilayer components interact with SLCs. Nevertheless, sporadic reports suggest that lipids are able to modulate the function of SLC transporters through direct interaction, either acting as inhibitory agents or as stabilizers that can potentiate transporter function. Reports of the development of bioactive lipid inhibitors can potentially open new therapeutic options [3]. However, a systematic study of lipid-binding sites in SLCs is still lacking. Here, we aim to discover novel SLC-lipid interactions using a combination of large-scale *in silico* tools and *in vitro* validation methods.

Method: We employ *in silico* coarse-grain (CG) molecular dynamics (MD) simulations to screen a large number of transporters for specific lipid-binding sites. Human protein structures based on AlphaFold predictions are immersed in three different model membranes: (1) an asymmetric native-like lipid mixture representing the 63 major constituents of mammalian plasma membranes; generic palmitoyl oleoyl phosphatidylcholine (POPC) bilayers containing either of the signaling lipids (2) sphingosine-1-phosphate (S1P) or (3) N-arachidonoylglycine (NAGly). After extensive MD simulations of 10+ μ s involving multiple replicas using the MARTINI CG forcefield [4], statistics on interactions between protein amino acid residues and various lipids, as well as on the spatial localization of lipid species are collected.

Results: Our methods have been applied to all proteins in the human SLC6 (neurotransmitter and amino acid transporter) and SLC39 (zinc/iron/manganese transporter) families, encompassing 33 proteins in total. Analyzing the binding patterns for cholesterol, a lipid species important for membrane protein stability, we have been able to reproduce cholesterol-binding sites previously observed for SLC6A3 and SLC6A4 transporters [5]. SLC38 transporters also show a conserved pattern of cholesterol binding, indicating that cholesterol might play an important role in the stability of these proteins. In addition, novel phosphatidyl inositol 4,5-phosphate (PIP2) binding sites, as well as non-conserved interaction patterns for NAGly and S1P have also been identified in several proteins.

Conclusion: Our systematic approach has uncovered previously unknown, potential transporter-lipid interactions that can be important in either regulating transporter stability and function, or in the involvement in lipid signaling networks. Currently, follow-up studies are planned using all-atom MD simulations to describe the identified transporter-lipid interactions in more detail. Experimental validation using functional assays or mass spectrometric analysis can be used to support the proposed interactions. Our results are planned to be expanded to other

SLC families and made publicly available for the research community, and can potentially aid the development of specific bioactive lipid inhibitors in the future.

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PCA036

In-silico Electrophysiological Analysis Reveals Hyperglycemia Enhances Detrusor Smooth Muscle Excitability

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Introduction and Objectives:

Detrusor overactivity (DO) is characterized by enhanced spontaneous contraction and action potential (AP) generation of the detrusor smooth muscle (DSM) cells [Mahapatra et al., 2018]. Although diabetes mellitus (DM) has been shown to alter DSM function in several ways, the exact mechanism is not yet understood. This in silico electrophysiological assessment study aims to explore the modulating effects of hyperglycaemia on DSM cell excitability.

Methods:

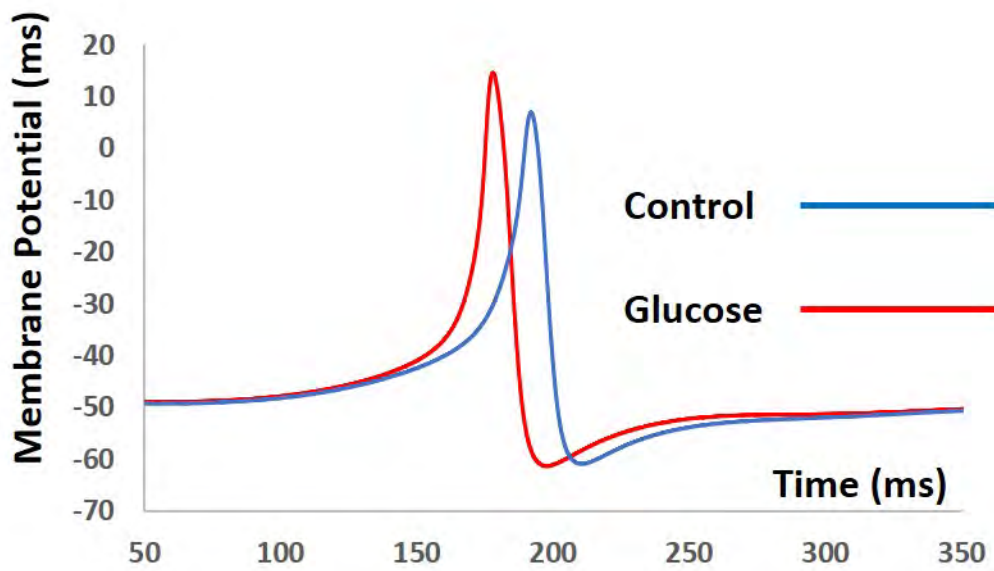
The in silico electrophysiological assessment is done with a single isolated DSM cell comprising two voltage-gated calcium channels, two voltage-gated potassium channels, three calcium-dependent potassium channels, one inward rectifier potassium channel, and one ATP-dependent one potassium channel (KATP), one leakage current across the cell membrane. The glucose of 0 to 10 micromolar (μM) is intracellularly induced, and the modulated KATP currents and APs are recorded with respect to both current and voltage clamp protocols.

Results:

The KATP currents are recorded from the DSM cell under the voltage clamp protocol. The holding and test potential are set at -80 mV and 60 mV with a step potential of 10 mV . It is shown that the KATP outward current is reduced by 50% after introducing a glucose concentration of $10\text{ }\mu\text{M}$. The total whole cell outward current is also reduced because of the elevated intracellular glucose concentration. Then, we implemented the current clamp ($0.001\text{ }\mu\text{A}$ amplitude, 10 ms duration) to evoke the AP with respect to control and hyperglycaemia (higher glucose) conditions (Figure 1). The RMP, AP peak, and AP duration are altered when the cell is exposed to a glucose concentration of $10\text{ }\mu\text{M}$. The RMP is shifted to a more positive potential by 1 mV , the AP peak is increased by 5 mV , and the AP duration is reduced by 5 ms .

Conclusions:

The involvement of active ion channels has been postulated for modulating the intracellular electrical activities in the DSM cell with DM. This study has shown that the DSM cell excitability is increased because of reduced whole cell outward current by the inactivation of KATP ion channels. The agonists of KATP ion channel could be considered the new pharmacological agents for DO with DM. In the future, the involvement of other ion channels can also be investigated to explore more electrophysiological evidence for DO under hyperglycaemia.



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PCA037

**Investigating lysosomal membrane proteins using SSM-based electrophysiology:
Improving amplification and accessibility**

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Introduction

Solid supported membrane-based electrophysiology (SSM-E) offers novel approaches for electrophysiological recordings from lysosomal membrane proteins such as transporters, ligand-gated and leak ion channels in their native membrane environment.

Method

The method of SSM-E relies on the adsorption of any membrane, native, cell culture-derived or organellar, to a lipid coated electrode, i.e the solid supported membrane, and the direct current read-out caused by the capacitive charging of the membranes. The 3 mm diameter electrode entails a >1000-fold amplification of the currents compared with conventional patch clamp, allowing for the measurements of low-conducting membrane proteins, such as transporters. The fact that also intracellular membranes can be accessed by SSM-E, their accessibility for investigation and characterization drastically improves.

Results & Conclusions

Here, we present a study on TMEM175 channels residing in lysosomes using SSM-E. We found an average permeability ratio between protons and potassium of $P_H/P_K = 48.500$ and similar conductivities for K^+ , Rb^+ , and Cs^+ . We also found that TMEM175 activity is downregulated to 30% of I_{max} upon cytosolic acidification with a $pK=7.0$, while TMEM175 is resistant to lysosomal acidification. We also investigated dose-dependent effects on TMEM175 ($n=8$ sensors) exerted by blockers, i.e. Zn^{2+} ($IC_{50} = 1.5 \pm 0.2$ mM) and 4-AP ($IC_{50} = 1.7 \pm 0.3$ mM), and enhancers, i.e. DCPIB ($EC_{50} = 10 \pm 5$ μ M; $E_{max} = 275 \pm 37$ %) and arachidonic acid ($EC_{50} = 2 \pm 0.4$ μ M; $E_{max} = 168 \pm 5$ %). As expected, the enhancer SC79 which acts via PKA has only little effects on TMEM175 activity ($E_{max} = 109 \pm 5$ %) in our in vitro assay.

PCA038

Age-dependent cellular changes in response to SARS-CoV-2 in nasal epithelial cells

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BACKGROUND: SARS-CoV-2 is the virus responsible for the ongoing COVID-19 pandemic. Although this virus affects people of all ages, studies have shown that the elderly are at a higher risk of severe disease and death from COVID-19 compared to children, who once infected with SARS-CoV-2 rarely progress to respiratory failure. We aimed to investigate this by studying how the cells lining the nose respond to SARS-CoV-2 infection in people of different ages.

METHODS: To do this, we cultured differentiated primary nasal epithelial cells (NECs) at air-liquid interface from three different age groups: paediatric (<14 years, n=11), adult (30-50 years, n=9), and elderly (>70 years, n=9) individuals. Ethical approval was given through the Living Airway Biobank (REC reference: 19/NW/0171). We then used a comprehensive, multidisciplinary approach using functional assays and scRNAseq to analyse the cellular landscape of the infected cultures and examined the replication of the virus within the different cell subtypes.

Study Population			Total cultures analysed (n)	Total cells for scRNAseq
Total n	29		251	
% Female	41%		38%	
Brushings	n	Age (mean ±SD)	n	
Paediatric (0-11y)	14	4.9 ±4.2	118	32,892
Adult (30-50y)	9	36.9 ±2.7	65	56,221
Elderly (70y+)	9	83.6 ±6.7	68	50,485
			Total cells	139,598

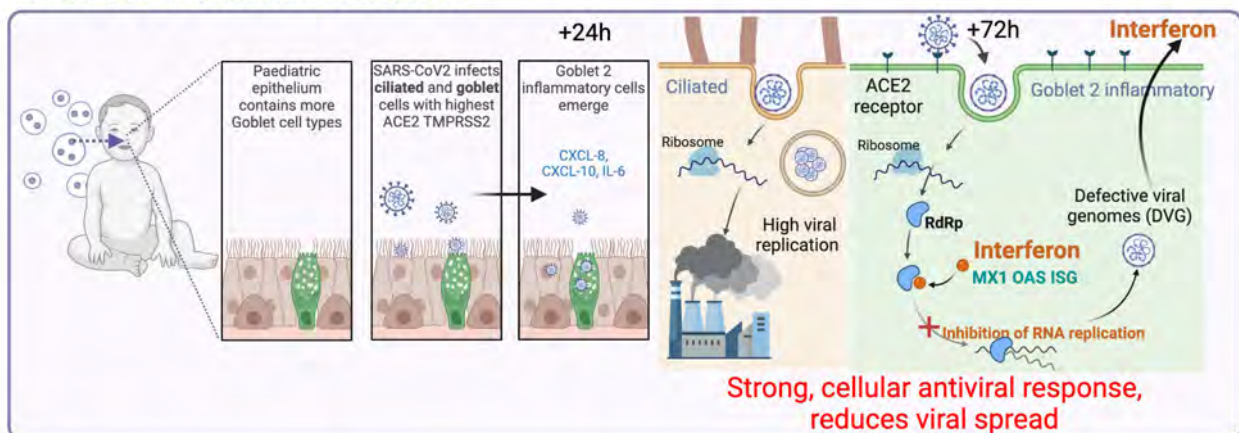
RESULTS: Our data revealed that nasal epithelial cell subtypes show different tropism to SARS-CoV-2, correlating with age and ACE2 and TMPRSS2 expression. For example, we found that ciliated cells are a viral replication centre across all age groups, but a distinct goblet inflammatory subtype emerges in infected paediatric cultures, identifiable by high expression of interferon-stimulated genes, truncated viral genomes, greater sub-genomic viral RNA, and less infectious progeny compared to older adult cultures. On the other hand, SARS-CoV-2 infected elderly secretory cells were shed, and cultures suffered greater epithelial damage with age. Dysfunctional repair pathways were stimulated, and there was an increase in basaloid-like cells

that are associated with fibrosis markers and greater viral spread. We hypothesized that SARS-CoV-2 infected nasal epithelial cells undergo reprogramming by these mechanisms in an age-dependent manner and that these processes contribute to COVID-19 pathogenesis by delaying disease resolution and enhancing viral spread.

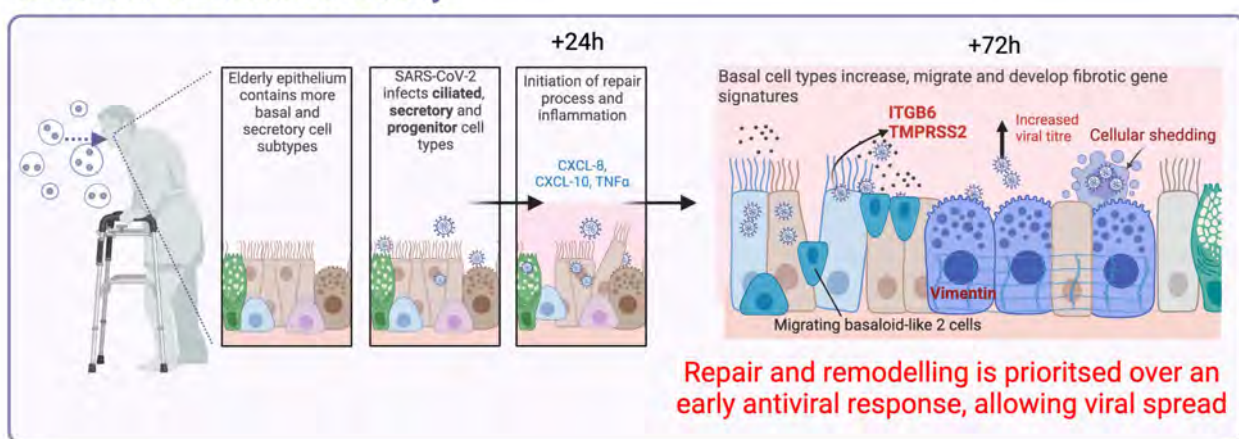
CONCLUSIONS: Our study provides new insights into age-associated COVID-19 pathogenesis. We found that SARS-CoV-2 exhibits differential tropism for nasal epithelial cells with age, with preferential infection of paediatric goblet or elderly secretory cell types. Infected paediatric goblet cells mount a robust innate antiviral response to SARS-CoV-2 dominated by interferon, which correlates with reduction in infectious viral load. In the elderly dysfunctional repair pathways are stimulated, and there is an increase in basaloid-like cells that are associated with fibrosis markers and greater viral spread. These insights could aid in the development of new treatments for COVID-19, particularly for older individuals who are at greater risk of severe infection.

This work is currently published as a preprint: <https://www.biorxiv.org/content/10.1101/2023.01.16.524211v2.full>

SARS-CoV-2 infection of children



SARS-CoV-2 infection of elderly



PCA039

Peripheral chemoreceptor sensitivity is elevated in patients with long COVID

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Post-COVID-19 syndrome (long COVID) describes ongoing symptoms for 12 or more weeks following infection with SARS-CoV-2. Respiratory-related symptoms including breathlessness [1], exercise intolerance and ventilatory dysfunction [2] are common in long COVID. Further, cells at the primary site of oxygen sensing (carotid bodies) are susceptible to SARS-CoV-2 infection [3]. We aimed to determine whether long COVID patients have altered carotid chemoreceptor sensitivity, by assessing resting hypoxic ventilatory response and ventilatory efficiency during exercise. Fourteen long COVID patients (3 male, 42 ± 12 years) and eight control participants (did not develop long COVID post-infection) gave written informed consent to participate in an ethically approved study (NHS REC). The control group was supplemented by six healthy individuals who undertook the same experimental procedures in the same laboratory, prior to the pandemic (in total; 2 male, 35 ± 13 years, $P=0.1195$ for age versus patient group). Participants did not have pre-existing respiratory or cardiovascular disease and resting lung function (spirometry) was similar between the groups. Transient hypoxia was achieved by supplementing inspired air with 100% N₂ for 5-8 short periods, lasting 5-30 seconds, reducing S_pO₂ to nadirs of between 65 and 99%. Breath-by-breath minute ventilation (V_E), tidal volume and breathing frequency, and beat-to-beat heart rate and blood pressure, were monitored during N₂ supplementation and for one minute afterwards. The 95th percentile of these variables and the nadir S_pO₂ were determined for each hypoxic period and entered into a simple linear regression, where the slope defined response to hypoxia (peripheral chemoreceptor sensitivity). The V_E response to hypoxia was greater in long COVID patients versus controls (-0.44 ± 0.23 versus -0.17 ± 0.13 L/min/S_pO₂, Figure; $P=0.0007$, independent samples T-test), demonstrating greater ventilation for a given fall in S_pO₂. This difference was caused by a larger tidal volume response, as breathing frequency response was similar. Heart rate and blood pressure responses were also unchanged (Figure). Participants performed a maximal exercise test (upright cycle ergometer, ramp protocol to volitional exhaustion). Long COVID patients had a lower peak VO₂ versus controls (18.6 ± 4.7 versus 26.7 ± 7.4 ml/kg/min, $P=0.0015$), despite reaching a similar peak V_E (66.4 ± 22.4 versus 71.5 ± 29.2 L/min, $P=0.6012$), respiratory exchange ratio (1.27 ± 0.09 versus 1.28 ± 0.11 , $P=0.7483$), and percentage of predicted heart rate (92 ± 9 versus 97 ± 6 %, $P=0.1326$) to the control group. V_E/VCO₂ slope (ventilation for a given expired CO₂ volume) was greater in the long COVID group versus controls (37.8 ± 4.4 versus 31.4 ± 4.8 , $P=0.0008$), indicating reduced ventilatory efficiency in the long COVID group. Furthermore, V_E/VCO₂ slope was correlated with hypoxic ventilatory response, such that enhanced chemoreflex sensitivity was associated with poorer ventilatory efficiency during exercise ($r=0.54$, $P=0.0037$, Pearson's correlation, $n=28$). These data demonstrate that long COVID is associated with high peripheral chemoreflex sensitivity

and reduced ventilatory efficiency during exercise. These changes could underlie the respiratory symptoms of long COVID such as dyspnoea and exercise intolerance. Targeting the carotid body to reduce its sensitivity may provide benefit to long COVID patients.

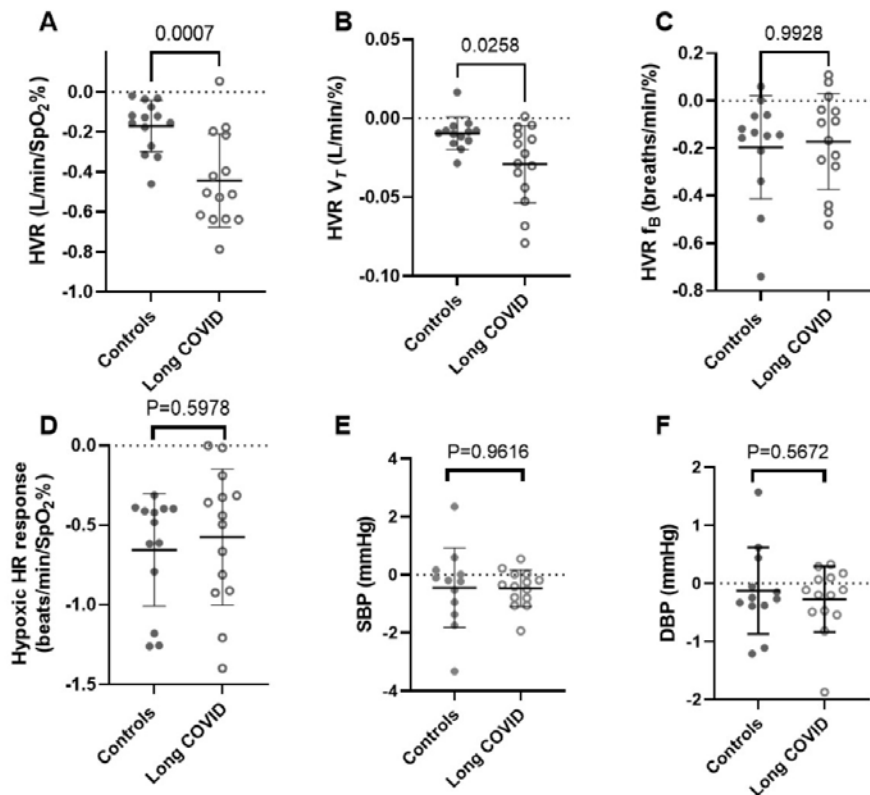


Figure. Hypoxic ventilatory and haemodynamic responses, assessed as (A) minute ventilation, (B) tidal volume (V_T), (C) breathing frequency (f_B), (D) heart rate (HR), (E) systolic blood pressure (SBP), and (F) diastolic blood pressure (DBP) response to hypoxia. HVR; hypoxic ventilatory response. Data are mean ± SD with group differences tested by independent samples T-test. N=14 long COVID and 14 control participants.

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PCA040

The influence of biological sex on oxygen uptake kinetics during moderate and heavy intensity exercise

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Rationale: The rate at which oxidative ATP provision can meet the energy demand of exercise is a determinant of contractile dysfunction [1]. Evidence suggests that females experience less contractile dysfunction than males for the same duration and intensity of exercise [2], however whether this is caused by bioenergetic differences or differences in the contractile properties of exercising muscle is unknown. Therefore, this study compared the pulmonary oxygen uptake ($\dot{V}O_2$) kinetics during moderate and heavy intensity exercise in males and females.

Methods: Sixteen healthy adults (8 of each sex, 27 ± 5 years) completed three experimental visits. First, participants completed a submaximal incremental exercise test (+25 W every 5 minutes) to identify lactate threshold (LT), then a maximal incremental exercise test (25 W.min⁻¹) to exhaustion to identify $\dot{V}O_{2peak}$ and power at $\dot{V}O_{2peak}$ (P_{max}). Visits two and three involved three six-minute cycling bouts at 80% of LT (moderate intensity), interspersed with six minutes of unloaded pedalling, and one 30-minute bout at a work rate 30% between LT and P_{max} (heavy intensity).

Data from the final two visits were filtered and linearly interpolated (1s intervals), then pooled to form a dataset of six moderate and two heavy intensity transitions. The first 20 s of each transition was removed. Thereafter, three minutes of pre-transition data and six (moderate) or two (heavy) minutes of post-transition data were fit with a mono-exponential curve to obtain the parameters of the phase II kinetics. The $\dot{V}O_2$ slow component was also quantified for the heavy intensity bouts.

Results: Absolute $\dot{V}O_{2peak}$ was greater in males (3.47 ± 0.58 vs 2.49 ± 0.44 L.min⁻¹, $p=0.002$), however relative values were not statistically different (46.2 ± 6.6 vs 40.5 ± 6.7 ml.kg⁻¹.min⁻¹, $p=0.111$). Males achieved greater power outputs at $\dot{V}O_{2peak}$ and LT ($p \leq 0.023$), meaning power outputs for subsequent bouts were 30% greater compared to females.

The primary amplitude of the moderate intensity transition was not different between male and females (24 ± 3 vs 24 ± 5 % $\dot{V}O_{2peak}$, $p=0.949$). The time constant was also not different (27.9 ± 7.5 vs 24.8 ± 6.6 s, $p=0.385$). Similarly, in the heavy intensity domain, neither the primary amplitude (43 ± 5 vs 38 ± 7 % $\dot{V}O_{2peak}$, $p=0.179$) or time constant (28.8 ± 7.9 vs 27.2 ± 7.1 s, $p=0.633$) were different. Likewise, the amplitude of the $\dot{V}O_2$ slow component was not different between sexes (12 ± 7 vs 11 ± 3 % $\dot{V}O_{2peak}$).

Conclusion: No sex differences were observed in the $\dot{V}O_2$ response to exercise in the moderate or heavy intensity domains, implying there was no sex difference in the bioenergetic stress experienced. Combined with evidence of no hormonal effect [3] on these parameters, this suggests females should not be excluded from studies of cardiopulmonary responses to

exercise. Deoxyhaemoglobin kinetics recorded via near infrared spectroscopy of the *vastus lateralis* will also be shared.

- [1] Goulding et al. (2021) *Exerc Sport Sci Rev* 49(4) [2] Ansdell et al. (2020) *J Physiol* 598(24)
[3] Mattu et al. (2020) *Scand J Med Sci Sport* 30(3)

PCA041

Role of concurrent endurance and strength training on disease expression of patients with hypertrophic cardiomyopathy

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INTRODUCTION: Recommendation on physical exercise in patients with hypertrophic cardiomyopathy (HCM) has evolved from a global stressor to a stimulus whose variables are controllable. Our group has recently reviewed and analysed the impact of medications, invasive procedures and also exercise in HCM, reaching the conclusion that physical exercise emerges as a coadjuvant therapy which is safe and associated with benefits on functional capacity^{1,2}. There are several clinical gaps on how to properly stimulate HCM patients with concurrent training to achieve the best dose-response, particularly regarding the optimal intensity or volume of the endurance component, and the type of exercises of the strength training.

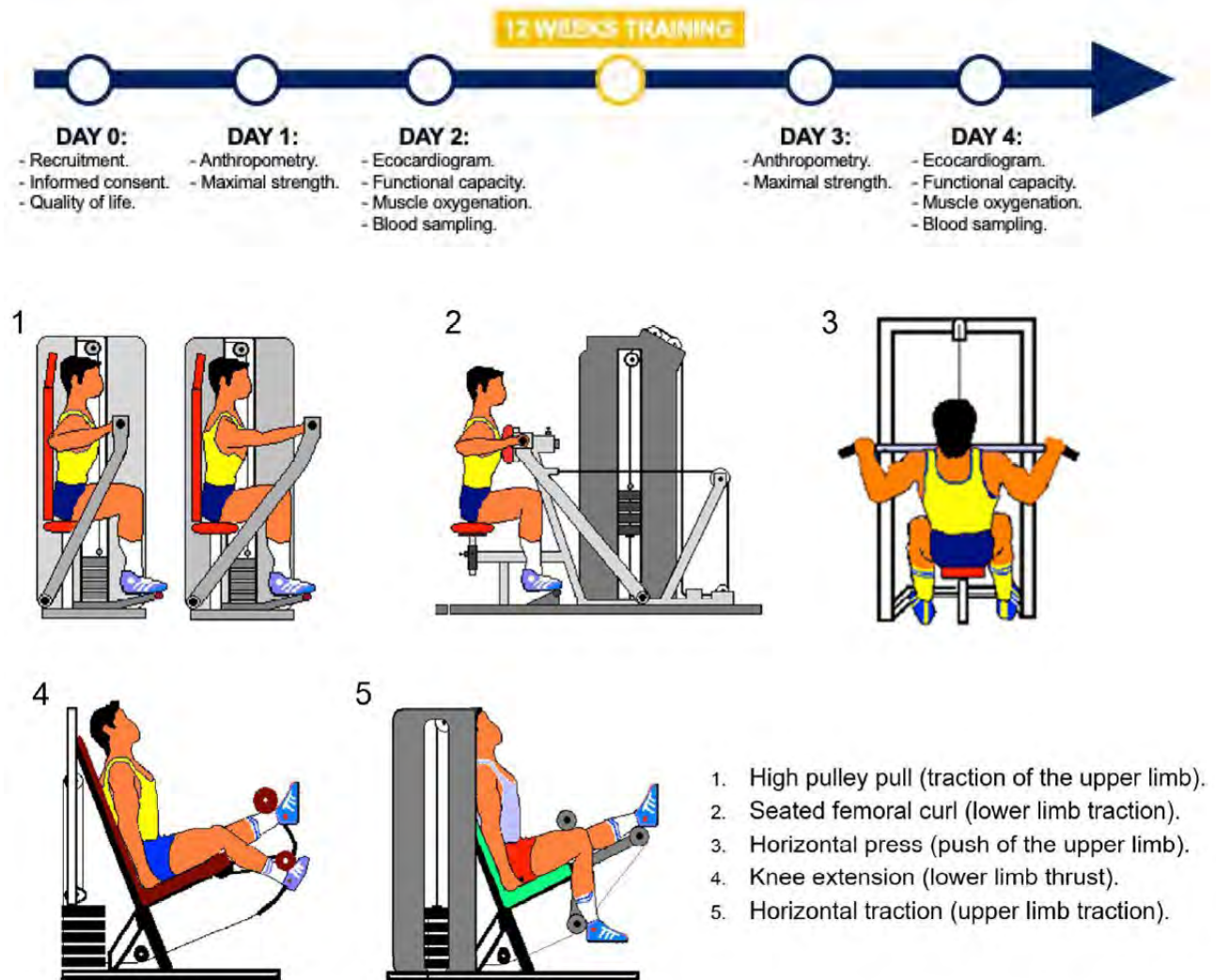
AIM AND OBJECTIVES: To examine the impact of a concurrent strength and endurance training on functional capacity by cardiopulmonary exercise test (CPET), serum biomarkers and clinical (ECG, advanced cardiac imaging) related variables in patients with HCM, and the safety training protocol.

METHODS: 40 adults HCM patients with obstructive and non-obstructive phenotype (1:1) and 20 healthy age and gender-matched controls will follow a 12-week training program supervised by sport scientists and cardiologists (Fig 1). All participants will be informed of the details of the aims and the protocol and should signed a dedicated consent form. The protocol has been approved by the local Ethical and Research Committee (2021-10-4-HCVA). All participants will undergo an initial and final assessment including anthropometry, quality of life survey, CPET, cardiac examination, ECG, echocardiogram, strength of the upper and lower body, muscle oxygenation, hemogram and blood tests including serum biomarkers analysis before and after the 12-week concurrent endurance and strenght-adapted (Fig 2) training protocol.

EXPECTED RESULTS: The training protocol proposed here will probably improve those previously proposed in terms of the selection and distribution of sessions and exercises, as well as the choice of intensity and adequate volume. In this sense, it is expected to achieve an increase in functional capacity substantially higher than those that have already been documented. Taking into account the results published by our group^{1,2}, an average improvement of 4.33 mL kg⁻¹ min⁻¹ is observed with the protocols used up to now, which represents an increase of 19.5% in functional capacity. Numerous studies establish a threshold value of functional capacity at 7 METs, from which functional HCM patients are considered functional. Therefore, an increase exceeding the threshold and reaching ~26.6 mL kg⁻¹·min⁻¹ of VO_{2max}. would make the average of trained HCM patients progress from "non-functionality" to the

functional condition. In addition, training is expected to increase the daily caloric expenditure of patients, thus promoting the achievement of a negative energy balance that facilitates weight loss and improve BMI, which have an independent impact on HCM³.

CONCLUSION: Therefore, in conclusion, it is expected that, as a result of training, patients will improve their symptomatic status and functional class by a safe protocol of training.



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PCA042

The exercise-mediated metabokine Beta-aminoisobutyric acid is an exercise mimetic driving skeletal muscle metabolic and functional adaptation

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Background: Skeletal muscle integrates many of the systemic signals, which contribute to the adaptive remodeling, and beneficial effects of exercise. One mechanism through which muscle mediates the systemic effects of exercise is through muscle-derived hormones known as myokines. We identified the metabolite β -aminoisobutyric acid (BAIBA) as an exercise-mediated small molecule myokine. BAIBA is secreted from muscle in response to increased expression of the transcriptional co-regulator PGC-1 α , a master regulator of the muscle adaptive response to exercise. However, how BAIBA functions to regulate the adaptive responses of skeletal muscle to exercise remains poorly understood.

Methods: 8-week-old male C57BL/6J mice (n=20) were randomly assigned to receive either a chow diet or a chow diet supplemented with 100mg/kg/day of β -aminoisobutyric acid (BAIBA) in their drinking water for 6 weeks. At the end of the study, the right soleus muscles were assessed *in situ* for force and fatigability; and for oxygen consumption using Oxygraph-2K high-resolution respirometers. The expression of genes associated with fibre-type was evaluated using RT-qPCR in fully differentiated primary human myotubes. The role of PPAR δ was investigated using siRNA techniques. Two way ANOVA was performed for statistical analysis. P-value nominal significance will be $p < 0.05$.

Results: We show that BAIBA improves muscle metabolism, exercise efficiency and performance in mice. Oxygen consumption (VO₂) and energy expenditure are increased in BAIBA-treated mice. Furthermore, BAIBA increased soleus *in situ* muscle contractile force ($p=0.0004$), fatigue resistance ($p=0.0063$), mitochondrial number and function. We found that BAIBA drives muscle fibre-type switching to an oxidative phenotype. BAIBA increased expression of PPAR δ ($p=0.0001$, $n=4$ /group), and genes determining muscle fibre-type, including Myosin Heavy Chain 7 (MYH7) (type I muscle) ($p=0.0001$ $n=4$ /group), Myosin Heavy Chain 2 (MYH2) (type IIA intermediate muscles) ($p=0.0046$, $n=4$ /group) in fully differentiated primary human myotubes. BAIBA regulates specific fibre-type gene expression in human myocytes through PPAR δ .

Conclusion: Our findings demonstrate that BAIBA is a key paracrine myokine, which, in part, regulates the effects of exercise to improve muscle function with resultant effects on exercise performance.

Keywords: BAIBA, skeletal muscle adaptation, exercise performance, PPAR δ , oxidative phenotype, fibre-type switching.

PCA043

The impact of the time of day on metabolic responses to resistance exercise in healthy adults: a randomised controlled trial

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Introduction:

Resistance exercise has many health benefits, including stimulation of glucose metabolism. However, the optimal time of day to perform resistance exercise is still unknown. The current study sought to determine the impact of the time of day on metabolic responses to resistance exercise in healthy adults in a randomised controlled trial.

Methods

We recruited 17 participants, inclusion criteria age between 18-45 years old with body mass index (BMI) >23.0kg/m². Exclusion criteria included having undergone surgery for weight loss, had prior history of heart, lung, cancer, kidney, endocrine, or liver disease. Participants were randomised into either a control, exercise in the morning (8:00-10am) or exercise in the evening (4:00-8:00pm) group. Those in the exercise group performed 8 resistance exercises (1 set to failure) 3 times over a one-week period, at their allocated time of day. Interstitial glucose responses were measured using a flash glucose monitoring (FGMs) for a one-week period prior to any exercise (habitual activity) and during the one-week exercise period with data compared between groups in time periods 6h post-exercise time and over 24h periods (both on exercise and non-exercise days), and over a 7-day period (exercise and non-exercise weeks).

Results

Participants were randomised to morning exercise n= 5; evening exercise; n= 6 or control group n=6. No time (exercise or no exercise days), group (control, morning or evening) or time*group interactions were seen for with mean glucose or glucose variability (measured as glucose standard deviation (SD)) in the 6h post-exercise period (all p>0.05). When comparing the 24h period no time (p=0.909), group (p=0.334) or time*group (p=0.911) interactions were seen for mean glucose. No time (p=0.537) or group (p=0.510) effects were seen for SD but a significant time*group interaction (p=0.008) was seen. Post-hoc tests revealed a lower (p=0.012) SD on exercise (0.65(0.05)) compared to no-exercise days (0.74(0.10)) within the evening group. No other differences were seen in post-hoc tests. Comparing data over a 7-day period no time, group or time*group (all p>0.05) interactions were seen for mean glucose and SD (all p>0.05).

Conclusion

The current data is part of an ongoing study, but our preliminary data indicates that there is no effect of the time of day mean glucose levels, although exercise may reduce measures of glucose variability.

PCA044

The effects of beta-lactoglobulin (BLG) versus whey protein isolate (WPI) on blood aminoacidemia and muscle protein synthesis in older males

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Background: Maximising the muscle anabolic response to dietary protein is crucial for muscle mass maintenance in older individuals, due to the presence of anabolic resistance contributing to sarcopenia (1). Within dietary protein, leucine is thought to be the most potent amino acid (AA) for stimulating muscle protein synthesis (MPS; 2, 3). Supplementing leucine-enriched essential AA and/or whey protein (WP) has previously been shown to be as anabolic as larger doses of protein (4), thus may negate the need for large protein meals, especially in clinical settings. Beta-lactoglobulin (BLG) is the most abundant WP in bovine milk and has a high content of branched chain amino acids (BCAA), especially leucine (5). We, therefore, investigated the impact of BLG *versus* WP isolate (WPI), at a suboptimal protein dose, on blood aminoacidemia and MPS in healthy older males.

Methods: In this double-blind cross-over trial, 10 healthy older males (69 ± 1 years, 181 ± 2 cm, 92 ± 5 kg) were randomised to receive either i) BLG (1.5 g leucine) or ii) WPI (1 g leucine), with supplements matched for protein content (10 g). A primed, constant intravenous infusion of [$1, 2^{13}\text{C}_2$] leucine was used to determine MPS at baseline and following feeding. Muscle biopsies were taken at 1.5 h after commencement of stable isotope tracer infusion (i.e., 0 h), immediately before a bolus feed (3 h; i.e., BLG or WPI) and 3 h post-feed (6 h). Serial arterialised blood samples were obtained to quantify plasma AA concentrations. Data are presented as mean $\pm$ SEM. MPS data were analysed using a two-way ANOVA (supplement $\times$ time) with a Šídák correction. Plasma AA data were analysed using a mixed-effects analysis (supplement $\times$ time) with a Šídák correction. All data analysis was performed using GraphPad Prism (GraphPad Software Inc, San Diego, CA). The alpha level of significance was set at $p < 0.05$.

Results: Blood plasma BCAA, leucine, isoleucine and valine concentrations significantly increased following feeding in response to BLG and WPI before returning to baseline, except plasma leucine which remained elevated in both groups. Peak plasma leucine concentrations were significantly greater following BLG (BLG: 422 ± 17 μM *versus* WPI: 364 ± 20 μM , $p = 0.0013$). Conversely, peak isoleucine concentrations were significantly greater following WPI (269 ± 34 μM *versus* BLG: 165 ± 14 μM , $p < 0.0001$). Myofibrillar MPS increased significantly following feeding (BLG; fasted: 0.048 ± 0.006 %/h, fed: 0.101 ± 0.012 %/h, $p < 0.0001$ *versus* WPI; fasted: 0.042 ± 0.006 %/h, fed: 0.075 ± 0.006 %/h, $p = 0.0032$), with a strong trend to BLG stimulating greater MPS than WPI post-feed ($p = 0.052$).

Conclusion: BLG exhibited greater peak leucinaemia compared to WPI, with both BLG and WPI significantly stimulating MPS following feeding. There was a strong trend to BLG stimulating greater MPS compared to WPI which may be physiologically significant, especially in larger trials. We conclude BLG containing a suboptimal dose of protein leads to an anabolic response to feeding in healthy older men.

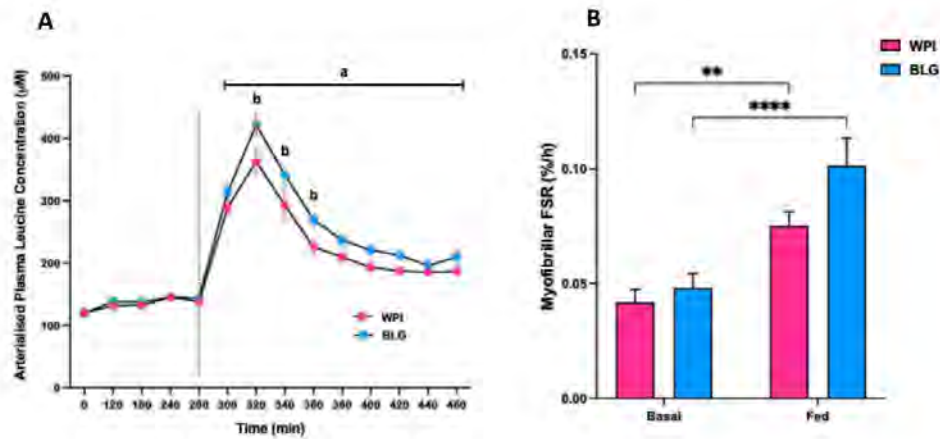


Figure 1. **A;** time-course effects of beta-lactoglobulin (BLG) and whey protein isolate (WPI) on arterialised plasma leucine concentrations. a: significantly different *versus* basal ($p < 0.05$), b: significantly different between groups ($p < 0.05$). **B;** the effects of BLG and WPI on myofibrillar fractional synthetic rate (FSR) following feeding. **: $p < 0.01$, ****: $p < 0.0001$. Data presented as mean $\pm$ SEM.

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PCA045

A healthy lifestyle and indoor air quality on cognitive performance of elementary school pupils

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Since the Covid-19 pandemic in 2020, indoor ventilation rates in classrooms became a major topic. However, the research on the effect of the indoor environment on occupants' health and cognition is much older. Studies have shown that high concentrations of carbon dioxide (CO₂) are negatively associated with the cognition performance of occupants (Du et al., 2020). Air pollution can also cause adverse health reactions (Fuentes-Leonarte et al., 2009). Elementary school children are a particularly vulnerable group because they are still in their growth phase and their school performance early on determines their success in higher education and the labour market later on. Past research found a negative relationship between classroom air quality and school performance (Wargocki et al., 2020). However, there are individual differences in the response to poor air quality which are still unknown. Overall health could be an important factor moderating individual resilience.

Therefore, this study examines if unhealthier school children in terms of overweight and higher sick leave days than their peers are less resilient towards the detrimental effects of indoor CO₂ on test scores. Data from two field studies have been merged. The first study found a negative effect of indoor CO₂ on test scores for pupils in schools in the south of The Netherlands (Palacios et al., 2022). From this dataset, 5 schools took part in an intervention study aiming to improve children's dietary behaviour and physical activity levels, which successfully reduced BMI levels (Bartelink et al., 2019). Thus, the health data of 1,149 children could be connected to their test scores and the indoor CO₂ concentration of 58 classrooms. The scores of a nationally standardized test have been collected in January 2019 and 2020.

Linear regression models with various fixed effect specifications on the testing period, test domain and classroom were conducted. Standard errors were clustered on classroom and period levels to account for observation dependency within a classroom. Preliminary results show that higher indoor CO₂ concentrations and being more often on sick leave are negatively associated with lower test scores ($p < .05$). Additionally, being overweight is negatively associated with test scores ($p < .05$), however, it partially mitigates the negative effect of CO₂ ($p < .05$), contradicting our hypothesis. Being part of an intervention school offsets the negative effects of CO₂ on test scores ($p < .001$). This countereffect is significant for the test domains of math and reading ($p < .01$) compared to spelling tasks. The regression models corrected for indoor temperature and relative humidity levels, indoor fine particles concentration, children characteristics (age, sex, absence other than due to sickness, parental socioeconomic status) and learning environment characteristics (class size, noise levels). In conclusion, the individual health of a child determined by BMI and sick leave days does not reduce the negative effect of CO₂ on academic performance. However, an intervention aiming at the physical activity level

and dietary behaviour can partially offset the negative impact of poor air quality on school performance.

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PCA046

An exercise-responsive candidate obesity gene with sexual dimorphism

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Obesity increases the risk for diabetes and cardiovascular disease. Genetic predisposition exacerbates environmental drivers of obesity such as energy dense diets and sedentary lifestyle. We have exploited divergently selected Fat (23% fat as bodyweight) and Lean (4% fat as bodyweight) lines of mice originating from a common base population to identify genes underlying divergent adiposity. A stratified approach using quantitative trait loci (QTL; heritable genetic intervals segregating with adiposity in Fat x Lean F2 populations), metabolic tissue transcriptomics and comparative cross-species bioinformatics identified candidate obesity and leanness genes in adipose tissue (Morton *et al.*, 2011, 2016). Using a similar approach, we have identified novel muscle-expressed genes that segregate with adiposity. A specific phospholipase A2 isoform (we name here PlaX), positioned in the Found in obesity (Fob)-1 QTL, exhibited ~5-fold elevated mRNA levels in the skeletal muscle of Fat mice compared to Lean mice.

Our aim in this study was to characterise PlaX's expression and regulation in skeletal muscle across different muscle beds, in response to exercise, and between sex.

To better understand the role of PlaX in skeletal muscle, PlaX expression was measured in different skeletal muscle beds in 8-week old male C57BL/6J mice. Relative mRNA expression of PlaX was detected in soleus, gastrocnemius, EDL and quadriceps muscle, and preferentially expressed in gastrocnemius and EDL muscle [$P < 0.05$; ($n = 4/\text{group}$)]. To test the translational relevance of our findings, PLAX expression was measured in human skeletal muscle. PLAX expression was measured in vastus lateralis biopsies from female and male participants with normal glucose tolerance (NGT) and those with Type 2 Diabetes (T2D) by microarray (NGT-female: $n = 45$, NGT-male: $n = 50$, T2D-female: $n = 40$ and T2D-male: $n = 50$). PLAX mRNA abundance was significantly higher in female skeletal muscle ($q\text{-value} < 10e-15$), with no difference between participants with T2D and NGT. We thus hypothesised that PlaX expression may be regulated by sex hormones. To test this, quadriceps muscle from 8-weeks old gonadectomised male C57BL/6 mice supplemented with vehicle, male sex hormone (dihydrotestosterone, DHT; 100 $\mu\text{g}/\text{day}$) or female sex hormone (estradiol, E2; 2 $\mu\text{g}/\text{day}$) by a subcutaneous minipump for 3 weeks were analysed to quantify PlaX expression. Relative PlaX mRNA abundance did not differ between the male or female sex hormone supplemented gonadectomised mice. However, PlaX expression was significantly increased after gonadectomy surgery [$*P < 0.05$; ($n = 9/\text{group}$)]. Expression levels were not rescued after sex hormone supplementation in male mice. Given the beneficial effects of exercise upon skeletal

muscle, we then aimed to determine whether PLAX expression is altered in human skeletal muscle in response to exercise. PLAX expression was analysed in MetaMex, an application to perform meta-analysis of skeletal muscle response to exercise (Pillon *et al.*, 2020). PLAX expression was significantly downregulated in vastus lateralis biopsies of young male and female healthy subjects after acute aerobic exercise ($\log_2FC = -0.23$, $FDR = 0.038$).

Our genetic strategy has identified a novel potential skeletal muscle driver of obesity that appears to display sexual dimorphism and is downregulated in response to exercise.

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PCA047

Altered skeletal muscle mitochondrial morphology in late middle-aged humans, despite maintained mitochondrial function and content

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Introduction: Ageing is typified by a decline in maximal oxygen uptake (VO_{2max}) (Conley et al., 2000a; Conley et al., 2000b), which can limit physical function and daily life activities. This decline in VO_{2max} is related to a lower skeletal muscle mitochondrial function and loss of muscle mass (Conley et al., 2000a; Conley et al., 2000b). However, data in humans are inconsistent, with many studies showing no difference in mitochondrial function between young and old or middle-aged groups (Rasmussen et al., 2003). Most human studies have relied on assessments of maximal mitochondrial function (i.e. enzyme activities or phosphocreatine recovery kinetics), which may conceal subtle changes in mitochondrial ultrastructure and function occurring prior to an overt reduction in maximal mitochondrial oxygen consumption.

Objectives: To determine aerobic exercise capacity, mitochondrial respiration, density, and ultrastructure in late middle-aged and younger adults, and systematically assess the interrelationships between VO_{2max} and muscle mitochondrial measures.

Methods: 12 healthy young (27 ± 5 years, 8 males) and 10 late middle-aged adults (55 ± 6 years, 5 males) were recruited. All participants performed a maximal ramp incremental test on a cycle ergometer to determine VO_{2max} . On a separate day, muscle biopsies were obtained from the *vastus lateralis* muscle. Mitochondrial respiration was assessed in permeabilized fibres using high-resolution respirometry. Transmission electron microscopy was to study mitochondrial density and ultrastructure. Succinate dehydrogenase (SDH) activity was assessed via histochemistry in sections.

Results: VO_{2max} was lower in the late middle-aged compared to the younger group (34 ± 5 vs. 45 ± 7 mL.kg⁻¹.min⁻¹, $P < 0.001$). Despite this, maximal oxidative phosphorylation capacity (old = 99 ± 27 vs. young = 99 ± 17 pmol O₂.s⁻¹.mg⁻¹, $P = 0.95$) and mitochondrial area density (old = 6.2 ± 1.5 vs. young = $6.0 \pm 0.5\%$, $P = 0.86$) did not differ between groups. SDH activity was lower in old versus young subjects (old = 0.97 ± 0.18 vs. young = $1.18 \pm 0.20 \times 10^{-5}$ $\Delta A_{660} \cdot \mu M^{-1} \cdot s^{-1}$, $P = 0.02$), an effect that appeared to be confined to the most oxidative fibres (old = 1.23 ± 0.22 vs. young = $1.65 \pm 0.39 \times 10^{-5}$ $\Delta A_{660} \cdot \mu M^{-1} \cdot s^{-1}$, $P = 0.008$) and not the least oxidative fibres (old =

0.71 ± 0.22 vs. young = $0.75 \pm 0.13 \times 10^{-5} \Delta A_{660} \cdot \mu M^{-1} \cdot s^{-1}$, $P=0.69$). Late middle-aged participants displayed smaller (old = 66 ± 5 vs. young = $95 \pm 17 \text{ nm}^2$, $P=0.001$), but more numerous (old = 0.71 ± 0.13 vs. young = $0.94 \pm 0.22 \text{ mitochondria} \cdot \mu m^{-2}$, $P=0.03$) mitochondria when compared to younger participants. The area of individual mitochondria correlated negatively, and the number of mitochondria per unit area of muscle correlated positively with age, respectively (Figure 1). Various indices of mitochondrial function were correlated with $VO_{2\max}$ in the younger but not the late middle-aged group (Figure 2).

Conclusion: These data demonstrate that late middle-aged individuals had smaller, more numerous mitochondria for the same mitochondrial density and maximal respiration. The lack of correlations between mitochondrial measures and $VO_{2\max}$ in this group suggests that $VO_{2\max}$ is more likely constrained by O_2 delivery-related processes in late middle-aged humans. The extent to which these mitochondrial structural alterations predispose skeletal muscle to ageing remains to be determined.

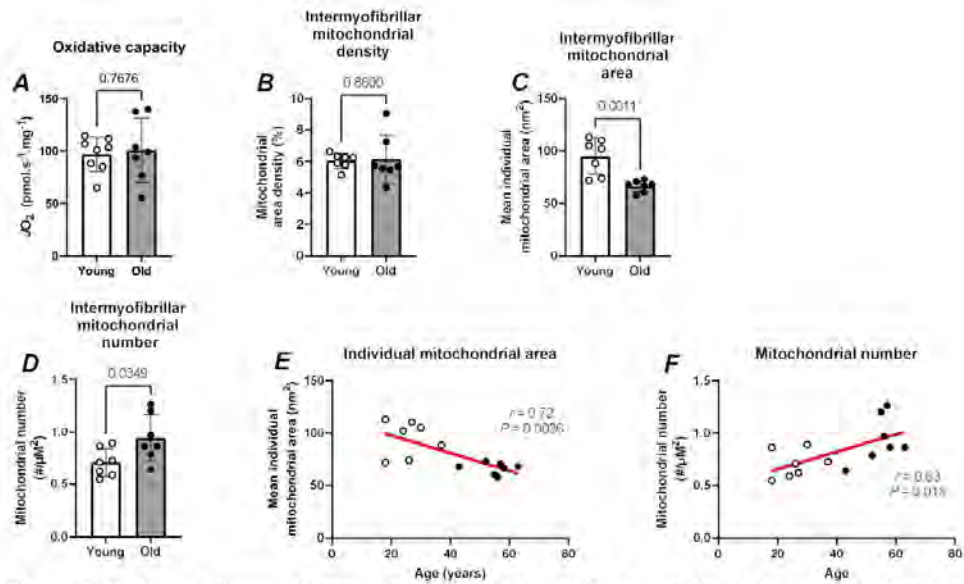


Figure 1. Similar mitochondrial respiration (**A**) and density (**B**) was achieved by smaller (**C**) and more numerous (**D**) mitochondria in late middle-aged compared to young participants. Mitochondrial area (**E**) correlated inversely & mitochondrial number (**F**) correlated positively with age.

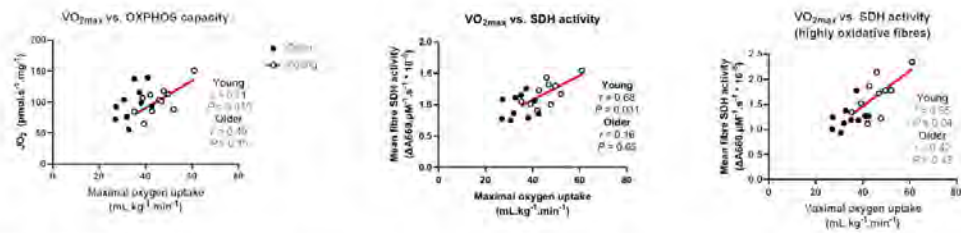


Figure 2. Maximal oxygen uptake correlated positively with maximal oxidative phosphorylation capacity, overall succinate dehydrogenase activity, and succinate dehydrogenase activity in oxidative fibres in young but not late middle-aged participants.

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PCA048

Effect of spermidine administration on denervation-induced skeletal muscle atrophy

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Introduction

In modern society, skeletal muscle atrophy is a serious problem. It is well known that in many types of skeletal muscle atrophy, autophagy, which is a degradation mechanism, is reduced and waste products accumulate (Sakuma et al., 2016). Spermidine (SP) is a kind of polyamine that is abundantly contained in foods such as soybeans, and known as an autophagy inducer (Madeo et al., 2019). Previous studies showed that spermidine administration during exercise suppresses pharmacological skeletal muscle atrophy (Fan et al., 2017). However, it is unclear whether SP administration suppresses denervation-induced skeletal muscle atrophy.

Aims

The aims of this study was to investigate the effects of SP on denervation-induced skeletal muscle atrophy and autophagy-related proteins.

Methods

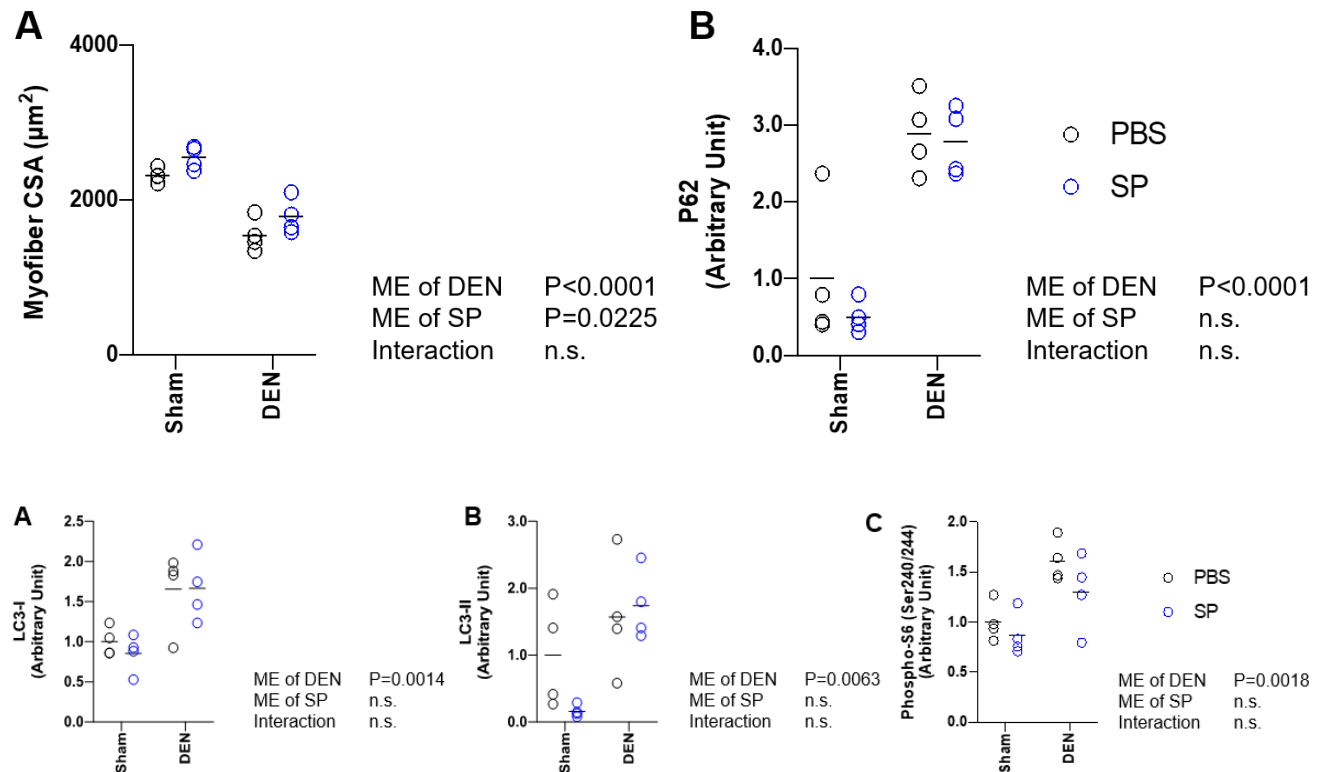
All the experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba, Japan (22-397). Male Institute of cancer research mice aged 7 weeks were used in this study (n = 4). After 1 week of acclimation, sciatic nerve transection-induced denervation (Den) was performed on the right leg of the mouse to induce skeletal muscle atrophy. A sham operation was performed on the left leg as a control. After the operation, spermidine 10 mg/kg BW was administered by intraperitoneal injection every other day. Tibialis anterior (TA) was collected two weeks after surgery. Two-way analysis of variance (ANOVA) was performed using the GraphPad Prism 8 (GraphPad, Inc.), and significance was set at $P < 0.05$ for all cases.

Results and discussion

We performed immunohistochemical staining of Laminin- $\alpha 2$ using frozen sections of TA. Significant main effects of Den and SP on muscle fiber size were identified (Fig. 1A). We evaluated protein expression levels by western blotting. Only a significant main effect of Den was identified on the protein expression related to autophagy (P62, LC3- I , LC3- II) (Fig. 1B, 2AB) and Protein synthesis (p-RPS6) (Fig. 2C). These data suggest that SP suppress denervation-induced skeletal muscle atrophy independent of autophagy pathway. SP has also been reported to relieve inflammation and oxidative stress, so further research is needed (Madeo et al., 2019).

Conclusion

SP suppressed denervation-induced skeletal muscle atrophy but did not affect key autophagy-related proteins.



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PCA049

Assessment of Cardiopulmonary Fitness and Physical Activity in Health Science Students: a Cross-sectional Study

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Background: Low levels of physical activity and cardiopulmonary fitness can have a negative impact on health and wellbeing, increasing the risk of chronic diseases and premature mortality. However, levels of physical activity in young people, including health science students, is usually low. Addressing this issue is important as health science students potentially affect the health of wider population.

Aims: This study aimed to assess the levels of cardiopulmonary fitness and physical activity among health science students at Karnali Academy of Health Sciences Jumla, a remote mountainous district of Nepal.

Methods: A cross-sectional study was conducted among the certificate and undergraduate level health science students of Karnali Academy of Health Sciences. All consenting apparently healthy students were included in the study, while those with conditions that might affect physical activity were excluded. Cardiopulmonary fitness was assessed by calculating VO₂max from the Queen's College Step Test. Physical activity levels were measured using the International Physical Activity Questionnaire, short form, to obtain the MET value. Descriptive (proportion, median, interquartile range) and inferential (Wilcoxon Rank-sum, Spearman correlation) statistics were used for data analysis using GNU-PSPP software. Multiple linear regression analysis was performed to determine the predictors of VO₂max.

Results: A total of 107 health science students (56 females) from Karnali Academy of Health Sciences in Jumla, Nepal were included in the study. Their age ranged from 18 to 37 years (median age 20). The median VO₂max of all students was 40.05 (IQR 35.68 – 50.85) ml/kg/min, with males having significantly higher value [51.69 (IQR 45.81 – 57.57)] than females [36.37 (IQR 34.90 – 38.58)] ($p < 0.001$, Wilcoxon Rank-sum test). The median weekly physical activity score was 1030 MET-minutes/week, with males reporting higher levels [1436 (962 – 2670)] than females [678 (414 – 1103)] ($p < 0.001$, Wilcoxon Rank-sum test). Moreover, only 20.5% of the students met the WHO's recommended levels of physical activity. There was a significant moderately positive correlation between VO₂max and total MET value per week (Spearman rho = 0.504, $p < 0.001$), but a negative correlation with body adiposity ($p < 0.02$). Physical activity level ($p < 0.001$), sex ($p < 0.001$), and BMI ($p = 0.004$) were significant predictors of VO₂max, but age was not a significant predictor ($p = 0.254$) according to multiple linear regression analysis.

Conclusion: Health science students at Karnali Academy, Jumla, have average levels of cardiopulmonary fitness, but a significant proportion did not meet the recommended levels of physical activity. The findings highlight the need for targeted interventions to improve the health and wellbeing of these students, especially among females, who showed significantly lower levels of physical activity than males. The education system should encourage physical activity and promote healthy lifestyle behaviors among students, as this could have significant implications for their future health and wellbeing, and potentially benefit the wider population.

However, it is important to note that this study is limited to a single center and is a cross-sectional study, therefore, causal associations between variables cannot be made. Future research should focus on identifying effective interventions that can promote physical activity and healthy lifestyle behaviors among health science students in this region.

ADDITIONAL TABLE FOR THE ABSTRACT
**“ASSESSMENT OF CARDIOPULMONARY FITNESS AND PHYSICAL ACTIVITY IN
 HEALTH SCIENCE STUDENTS: A CROSS-SECTIONAL STUDY”**

Table 1. Comparison of physical activity or fitness categories with sex

Category	Male frequency (%)	Female frequency (%)	Chi Square value ^a	P value
IPAQ category of activity				
Inactive	6 (11.76)	24 (42.86)	29.704	<0.001*
Minimally active	24 (47.06)	31 (55.36)		
HEPA active	21 (41.18)	1 (1.78)		
Total	51 (100)	56 (100)		
ACSM category of fitness				
Poor	6 (11.76)	12 (21.43)	8.512	0.04*
Fair	13 (25.49)	10 (17.86)		
Good	16 (31.37)	27 (48.21)		
Excellent	16 (31.37)	7 (12.5)		
Total	51 (100)	56 (100)		
* Significant at 95% CI; ^a Fisher Exact test				

Table 2. Correlations of different health-related parameters (Spearman Correlation)

Parameter 1	Parameter 2	Spearman Rho	P value
VO _{2max}	BMI	-0.323	0.001 *
VO _{2max}	WHR (in males)	-0.336	0.016 *
VO _{2max}	WHR (in females)	-0.045	0.741
VO _{2max}	METtotal	0.504	<0.001 *
VO _{2max} Percentile	BMI	-0.365	<0.001 *
VO _{2max} Percentile	DBP	-0.198	0.04 *
VO _{2max} Percentile	METtotal	0.27	0.005 *
VO _{2max} Percentile	Age	-0.077	0.43
MET Total	BMI	-0.172	0.076
MET Total	WHR (in males)	-0.264	0.061
MET Total	WHR (in females)	-0.095	0.486
BMI	WHR (in males)	0.542	<0.001 *
BMI	WHR (in females)	0.336	0.011 *
* Significant at CI 95%			

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PCA050

Withania somnifera (L.) Leaf Extracts Therapeutic properties of Wound Healing in Experimental Male Rats

undefined Al-shoaibi¹, Lamis Kaddam⁴

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Introduction: *Withania somnifera* (L.), is one of the important medicinal plants and have a wide range of medicinal properties and bioactive compounds.

The present study aimed to investigate chemical profile and wound healing activity of methanol:chloroform(1:1) extract of *Withania somnifera* leaves on coetaneous wounds in the experimental male rats model.

Methods: Phytochemical screening for methanol:chloroform(1:1) extract of

Withania somnifera leaves was carried out using different standard methods to show the chemical profile of active components in extract.

Wound healing assay:

20 Male rats weighing 150 to 200 g were housed under normal conditions of light, room temperature and humidity.

The dorsal skin was shaved and cleaned with bethedine under anesthesia, and one open full-thickness wound that approximately 1.5 × 1.5 cm long was incised up to the level of subcutaneous adipose tissue by means of a surgical blade.

After the wounding process, each mouse was housed in a sterilized cage and given autoclaved food with redistilled water in order to prevent bacterial infection.

. The first group was left without treatment (control group), the second was treated with Povidone iodine (standard drug), the third group received 100 mg concentration of methanol:chloroform extract of *W. somnifera* and the forth group received 200 mg concentration of methanol:chloroform extract of *W. somnifera*. The wounds in the control and experimental groups were treated topically twice daily. The area of wounds was measured every day, from the first day to 12th day.

Results: The results present study revealed that Phytochemical screening for methanol:chloroform (1:1) extract of *Withania somnifera* leaves indicated the presence of various phytoconistituants like alkaloids, flavonoids, tannins, phenols, terpenoids and steroids.

application of methanol:chloroform (1:1) extract of *W. somnifera* leaves at two concentrations 100 and 200 mg improved wound healing at all times beginning from first day of treatment to 9th day when the wounds were completely healed compare to wounds treated with standard drug (Povidone iodine) which were not completely healing after 12th day of treatment. Therefore methanol:chloroform (1:1) extract of *W. somnifera* leaves at two concentrations 100 and 200 mg showed significantly wound healing activity more than that of Povidone iodine ($p<0.05$).

The concentration of 200 mg from extract showed high significant of wound healing was not significantly compare to the effect of 100 mg of extract ($p<0.05$). Interestingly, during this study w the zone of wounds in rats treated with plant extracts returned to normal appearance and the hair had grown faster compared to positive and negative control

Conclusion: Methanol:chloroform (1:1) extract of *W.somnifera* leaves at two concentrations 100 and 200 mg showed significantly wound healing activity in experimental rats model more than that of Povidone iodine ($p<0.05$)

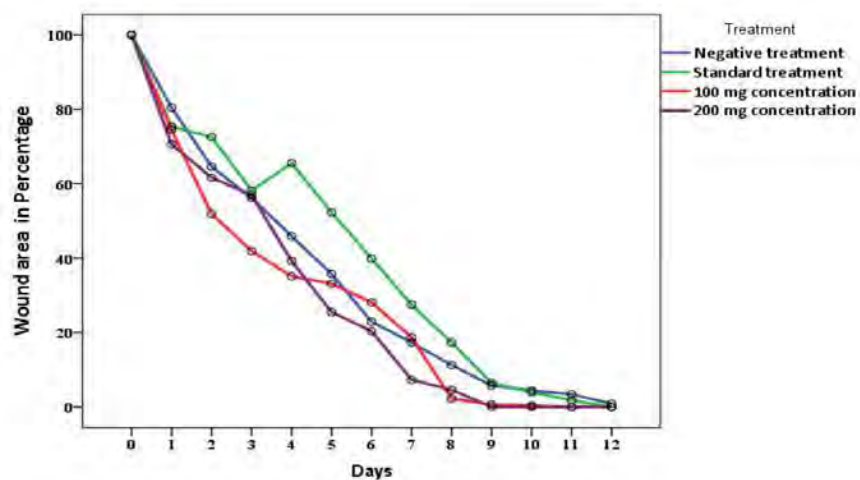


Figure (III). The effect of methanol:chloroform (1:1) extract of *Withania somnifera* leaves on coetaneous wounds made on the dorsal skin of male rats.

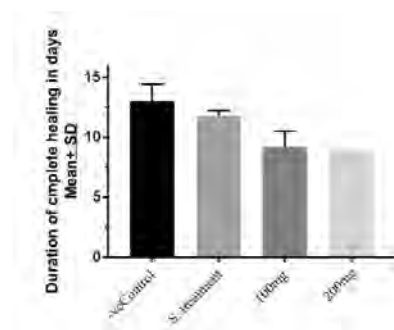


Figure (1). The effect of methanol:chloroform (1:1) extract of *Withania somnifera* leaves on time of complete healing

Table(1) Screening for secondary metabolites of methanol:chloroform(1:1) extract of *W. somnifera* leaves.)

Test	Reagent	Result	Observation
Alkaloids	Dragendorff's	+	yellow precipitate
Phenolic compounds	FeCl ₃ (5%)	+	bluish black color
Tannins	FeCl ₃ (1%)	+	bluish black color
Flavonoids	NaOH	+	yellow color
Steroids and Terpenoids	Salkowski's test	+	reddish brown color ring

Key: (+) Positive Test



Figure (II) appearance of coetaneous wounds in the experimental male rats treated with:

- (A) 100 mg of methanol:chloroform (1:1) extract of *w. somnifera* leaves [day 8].**
- (B) 200 mg of methanol:chloroform (1:1) extract of *w. somnifera* leaves [day 9].**
- (C) Standard drug (Povidone iodine)[day 11].**
- (D) Control[day 12].**

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PCA052

The metabolic cost of inspiratory muscle training in healthy adults

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Introduction: Inspiratory muscle training (IMT) is an intervention used in various clinical settings, including in critical care to ameliorate the effects of ventilator-induced diaphragm dysfunction. Metabolic oxygen consumption during IMT has not previously been explored. A recent study by our group explored the metabolic cost of IMT in mechanically ventilated individuals. We sought to supplement this with an understanding of the response of the healthy respiratory system to IMT.

Methods: The study conformed to the principles of the Declaration of Helsinki. Maximum inspiratory pressure (P_Imax) was measured with a differential pressure transducer during a sustained maximal inspiratory effort against an occlusion and calculated as the greatest one-second mean pressure.

IMT was applied at 4cmH₂O ("sham") and at 30%, 50% and 80% of each participant's P_Imax using an inspiratory threshold loading device (POWERbreathe Plus IMT). Sham training was delivered using an inverted Philips Threshold PEP device. Breath-by-breath oxygen consumption (VO₂) was measured using the Beacon Caresystem (Mermaid care A/S, Noerresundby, Denmark). Pressure at the airway opening was measured continuously using a port in the bacterioviral filter attached between the facemask and the IMT device.

IMT was applied for twelve breaths at each load with five minutes of tidal breathing between loads; the order of loads was randomised. VO₂ was recorded as a mean of the two minutes immediately following each IMT dose (recording during IMT bouts was found to be unfeasible due to the substantial negative pressures generated). Tension-time index of the respiratory muscles (TT_{mus}) was calculated as mean airway pressure divided by P_Imax, multiplied by the respiratory duty cycle (P_I/P_Imax x T_i/T_{tot}). Friedman's ANOVA was used to examine whether VO₂ differed with IMT dose, with Dunn's post hoc testing (using Bonferroni correction for multiple comparisons) for differences in VO₂ at individual doses. Linear mixed effects modelling (LMM) was used to quantify the relationship between IMT dose and VO₂ and between TT_{mus} and VO₂.

Results: Thirty healthy adults (eighteen female) were studied (median (IQR) age 32.0 (24.3 – 44.5) years, mean (SD) P_Imax 119 (48)cmH₂O. Distribution of VO₂ differed significantly with IMT dose (p<0.001). Baseline median (IQR) VO₂ (4.42 (4.81 – 6.50) ml/min/kg) was not significantly different to sham (4.90 (4.11 – 5.03), p=0.305) or 30% (4.38 (3.69 – 5.23), p=1.000) but 50% (4.64 (4.09 – 5.28)) and 80% (5.09 (4.81 – 6.50)) IMT doses were significantly higher (p=0.043 and p<0.001 respectively). VO₂ at 30% and 80%, sham and 80%, and 50% and 80% doses were significantly different (p<0.001, p=0.004 and p=0.043 respectively). LMM showed a significant dose-response relationship between IMT dose and VO₂ (slope (95% confidence interval): 0.013 (0.009 – 0.018)ml/min/kg per %P_Imax increase in IMT dose, p<0.001). VO₂ was

also significantly related to TTmus: slope (95% CI) 3.74 (2.67 – 4.81)ml/min/kg per unit increase in TTmus ($p < 0.001$).

Conclusion: Oxygen consumption during inspiratory muscle training exhibits a significant positive dose-response relationship. There is also a significant positive relationship between VO_2 and respiratory muscle effort relative to capacity. Examining the metabolic cost of breathing may offer an option to guide prescription of IMT.

PCA053

Phase angle, hydration and quality muscle index. Standardisation parameters of muscle quality in professional football players

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Introduction

Traditionally, muscle quality has been defined as the strength generated by muscle mass. Nowadays, we are able to look more deeply into the physiological and body composition mechanisms that determine muscle quality¹.

Intracellular water content (ICW) has been shown to be a useful indicator of strength-related muscle quality in the elderly, as this is the population group that is the most compromised at the water level². In high-performance sport, ICW has been found to be closely related to physical performance³. Phase angle (PhA) has also become an objective indicator of cellular health and functionality⁴.

For this reason, the aim is to obtain bioelectrical impedance (BIA) values of both PhA and muscle quality related to hydric parameters.

Methods

The sample of this longitudinal study was 33 professional football players (Table 1), from the first team of Watford FC (England). The measurements were taken during the competitive stage of the 22/23 season (year 2022).

Table 1. Characteristics of the players assessed. n=33.

Variable	Mean ± SD
Age (years)	26,33 ± 4,26
Weight (kg)	80,90 ± 8,69
Height (cm)	183,16 ± 7,96

Players were assessed by multifrequency BIA model Tanita MC-780 MA under fasting conditions, without having exercised in the previous twelve hours and having emptied the bladder before the test. A total of 247 measurements were obtained where the water component and muscle quality were analysed using the AIC/ACT index (intracellular water/total body water) and lower limb PhA, both at 50 KhZ. Injured players were excluded from the total sample.

This work was approved by the Ethics Committee of the Pablo de Olavide University.

Results

In our group, we obtained mean reference values for the AIC/ACT index of 0.661 ± 0.017 , and for the lower limb PhA of $7.8^\circ \pm 0.72^\circ$. Approximately 70% of the players are in the AIC/ACT ($x \pm SD$) range of 0.644-0.678 and PhA of 7.08-8.52°, below which we estimate muscle quality deficits in relation to hydration and muscle quality. Approximately 96% of our players are in the interval of AIC/ACT ($x \pm 2SD$) of 0.627-0.695 and PhA of 6.36-9.44°, below which we estimate as low muscle quality.

The evolution of this index and PhA during the 22/23 season is shown in table 2.

Table 2. BIA measurements season 22/23 (year 2022) n=33.

	ICW/TBW and PhA LL	
Period	July – August Preseason	September to December
Number of measures	153	94
ICW/BTW		
Mean $\pm$ SD	$0,658 \pm 0,018$	$0,660 \pm 0,016$
PhA LL		
Mean $\pm$ SD	$7,84^\circ \pm 0,89^\circ$	$7,96^\circ \pm 0,66^\circ$

LL: Lower limbs. Frequency BIA at 50 kHz

Conclusions

We consider the muscle quality index and the PhA to be very useful for analysis by the technical and health staff of a professional football club. Our data on the 70% range can serve as a reference, below which there is a deficit in muscle quality. However, it would be advisable for each club to obtain the average values of the index and PhA in order to be able to compare and analyse their athletes.

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LL: Lower limbs. Frequency BIA at 50 kHz

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PCA054

Supplementation with quercetin: cortisol and immunoglobulin values in professional soccer players

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Introduction

Quercetin is one of the most widely consumed flavonoids in the human diet and is widely distributed in fruits and vegetables¹. It is the most potent remover of Reactive Oxygen Metabolites (ROMs) and enhances the body's antioxidant capacity by modulating glutathione levels².

This flavonoid has been shown to promote post-exercise recovery³, reduce oxidative stress and muscle damage, and accelerate recovery after intense exercise⁴.

Methods

Longitudinal study with 27 players from Udinese Calcio of Italy (Table 1), in which two evaluations were carried out, November 2021 and March 2022.

The impact of quercetin supplementation on post-match recovery was studied, measuring cortisol and salivary immunoglobulin (IgA) values at 60-72 hours post-match.

Table 1. Characteristics of the evaluated players n=27.

Variable	Mean ± SD
Age (years)	25,24 ± 6,8
Weight (kg)	86,33 ± 9,76
Height (cm)	184,5 ± 8,3

The competition started on 22nd August. From August to November the players did not take quercetin and from December to March they took 500 mg quercetin the day after each of the official matches. In the first and second evaluations, IgA and cortisol values were measured 60-72 hours post-match in order to compare the values obtained at both times.

The players performed the cortisol and IgA tests on an empty stomach, always supervised by the nutritionist. Once the sample was obtained, it was analysed immediately. To assess salivary cortisol and IgA we used the SOMA Bioscience system, a non-invasive, fast and easy-to-use system.

This work was approved by the Ethics Committee of the Pablo de Olavide University.

Results

The average values obtained are shown in table 2.

Table 2. Cortisol and IgA values after quercetin supplementation.

	1st assessment	2nd assessment
	30th November 21	22nd March 22
Cortisol (µg/ml)	3,84 ± 1,45	3,29 ± 2,17 *
IgA (µg/ml)	304,93 ± 206,1	125,72 ± 74,76 **
Number of matches	16	19
*Significant differences between November and March. * p < 0.05, ** p < 0.01.		

We found that post-match quercetin administration significantly reduced cortisol values and very significantly reduced IgA values, which could be related to better recovery, as argued in another research.

Conclusions

The administration of 500 mg quercetin the day after the match is a good recovery strategy in football, as it improves the immune response and adaptation to the stress produced by the competitive event, which we believe leads secondarily to injury prevention.

Table 1. Characteristics of the evaluated players n=27.

Variable	Mean $\pm$ SD
Age (years)	25,24 $\pm$ 6,8
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PCA055

Risks, Comorbidities, and Diagnostic Assessment Tools of Chronic Obstructive Pulmonary Diseases (COPD): A Systematic Review and meta-analysis

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Background:

Chronic obstructive pulmonary disease (COPD) is a respiratory condition associated with chronic and/or acute respiratory insufficiency. According to the Global Initiative for Chronic Obstructive Lung Disease, COPD classification is based on a combination of spirometry variables, symptom scores, and history of exacerbations/ hospitalizations. Recent epidemiological studies have shown that COPD often coexists with other diseases. Although some comorbidities arise independently of COPD, others are causally related, either through shared risk factors (i.e smoking, aging), genetic factors, or the low-grade inflammation characteristic of COPD. The distinction between comorbidities and systemic manifestations of COPD is unclear. Cardiovascular disease, metabolic syndrome, osteoporosis, depression, anxiety, and lung cancer are all highly prevalent comorbidities that exist regardless of COPD severity. Cachexia, skeletal muscle dysfunction, and anemia among many others may be viewed as systemic manifestations of COPD. Cross-sectional studies have shown that comorbidities are more common in persons with advanced COPD, thus resulting in poorer clinical outcomes. Hence, there is an overall growing need for improved characterization of COPD interplay with comorbidities and its association with long-term outcomes.

Methods:

Literature was searched in several electronic databases including Embase, Google Scholar, Ovid SP, MEDLINE-PubMed, and the European Respiratory Journal. The electronic databases were systematically searched till 31 Dec 2022. Thirteen studies with a total of n= 8,333,023 COPD and non-COPD subjects met the precise inclusion criteria. To pool the results, meta-analyses of odds ratios (ORs) were carried out with subgroup and sensitivity analyses under the random effects model.

Results:

The thirteen studies (n=4,110,161 COPD and n=4,222,862 non-COPD control patient data) were used for meta-analysis. The average age of COPD patients included in the studies ranged from 40.9 to ≥ 85 years of whom 52.3 to 83.8%. were male. The prevalence of cardiovascular comorbidities (OR 1.93, 95% confidence interval, CI 1.73-2.15; P value <0.00001), cerebrovascular incidents (OR 1.88, 95% confidence interval CI 0.72–4.95; P value <0.00001), hypertension (OR 1.62, 95% confidence interval, CI 1.47-1.78; P<0.00001), diabetes (OR 1.50, 95% confidence interval, CI 1.24-1.82; P value <0.00001) and osteoporosis (OR 1.90, 95% confidence interval CI 1.37–2.64; P value <0.00001) were all considerably higher in COPD patients in comparison to non-COPD patients when adjusted for covariance. Additionally, men suffering from COPD were significantly more likely to develop cardiovascular comorbidities (OR 1.17, 95% confidence interval CI 0.98–1.39; P value <0.00001) and pulmonary malignancies

(OR 2.15, 95% confidence interval CI 0.48–9.60; P value <0.00001) than their counterparts. In contrast, women with COPD had a significantly higher risk of developing neurotic disorders, depressive disorders, and other non-psychotic mental disorders, than their counterparts.

Conclusion:

COPD is associated with a significantly higher prevalence of comorbidities including diabetes, hypertension, cardiovascular disease, cerebrovascular diseases, and osteoporosis. Risk factors such as male gender, older age, current smoking, and obesity all exacerbate the risk of comorbidity development. These findings should be considered in COPD research, control strategies, and prevention.

Keywords: chronic obstructive pulmonary disease, lung cancer, mortality, cardiovascular disease, cerebrovascular incidence,

PCA056

The Impact of Wim Hof Breathing on Cardiovascular, Respiratory and Metabolic Response to Maximal Aerobic Capacity Test in Healthy Adults

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Introduction: Wim-Hof breathing (WHB) combines periods of hyperventilation (HV) followed by voluntary breath holding (BH) at low lung volumes until one can hold it. It is increasingly used by recreational and professional athletes to improve physical performance. **Aim:** The purpose of our study was to compare the acute metabolic, cardiovascular, and respiratory response to maximal aerobic capacity test (MACT) performed after WHB to MACT response after spontaneous breathing. **Methods:** Fourteen healthy recreational athletes participated in our crossover study, designed in accordance with the Declaration of Helsinki and approved by the national Ethics Committee. After baseline measurements, the MAPT was performed with a randomly selected breathing pattern applied immediately prior to exercise: spontaneous breathing (control) or WHB. For WHB, participants followed the breathing instructions of the mobile application of the WHB method: HV (30 deep breaths at 0.32 Hz), BH, and deep inhalation held for 15 seconds, repeated three times. MAPT consisted of a 1-minute warm-up at 30 W followed by gradual cycling with a power increase of 30 W per minute to exhaustion. The Cosmed Quark PFT was used to measure cardiac, respiratory, and metabolic parameters before and during MAPT: heart rate, end-tidal partial pressure of carbon dioxide and oxygen (PetCO₂ and PetO₂), oxygen consumption (VO₂), carbon dioxide production (VCO₂), pulmonary ventilation (VE), workload and derived parameters such as the ratio of VO₂ to workload before reaching the anaerobic threshold (VO₂/WR slope), ventilatory equivalents, oxygen pulse ect. Participants rated their perception of exertion during MAPT using the Borg scale (RPE). ANOVA for repeated measures was performed in SPSS, and $p < 0.05$ was considered evident for significant differences between the two trials. **Results:** Analysis showed positive effects of WHB practice prior to MAPT on VO₂/WR slope and RPE. We found lower VO₂/WR slope in WHB compared to control ($p=0.016$) and lower RPE in WHB compared to control ($p=0.015$). WHB decreased PetO₂ ($p=0.032$), PetCO₂ ($p=0.002$), and respiratory quotient ($p=0.01$) prior to exercise compared to spontaneous breathing. No significant differences were found in other measured parameters, including peak oxygen consumption and peak power, between the control and WHB trial. **Conclusions:** Our results suggest that athletes may benefit from performing WHB pre-exercise as less physical exertion is perceived compared to no WHB, at least when performing graded exercise of short duration. A lower VO₂/WR slope after WHB may be either a positive or negative adaptation to WHB: it may represent better oxygen uptake efficiency or lower oxygen availability in active muscles. However, none of the possible adaptations were associated with differences in peak aerobic power and peak oxygen consumption, suggesting that they are shortlasting or limited to aerobic metabolism. Measurement of the slope of oxygen uptake efficiency (OUES) or blood lactate concentration during MAPT would be useful to address potential adaptation to WHB.

PCA057

Relationship between Body Mass Index and Emotional Eating among Indian and Indonesian Population: A cross-sectional study

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Introduction: Obesity is a major social issue with increased prevalence globally over the decades. Emotional eating has been considered as one of the factors leading to weight gain and subsequent obesity. Literature shows potentially addictive properties of hyperpalatable foods, and the existence of food addiction. This study aims to classify individuals as a function of the relation between food intake and emotions in Indian and Indonesian population using Emotional Eater Questionnaire (EEQ) developed by Garaulet et al¹.

Method: Voluntarily consenting 69 Indians (25 males, 44 females) and 71 Indonesian adults (23 males and 48 females) in age range 20-45 years were selected to observe and compare the relationship between emotional eating behaviour. The subjects had statistically matched body mass index (BMI). They answered EEQ as a google form that included demographic variables and eating attitude-related questions. Subjects diagnosed with hypertension, diabetes, cancer, renal or heart diseases, endocrinal disorders and patients having any severe mood disorder on medication were excluded. The BMI were measured using Quetelet Index in kg/m² and subjects were classified as non-obese (BMI < 23 kg/m²) and obese (BMI > 23 kg/m²).

Results: Among Indians, 12 males and 24 females and among Indonesians 16 males and 25 females were found to be obese. BMI of the subjects was compared statistically intra and inter categorically using Spearman correlation. Depending on their EEQ scores, the subjects were categorized as non-emotional (EEQ score 0-5), low-emotional (EEQ score 6-10), emotional (EEQ score 11-20) and high-emotional (EEQ score >21) eaters. Obtained results indicate that subjects with higher BMI have higher EEQ scores. Spearman coefficient showed significant association between the various categories of EEQ and had higher BMI. MannWhitney test was done to compare scores of EEQ between Obese and Non-obese subjects which showed a strong, positive correlation, which was statistically significant ($p < 0.00001$) for both Indian as well as Indonesian subjects. One way ANOVA applied between EEQ categories and BMI was also found to be statistically significant ($p < 0.05$) for both nationalities.

Discussion: Overeating and obesity stems from many biological factors engaging both central and peripheral systems in a bi-directional manner involving food and emotions. It was observed

that the proportion of emotional eating is more in obese than in non-obese subjects. Pleasure associated with food consumption leads to Dopamine production, causing activation of brain reward pathways that overrides other signals of satiety and hunger. Thus, a gratification habit through a favorable food leads to overeating and obesity. Obtained results support the hypothesis that emotional eating is one behavioral mechanism between over-eating and subsequent development of obesity.

Conclusion: Emotional eating behavior can be quantified using EEQ score in Indian and Indonesian population. Participants with higher BMI showed higher EEQ scores suggesting emotional eating as one of the contributing factors of obesity. Identifying and developing quantifiable measures to assess behavioral adaptations associated with emotional eating could provide a means of holistic approach to obesity management and prevention.

1. Garaulet et al. Validation of a questionnaire on emotional eating for use in cases of obesity; the Emotional Eater Questionnaire (EEQ) (Nutr Hosp. 2012;27:645-651)

PCA058

Musculoskeletal limitation is prevalent in preoperative cardiopulmonary exercise testing and favours use of submaximal metrics: implications for surgical risk assessment

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Background: Cardiopulmonary exercise testing (CPET) is used to determine cardiorespiratory fitness (CRF) in patients prior to major surgery given its capacity to predict post-operative survival [1]. Low CRF is attributed to 17% of postoperative mortality, presents greater risk than traditional measures of cardiovascular disease, and unfit patients experience a 5-fold greater mortality hazard [2]. CPET is preferably conducted to the limit of tolerance and primary metrics include, anaerobic threshold (AT), peak oxygen consumption ($\dot{V}O_2$ peak), and ventilatory equivalent for carbon dioxide at AT ($\dot{V}_E/\dot{V}CO_{2-AT}$) [3]. In non-surgical populations, musculoskeletal (MSK) conditions affect up to 1 in 3 people and are associated with a two-fold likelihood of being physically inactive [4]. In this study we observed a large cohort of patients who underwent CPET prior to colorectal surgery to identify the prevalence of MSK limitation. We then compared to what extent potential inability to provide authentic maximal CPET effort may inform metrics used for surgical risk stratification in this population.

Methods: A consecutive sample of 640 patients scheduled for elective colorectal surgery who attended CPET testing were retrospectively examined. CPET was conducted in accordance with consensus clinical guidelines [5] using cycle ergometry (Lode, Gronigen, The Netherlands) and a Medgraphics Ultima metabolic cart (MedGraphics™, Gloucester, UK). Patients exercised to their limit of tolerance to provide information for prognostic and diagnostic utility. The Medgraphics Breeze™ software automatically determined $\dot{V}O_2$ peak and respiratory exchange ratio (RER). The AT was manually interpreted using the V-slope method and $\dot{V}_E/\dot{V}CO_{2-AT}$ calculated. Immediately following test termination patients were asked why they stopped, and responses used to stratify groups by prevalence of MSK pain (MSK+ or MSK-). Following confirmation of distribution normality (Shapiro W Wilks tests), data were analysed using independent samples *t*-tests for continuous data, or χ^2 tests for frequency counts. Data are expressed as mean $\pm$ SD and significance established at $P < 0.05$.

Results: Not all patients completed CPET. Seventy seven of 619 patients (12%) who completed CPET reported MSK pain as the reason for terminating prematurely, whilst 13 of the 21 (62%) patients unable to perform CPET were prevented in doing so by MSK limitations. Patients who reported MSK pain as the cause for premature test termination exhibited lower $\dot{V}O_2$ peak whereas the submaximal AT metrics were comparable (Table 1).

Conclusions: Exercise limitation due to MSK pain is prevalent in patients undergoing preoperative CPET. Patients unable to CPET have high risk of postoperative mortality and MSK limitation is often what prevents them from performing a test. Of MSK+ patients able to CPET, reduced $\dot{V}O_2$ peak is likely, and caution should be applied to this metric. Clinicians should consider metrics, such as the AT, that do not require maximal effort and investigate alternatives like the oxygen uptake efficiency slope allowing opportunity to account for disruption by early termination of exercise in these patients.

Table 1. Preoperative cardiorespiratory fitness of 640 patients undergoing colorectal surgery. Patients were compared by ability to perform a cardiopulmonary exercise test, or those who terminated a test because of musculoskeletal pain (MSK+) against those who did not (MSK-).

	<i>Whole cohort</i>	<i>MSK+ (n = 90)</i>	<i>MSK- (n = 550)</i>	<i>P-value</i>
Able to CPET*	619 (97)	77 (12)	542 (84)	
Unable to CPET*	21 (3)	13 (62)	8 (38)	0.000
Maximal variables				
Workload-peak (W)		88 (39)	98 (43)	0.068
$\dot{V}O_2$ peak (mL.kg ⁻¹ .min ⁻¹)		15.4 (4.2)	16.5 (4.8)	0.045
RER- $\dot{V}O_2$ peak		1.10 (0.1)	1.12 (0.1)	0.106
HR-peak (b.min ⁻¹)		125 (21)	128 (27)	0.367
Submaximal variables				
AT (mL O ₂ .kg ⁻¹ .min ⁻¹)		10.9 (2.1)	11.0 (2.5)	0.777
$\dot{V}_E/\dot{V}CO_2$ -AT		34 (5)	34 (6)	0.806
OUES (mL O ₂ .min ⁻¹ /L.min ⁻¹ $\dot{V}_E$)		1647 (456)	1639 (533)	0.894

Data are shown as mean (± SD) or *number (%). Definitions: AT, anaerobic threshold; CPET, cardiopulmonary exercise test; HR-peak, heart rate at peak; OUES, oxygen uptake efficiency slope; RER, respiratory exchange ratio; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide; $\dot{V}O_2$ peak, peak oxygen consumption; Work load-peak, work load at peak oxygen uptake.

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PCA059

The venoarteriolar reflex revisited – a pilot study

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The venoarteriolar reflex (VAR) is one of the several physiological responses that contribute to maintain hemodynamic stability, especially in dependent regions of the body. VAR consists on arteriolar constriction in response to an increase in venous transmural pressure, as occurring in postural changes, therefore preventing the formation of edema. A recent study has demonstrated, in the lower limb, the existence of a vascular response in the contralateral limb that occurs simultaneously to VAR. However, it remains unclear whether this contralateral response is also observed in the upper limb. This study aimed to quantify the microvascular changes to a postural modification designed to evoke VAR in the upper limb. Ten young healthy subjects (23.4 ± 4.9 y.o.; 6 females, 4 males) participated in this study after giving informed consent. After acclimatization, subjects performed a postural modification while sitting upright, as follows – 7 min with both hands at heart level (baseline phase), 5 min with one random hand (test) placed 40 cm below the heart (challenge phase), and 7 min in the initial position (recovery phase). Local blood flow and skin temperature were measured in the second finger of both hands. Galvanic skin response was measured in the third and fourth fingers of the unmoved (control) hand in order to assess sympathetic cutaneous activity. These variables were compared between the different phases of the protocol with the Wilcoxon signed rank test ($p < 0.05$). During the challenge phase a significant decrease in local blood flow was observed for the test hand. Similarly, there was a decrease in blood flow in the control hand, although not statistically significant. Skin temperature did not show any statistical differences between phases, guaranteeing that blood flow changes were not related to thermoregulatory phenomena. Also, no significant changes were found for galvanic skin response, suggesting that the sympathetic nervous activity is not involved in the observed vascular responses. These results suggest that the contralateral vasoconstrictor response to VAR, previously demonstrated in the lower limb, is also detected in the upper limb. The specific nature of this contralateral response should be investigated in future studies.

PCA060

Cardiovascular autonomic dynamics - an insight from the spectral organization of electrodermal activity signals

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Suprasystolic limb occlusion (SLO) is an important manoeuvre for blood pressure measurement, being also used as a challenge for the assessment of endothelial activity. It is commonly carried out by inflating a pneumatic pressure cuff above systolic pressure for a certain period of time. Although very useful, the magnitude and duration of occlusion are critical experimental aspects because of the mild discomfort it may cause to the subject. This perceived discomfort is likely to increase the activity of sympathetic nervous system and to have a systemic impact in hemodynamics. This study aims to further expand the knowledge on the physiological response to SLO by exploring the dynamics of autonomic regulation. Ten healthy male subjects (mean 20.2 ± 2.3 y.o.) participated in this study after giving informed written consent. After acclimatization, subjects performed a standard SLO protocol on a random upper limb while sitting upright, as follows: 10 min resting with both arms at heart level, 5 min arm occlusion (200 mmHg) and 10 min recovery in the initial position. Photoplethysmography (PPG) signals were acquired from the second finger of the occluded and non-occluded arms, with the latter being used for pulse rate variability (PRV) analysis, a surrogate of the well-known heart rate variability (HRV). The power of the high (HF), low (LF) and very low frequency components of the PPG signals were determined in all phases, as well as the LF/HF ratio. The electrodermal activity (EDA) was also acquired from the third and fourth fingers of both hands. The PPG and EDA signals were then decomposed with the wavelet transform in order to obtain their frequency spectra. During occlusion a significant decrease in the LF/HF ratio was noted, suggesting a decrease in cardiac sympathetic activity. In contrast, a significant increase in EDA was noted in both hands, suggesting an increase in cutaneous sympathetic activity. Also noteworthy, the EDA signals resembled one another very closely during the entire procedure, showing that the mechanical compression of the arm did not affect the magnitude of the signals. The spectral organization of the PPG signals has already been proposed to contain several components - cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent and endothelial NO-independent. The spectra of the EDA signals revealed a high frequency component that was aligned with the PPG cardiac component, together with several low frequency components which were only partially aligned with the sympathetic PPG component. These results suggest that the autonomic response to a SLO manoeuvre is complex and probably organ-dependent. This complexity is apparent from the EDA signal spectra, whose usefulness should be better investigated in the future.

PCA061

Spectral organization of skin temperature signals

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The human cutaneous microcirculation has been considered as a useful “window” to assess microvascular function, especially because of the possibility of using non-invasive technologies. Whenever measuring skin perfusion, it is advisable to measure skin temperature as well, in order to control the interference of thermoregulatory phenomena due to changes in ambient temperature. When performing measurements in an environment of stable external temperature, changes in skin temperature should reflect changes in skin perfusion. In fact, depending on the sensitivity of the sensors, it is possible that changes in skin temperature may reflect physiological mechanisms regulating perfusion. However, the spectral organization of continuous skin temperature signals remains poorly understood. Therefore, this study aimed to compare the frequency spectra of skin perfusion and skin temperature signals obtained from healthy subjects. Twenty healthy subjects (21.0 ± 3.0 y.o., both sexes) participated in this study after giving informed consent. After acclimatizing to room conditions, subjects performed one of two protocols while sitting upright – 10 subjects were subjected to a suprasystolic limb occlusion protocol (SLO, 5 min baseline, 5 min occlusion of a random arm, 5 min recovery); the other 10 subjects performed a postural modification (5 min with both hands at heart level, 5 min with a random hand placed 40 cm below heart level, 5 min recovery in the initial position). In both protocols, two variables were quantified in the index finger of the tested (occluded or lowered) limb - skin perfusion was quantified in the distal phalanx with a photoplethysmography (PPG) sensor, and skin temperature was quantified in the middle phalanx with a negative temperature coefficient (NTC) thermistor. Nonparametric statistics were used for comparisons between phases and signals ($p < 0.05$). In the SLO protocol both perfusion and temperature decreased significantly due to the mechanical compression of the brachial artery. In the hand lowering protocol, however, perfusion decreased significantly due to the venoarteriolar reflex, but not significant changes were observed for skin temperature. These results show that the NTC thermistor was less sensitive to the physiological challenges than the PPG sensor. Both signals were decomposed with the wavelet transform to obtain their respective frequency spectra. The spectral organization of the PPG signal has already been proposed to contain several components - cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent and endothelial NO-independent. The spectra of the temperature signal revealed components in the same frequency intervals as the PPG signal. In fact, the dominant frequency of each observed component was generally coincident between signals, although appreciable differences in terms of skewness and kurtosis were identified for the regions of cardiac and respiratory components. These results suggest that skin temperature signals might have the same physiological origins

than PPG signals and, consequently, might be useful to explore the dynamics of perfusion regulation.

PCA062

Study of the impact of body surface area on functional exercise capacity and disease progression in patients of silicosis

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Introduction: Silicosis is one of the oldest occupational lung diseases, occurring gradually over a period of 12-15 years in workers exposed to silica dust. However, there are very few studies identifying anthropometric variables associated with silicosis and their impact on disease progression. It would be of value to consider the physical characteristics of individuals as risk factors for developing occupational lung disease.

Aim: The objective of present study was to study the association between body surface area (BSA), pulmonary function indices and 6 minute-walk distance(6MWD) in patients of silicosis.

Materials and Methods: A cross-sectional study was conducted on 102 male patients diagnosed with silicosis. Height and weight were measured to calculate BSA. The subjects were divided into three groups on basis of BSA (square metre) - Group I with BSA < 1.6 sq. m., Group II with BSA = 1.6- 1.9 sq. m. and Group III with BSA > 1.9 sq. m. Each group was further subdivided into three subgroups according to years of exposure to silica dust, subgroups being Ia, IIa and IIIa (10-15 years of exposure), Ib, IIb and IIIb (15-20 years of exposure) and Ic, IIc and IIIc (>20 years of exposure). Spirometry and 6MWD were performed on all groups and subgroups. Data was expressed as mean and standard deviation. Statistical analysis was done using Epi info V7 software. The outcome variables were Forced expiratory volume in first second (FEV1), Forced vital capacity (FVC), FEV1/FVC ratio and Peak expiratory flow rate (PEFR). Student's t test of significance (ANOVA) was applied to test the difference between means. Level of significance was set at 5%.

Results: Average age of subjects was 43.80±8.8 years. The average duration of exposure to silica dust was 21.25±6.35 years. 6MWD showed no significant changes with years of exposure and BSA. Except for FVC [2.80±0.76, 3.12± 0.51, 2.62 ± 0.69], the total mean of all other pulmonary function indices showed a statistically significant decrease as we move from subgroup 'a' (10-15 years of exposure) to subgroup 'c' (> 20 years of exposure) in each group [FEV1- 2.27±0.69, 2.44± 0.44, 1.91± 0.59 ; FEV1/FVC(%) - 80.14±5.84, 78.13 ± 5.15, 72.17± 10.45; PEFR(L/s) - 5.18±1.27, 6.15 ±1.29, 5.06 ± 1.88 respectively in each subgroup a, b, c]. In group III, only FEV1 and FVC showed statistically significant decrease with increase in years of exposure [FEV1-3.05 ± 0.09(IIIa), 2.93 ± 0.66(IIIb), 2.23 ± 0.37(IIIc) (p<0.001) & FVC- 3.66 ± 0.31(IIIa), 3.5± 0.81(IIIb), 2.90 ± 0.20(IIIc)]. The spirometric indices were higher in group III compared to group I and group II. Statistically significant higher values of FEV1 [2.73 ± 0.37(p=0.03)] and FVC [3.35 ± 0.44 (p=0.01)] were observed in group III patients in all subcategories of exposure.

Conclusion: Patients of silicosis with BSA > 1.9 sq. m. had higher values of pulmonary function indices, irrespective of period of exposure to silica dust. Large body size may be of value in protection from developing occupational lung disease.

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PCA063

Acute cardiovascular responses to a single bout of high intensity inspiratory muscle strength training in smoking and non-smoking adults

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The use of cigarettes and e-cigarettes both increase blood pressure. Inspiratory muscle strength training (IMST) can lower blood pressure in healthy subjects (De Lucia et al, 2018). The aim of this study is to investigate whether IMST affects blood pressure and heart rate variability (HRV), a marker of cardiac autonomic function, in a group of smokers and non-smokers.

15 participants volunteered for this study (11 female). 5 participants smoked both cigarettes and e-cigarettes. Ethical approval was granted by the Science & Engineering Research Ethics committee, University of Plymouth, in accordance with the Declaration of Helsinki. All participants gave informed written consent. Participants completed a health questionnaire including information on exercise and smoking. Anthropometry, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were initially recorded, along with respiratory muscle strength (RMS); using the mean of maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP). 50% of the MIP reading was used to determine the resistance on two different brands of IMST (Threshold and Breather devices). Subjects were seated and five, 1-minute experiments were conducted; 1-minute rest, 1-minute IMST Device 1, 1-minute rest 2, 1-minute IMST Device 2 and 1-minute rest 3. IMST device order was randomised. Blood pressure, oxygen saturation (SpO₂), Borg scale for breathlessness, chest plethysmography and ECG were recorded for all experiments, with a nose-clip being worn. LabChart software and a PowerLab were used for data acquisition. HRV was analysed using time domain (heart rate - HR, standard deviation of the RR interval - SDRR), frequency domain (low and high frequency - LF and HF) and Poincaré analysis (SD1 and SD2). Repeated measures ANOVA (RMANOVA) and SPSS software was used to analyse the data (n=15). The probability of <0.05 was taken as statistically significant. Post-hoc analysis using pairwise comparisons between means was performed.

SDRR, SD1, Borg and DBP were all significantly different across all five interventions (Table 1). SDRR and SD1 were higher with both IMST devices, Borg was only higher with Device 2 (Threshold), while DBP fell with Device 1 (Breather). HR (p=0.45), LF (p=0.95), HF (p=0.96), SBP (p=0.12) and SpO₂ (p=0.18) did not show any significant changes over the five interventions. No significant differences were noted between smokers (n=5) and non-smokers (n=10).

The main findings from this study are the IMST devices resulted in time domain HRV parameters, breathlessness and DBP to differ. Unlike the work of De Lucia et al. (2018), which showed a drop in both SBP and DBP, our study only showed a drop in DBP. Reasons for this difference could include the acute duration of IMST and the fact we used a lower percentage of MIP when giving IMST. A low statistical power was most likely the reason no difference in smokers was found in the current study.

Table 1. Mean (SD) HRV and blood pressure data across all five experiments, with p values (n=15).

Variable	Rest 1	Breather	Rest 2	Threshold	Rest 3	P value
SDRR	69.4 ± 33.5	92.5 ± 42.9	78.2 ± 41.1	101.6 ± 47.0	86.6 ± 46.1	0.001
SDI	36.9 ± 24.4	59.5 ± 42.7	48.2 ± 43.6	68.2 ± 39.4	54.2 ± 52.7	0.038
Borg	0.4 ± 0.7	0.8 ± 0.9	0.4 ± 0.6	1.3 ± 1.4	0.4 ± 0.6	0.038
DBP	69.1 ± 8.1	62.51 ± 11.1	67.9 ± 8.1	70.7 ± 7.3	66.1 ± 11.1	0.0003

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PCA064

High intensity muscle contraction under caloric restriction promotes Irisin secretion in mice

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Introduction

It is well known that combining diet and physical activity are more effective for health than interventions of diet or physical activity alone (Faught *et al.*, 2017). Recent research show that skeletal muscle secretes myokines in response to exercise, which contribute to the adaptation of exercise in other organs (Severinsen & Pedersen, 2020). Therefore, we hypothesized that muscle adaptation by calorie restriction (CR) might enhance myokine responses by exercise. We have already reported that Irisin, one of myokines that contributes to metabolic activation of adipose tissue and weight loss, is secreted in response to muscle contraction (Tanimura *et al.*, 2022), and we combined this model with CR in this study.

Objective

The aim of this study was to investigate that effects of muscle contraction and CR on Irisin secretion.

Methods

All the experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba, Japan (22-397). Male ICR mice aged 7 weeks were used in this study. After 1 week of acclimation, mice in the CR group were given 60% of the average amount of food eaten by each mouse for 1 week acclimation periods. After 2weeks CR or *Ad libitum* (AL), we conducted electrical stimulation (ES) as a model to induce high intensity muscle contractions (Tanimura *et al.*, 2022). Gastrocnemius muscle and blood were obtained immediately after single bout of ES (n = 5-6 in each groups). We used Western blotting as the method of analysis for protein expressions in skeletal muscle and blood. One-way analysis of variance (ANOVA) or Two-way ANOVA were performed using the GraphPad Prism 8 (GraphPad, Inc.), and significance was set at $P < 0.05$ for all cases.

Results and Discussion

Body weight and gastrocnemius mass were significantly decreased in CR groups than AL groups (Fig.1). Thus, CR affected not only weight but also muscle condition. Blood Irisin levels were increased by both CR and ES, and an interaction was observed in the CR+ES group (Fig.2). Therefore, CR enhances Irisin secretion by exercise. One of the upstream molecules of Irisin, PGC-1 α , may be involved in this interaction.

Conclusion

CR and muscle contraction promote Irisin secretion synergistically.

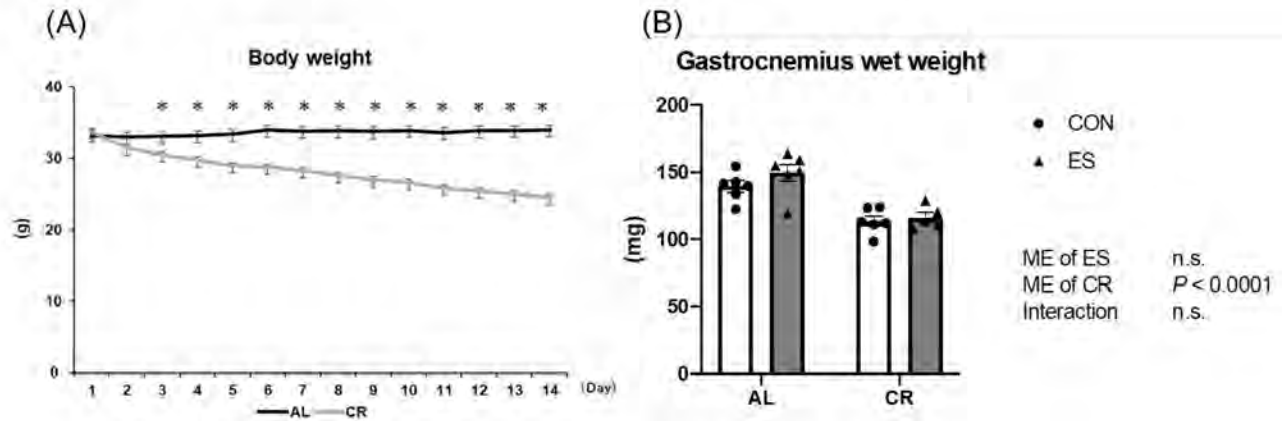


Fig1. Animal characteristics. (A) body weight and (B) gastrocnemius weight. Values represent mean $\pm$ SE ($n = 5-6$ per group). * $P < 0.05$ vs. AL

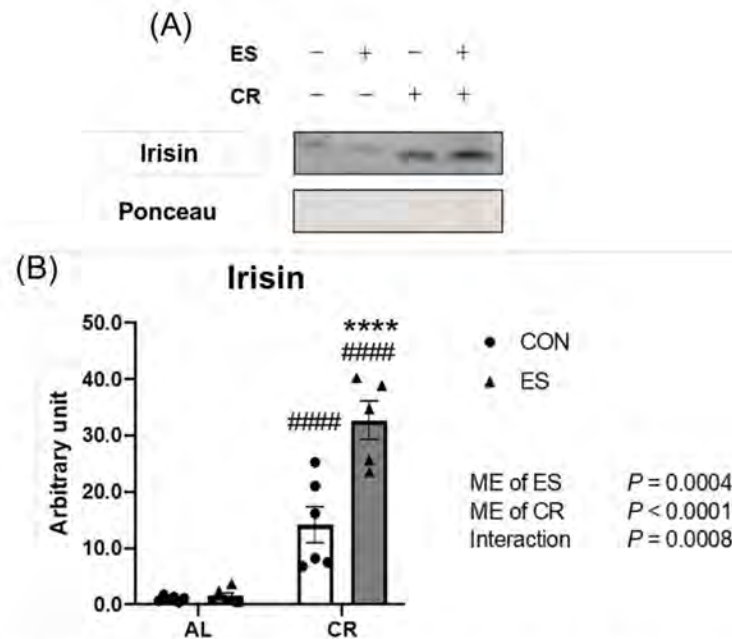


Fig 2. Irisin expression level in plasma. (A) Image of Irisin in western blotting and (B) Quantitative amount of this image. Values represent mean $\pm$ SE ($n = 5-6$ per group).

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PCA065

The effects of skin hydration levels on local skin wetness perception at the underarm

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Introduction:

The perception of skin wetness plays an important role in behavioural thermoregulation and thermal comfort (e.g. the onset of sweating). Whilst our understanding of the neurophysiology of skin wetness perception (WP) is expanding [1], research is lacking on how the biophysical status of the skin, such as its hydration levels, impacts local wetness sensitivity. Skin hydration levels can vary individually due to intrinsic (e.g. sex, age) and extrinsic factors (e.g. environment). Changes in the skin's stratum corneum hydration could alter the skin's properties leading to increased or decreased wetness sensitivity [2]. This study aimed to investigate the effect of skin hydration levels on local WP and its individual variability.

Method:

Ten male ($n=5$) and female ($n=5$) participants (28.8 ± 7.2 y; 171.3 ± 9.5 cm; 78.1 ± 18.2 kg) took part in two separate experimental trials, during which they underwent a quantitative sensory test (QST) of WP at baseline and following localised overhydration [i.e. OVH; $+22 \pm 20\%$ from baseline] or dehydration [i.e. DEH; $-44 \pm 20\%$ from baseline] of the underarm's skin. Participants reported on a 100-mm visual analogue scale the perceived magnitude of WP (anchor points: 0=dry; 100=completely wet) from the short-duration (i.e. 10s) static application of a cold-wet (i.e. 5°C below local skin temperature), neutral-wet (i.e. equal to local skin temperature) and warm-wet (i.e. 5°C above local skin temperature). Before the QST(s), local tactile sensitivity, skin temperature, stratum corneum hydration, and skin surface roughness were measured. Individual participants' perceptual responses to each temperature stimuli were coded as either A) a change in WP (i.e. ≥ 10 -mm difference in WP from baseline to post OVH or DEH); or B) no change (i.e. < 10 -mm difference). Pearson's chi-squared tests of independence were used to examine the association between changes in WP from baseline and skin hydration status (i.e. OVH or DEH) for each temperature stimulus.

Results:

We found a statistically significant association [$\chi^2 (2)=6.9$, $p= 0.03$] between skin hydration status and changes in WP during neutral-wet stimulation. Specifically, 60% of participants reported an increase in WP following OVH, whilst 30% reported a decrease following DEH (Fig. 1A). A similar trend was observed during cold-wet stimulation [$\chi^2 (2)= 5.4$, $p= 0.07$] whereby 50% of participants reported an increase in WP following OVH, whilst 40% reported a decrease following DEH (Fig. 1B). No significant association was found between changes in WP and skin hydration status during warm-wet stimulation ($\chi^2 (2)= 0.3$, $p= 0.865$)(Fig.1C). Differences in the changes in WP following manipulation in hydration status were not explained by sex (Fig. 1).

Conclusion:

The study found that skin hydration levels may influence WP, although this effect is dependent on stimulus temperature. Furthermore, hydration-dependent changes in WP were observed in ~50% of the sample only. The response to the change in skin hydration state may be divided into subgroups of responders and non-responders, with individual variability modifying the effect of skin hydration levels on WP to a larger extent than sex-related differences.

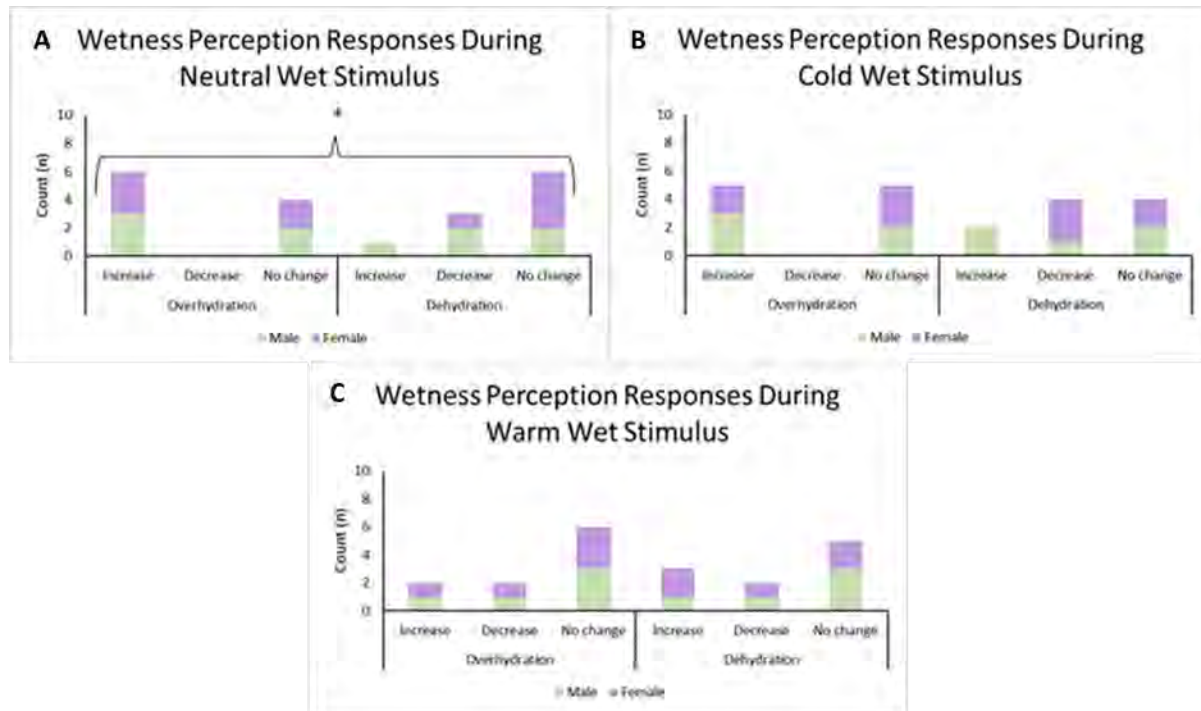


Figure 1. Proportion of males (i.e green) and female (i.e purple) participants (N; total sample size= 10) reporting an increase, decrease, or no change in WP during the application of neutral-wet (A), cold-wet (B), and warm-wet (C) stimuli following local over- or -de-hydration of the underarms' skin. *denotes statistically significant association between changes in WP from

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PCA066

Effective dose regimen of STZ for STZ-induced diabetes in a rat model

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Introduction: Diabetes mellitus (DM) is a metabolic illness, defined by a high level of blood sugar because of a problem with insulin synthesis, action, or both. Various clinical signs follow DM, majorly hyperglycaemia, polydipsia, polyuria, and polyphagia. Worldwide prevalence is high and predicted to rise to 592 million by 2035. Animal models are used in the study of diabetes due to ethical issues. Streptozotocin (STZ) model is frequently used but has poor dependability due to unexplained acute toxicity and effective dose variability. This research was carried out to determine the effective dose regimen of STZ for inducing diabetes in locally reared Wistar rats in Abeokuta.

Methodology: 28 male Wistar rats (160 - 190 g) were randomly divided into 4 groups (n=7) and monitored for 21 days after diabetes induction with STZ: Control (CTR), diabetics: DIA1 (60 mg/kg STZ), DIA2 (60 mg/kg STZ twice at 0 and 24 hours), and DIA3 (60 mg/kg STZ thrice at 0, 24 and 48 hours). Plasma glucose was determined with a glucometer. Body weights, feed intake, and faecal output were weighed with a digital balance, while water intake and urine output were measured with a measuring cylinder. Analyses of the data obtained were performed using a One-way ANOVA and Tukey's test at $p < 0.05$ for significance. The ethical rules set forth by the Federal University of Agriculture, Abeokuta's committee on animal care ethics and usage (FUNAAB/COLVET/CREC/2022/02/03) were adhered to.

Results: There was a significant ($p < 0.05$) decrease change in the percentage body weight of the diabetics (-15.53 ± 1.2 , -26.8 ± 1.2 , $-28.5 \pm 1.9\%$) compared to the CTR ($10.5 \pm 2.5\%$). Also, there was a significant ($p < 0.05$) increase in fasting blood glucose concentrations (135.2 ± 9.0 , 273.2 ± 6.5 , 257.0 ± 5.3 mg/dL) in the diabetics compared to the CTR (79.3 ± 1.1 mg/dL). Furthermore, water intake (56.9 ± 0.9 , 72.1 ± 1.7 , 77.8 ± 5.5 mL), feed intake (19.4 ± 0.6 , 23.3 ± 1.9 , 42.1 ± 2.1 g), voided urine (6.34 ± 0.1 , 8.39 ± 0.88 , 9.58 ± 0.50 mL) and voided faeces (10.4 ± 0.26 , 11.7 ± 0.43 , 8.5 ± 0.17 g) in the diabetics increased significantly ($p < 0.05$) when compared to the CTR (26.5 ± 0.8 mL, 13.4 ± 0.3 g, 1.84 ± 0.08 mL, and 6.5 ± 0.33 g respectively).

Conclusion: This study showed that the dose regimen of 60 mg/kg STZ administered intraperitoneally twice (24 hours apart) sustained diabetes for 21 days. We recommend that this dose regimen be adopted in STZ-induced diabetic studies in male locally reared Wistar rats in.

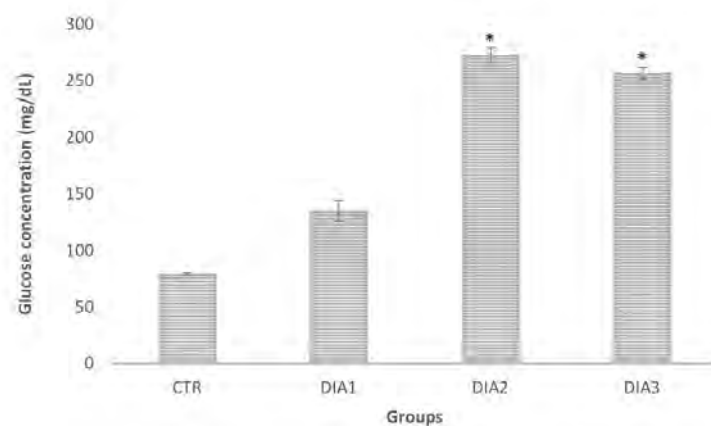


Figure 1: Fasting blood glucose level of control and test rats in (mg/dL), N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DIA2 = 60 mg/kg STZ consecutively, DIA3 = 60 mg/kg STZ three days consecutively

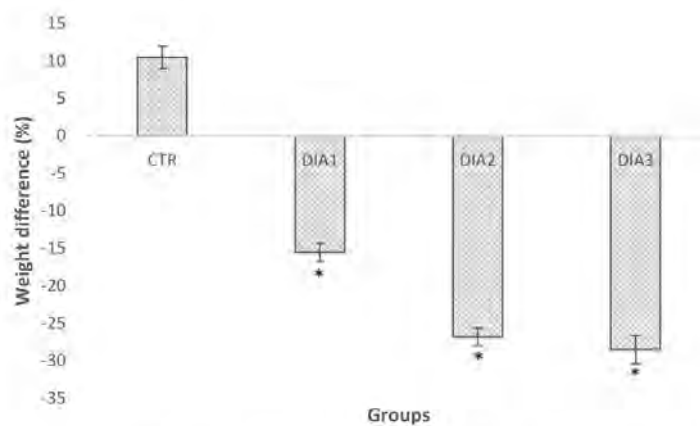


Figure 2: Weight difference of control and test rats in (%), N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DIA2 = 60 mg/kg STZ consecutively, DIA3 = 60 mg/kg STZ three days consecutively

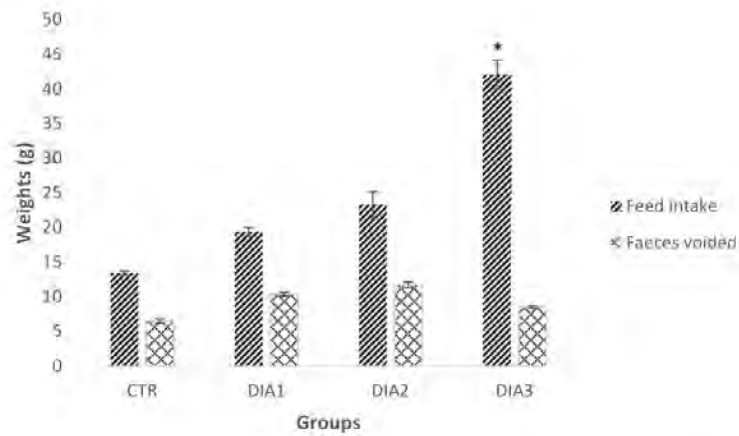


Figure 3: Feed intake and voided faeces of control and test rats in (g), N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DIA2 = 60 mg/kg STZ consecutively, DIA3 = 60 mg/kg STZ three days consecutively

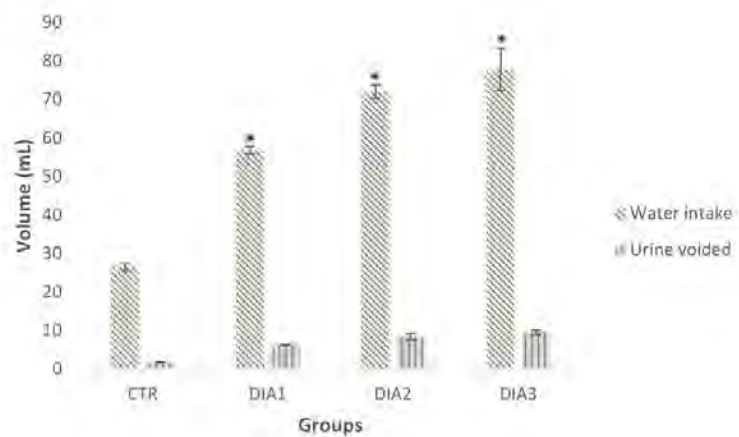


Figure 4: Fluid intake and output of control and test rats in (mL), N = 7, *P < 0.05 from CTR. CTR = Control, DIA1 = 60 mg/kg STZ once, DIA2 = 60 mg/kg STZ consecutively, DIA3 = 60 mg/kg STZ three days consecutively

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PCA067

Placental Mitochondrial Respiratory Function at High-Altitude: Studies of Growth Restriction in Andean Highlanders

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Objectives:

Chronic exposure to hypobaric hypoxia during pregnancy is associated with low birthweight and suppression of electron transfer system (ETS) proteins in placental mitochondria. Some resistance to growth restriction is seen in native high-altitude populations, which may be associated with placental metabolic changes protecting infant growth. It remains unclear whether such alterations in placental metabolism occur in intrauterine growth restriction (IUGR) pregnancies in highland Andeans at altitude. This study aimed to determine the relationship between fetal growth and placental mitochondrial respiration and enzyme activity in highland Andeans at high altitudes.

Methods:

Placental tissue and umbilical cord blood were collected from 50 Andean maternal-infant pairs living in La Paz, Bolivia (~3850m) after scheduled Cesarean delivery. Within this cohort, 26 had infants that were diagnosed with IUGR on prenatal ultrasound and the other 24 had infants of normal growth. Placental tissue was cryopreserved and analyzed using high-resolution respirometry and a substrate-uncoupler-inhibitor titration to assess for oxidative phosphorylation (OXPHOS) and ETS capacity supported by Complexes I, II, and IV. Spectrophotometric enzyme activity assays (EAAs) were used to measure citrate synthase, hydroxyacyl-CoA dehydrogenase (HOAD), lactate dehydrogenase, and hexokinase activities. Respiratory states and EAAs between groups were compared with a Student's t-test and contextualized with clinical data through simple linear regression or one-way ANOVA. These studies were approved by the University of Colorado IRB (Approval No. 14–2178 and 17-1529) and the Ethics Review Boards for the Caja Nacional de Salud and Hospital Materno-Infantil in La Paz, Bolivia.

Results:

There was no significant difference in mass-specific respiratory capacity in placentas from IUGR and non-IUGR infants in LEAK state, Complex I, Complex II, or Complex IV supported respiration (Figure 1). However, when only term pregnancies were considered, maximal respiratory capacity supported by substrates for Complexes I+II and max ETC were lower ($p < 0.05$) in the IUGR cohort (Figure 2). The IUGR placentas also had a 34.5% smaller surface

area ($p < 0.01$). Although the umbilical vein PO_2 did not differ by IUGR status, the change in arteriovenous O_2 difference ($v-aO_2$) between umbilical vein and artery was 56.1% larger ($p < 0.01$) in the IUGR cohort when corrected for infant birthweight. Cord blood hemoglobin ($p < 0.05$), hematocrit ($p < 0.05$), and red blood cells ($p < 0.05$) were also higher in the IUGR cohort. There was no significant difference in the activity of any enzyme measured.

Conclusions:

Within highland Andeans at altitude, there was a mild suppression of mass-specific oxidative phosphorylation in the placenta from IUGR pregnancies, and smaller placental surface areas. The mitochondrial suppression may conserve oxygen and thereby compensate for the reduced surface area for gas/nutrient exchange in the IUGR cohort. It is possible that the influence of altitude or pre-term status has masked the full effect of metabolic changes in IUGR pregnancies. The elevated change in $v-aO_2$ per birthweight in the IUGR cohort suggests a fetal hypoxia response despite a similar PO_2 in the umbilical vein.

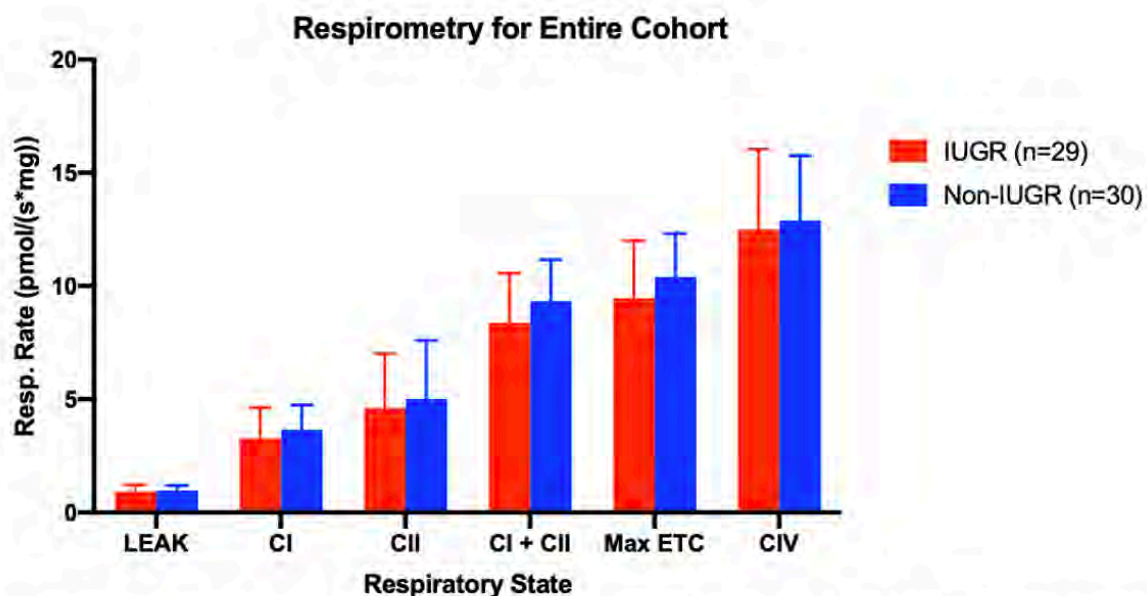


Figure 1: Placental mitochondrial respiratory capacity for entire cohort. Mass corrected oxygen consumption for placental tissue collected from the IUGR (red) and non-IUGR (blue) cohorts for oxidative phosphorylation (OXPHOS) supported by substrates for Complexes I (CI), II (CII), and IV (CIV) and Max ETC. Bars represent mean + SD. ** $p \leq 0.01$, *** $p \leq 0.001$, student's t-test.

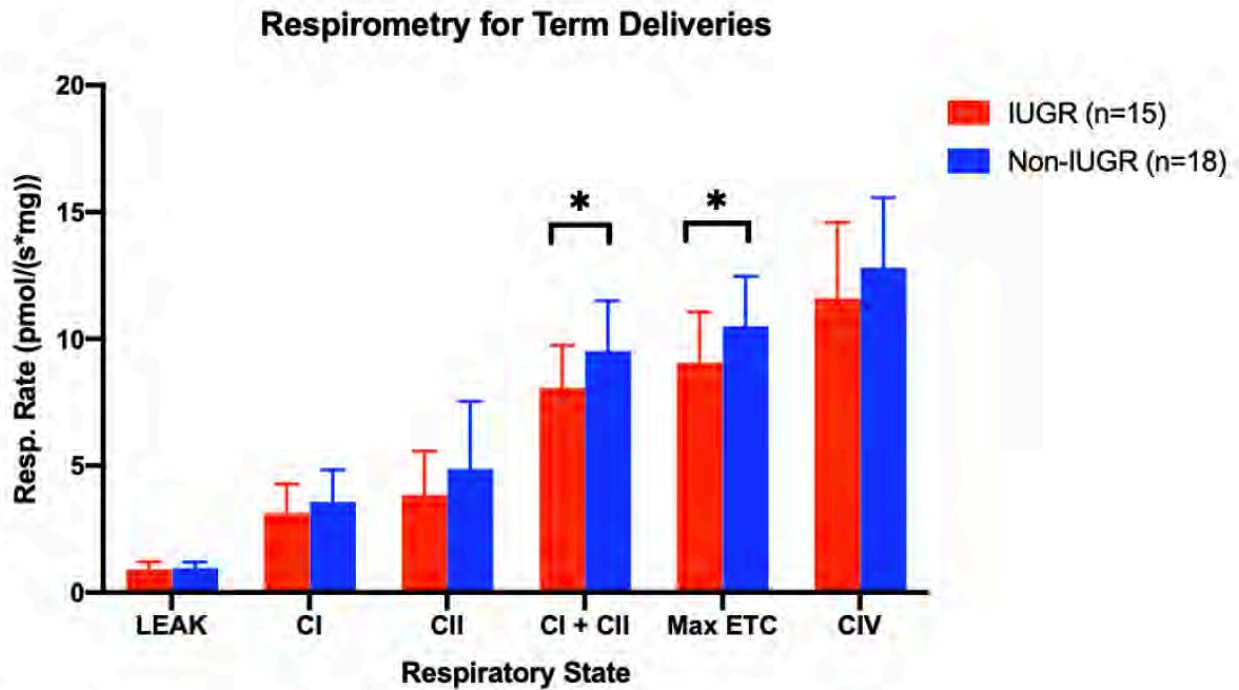


Figure 2: Placental mitochondrial respiratory capacity for term deliveries. Mass corrected oxygen consumption for placental tissue collected from term deliveries from IUGR (red) and non-IUGR (blue) cohorts for oxidative phosphorylation (OXPHOS) supported by substrates for Complexes I (CI), II (CII), and IV (CIV) and Max ETC. Bars represent mean + SD. ** $p \leq 0.01$, *** $p \leq 0.001$, student's t-test.

PCA068

Programming of impaired hepatic drug metabolism during pregnancy in sheep: Effects of hypoxic pregnancy and antioxidant treatment in fetuses and adolescent offspring

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Intro: Fetal growth restriction (FGR) affects 10% of pregnancies globally. FGR results from chronic fetal hypoxia which promotes oxidative stress *in utero* and increases the risk of chronic disease in later life (1). These chronic diseases generally require long-term drug treatment where tight control of drug-plasma concentrations within a therapeutic range is essential. Hepatic cytochrome P450 (CYP) enzymes metabolise 70-80% of all clinical drugs – with CYP3A metabolising more than 30% – and their activity is impacted by acute and chronic changes in oxygen and glucose availability (2). Understanding programmed changes in hepatic CYP activity can improve therapeutic outcomes; however, programming of hepatic drug metabolism in hypoxic pregnancy has not been investigated. We have shown that hepatic CYP3A activity is reduced in 21d old lambs born FGR (3), although what is driving this alteration is unknown. Mitochondria-targeted antioxidant treatment (MitoQ) in FGR animal models protects against programmed hypertension in adult offspring (4). Whether this protection extends to the programming of altered CYP activity in hypoxic pregnancy has not been investigated.

Aim: To determine if hypoxic pregnancy impairs hepatic CYP activity in offspring and if maternal MitoQ treatment is protective.

Methods: At 100±1 day of gestational age (dGA; term is 145 days) Welsh mountain ewes carrying singletons were catheterized under general anaesthesia (1.5-2.5mg/kg IV alfaxalone, Alfaxan; maintained with 1.5-2% isoflurane in 60:40 O₂:N₂O) with analgesic administered prior to surgery (1.4 mg/kg SC carprofen). Ewes were randomly allocated to normoxic (21% O₂: n=34) or hypoxic (isobaric chambers, 11% O₂: n=36) pregnancy with MitoQ (MS010 IV, 6mg/kg) or control (saline IV) treatment from 105-138 dGA. Ewes carrying male fetuses were humanely killed with an overdose of sodium pentobarbitone (0.4ml/kg IV, Pentoject) at ~138 dGA. Ewes carrying female fetuses lambed spontaneously and offspring were humanely killed (as per ewes) at 9 months of age (sexual maturity). Activity of 3 and 7 CYP enzymes in fetal and adolescent offspring respectively was determined in isolated hepatic microsomes using established functional assays (5). Data are presented as mean ± SEM and analysed using two-way ANOVA with the Tukey *post hoc* test.

Results: In 9-month-old lambs, hepatic CYP2B6 and CYP2E1 activity was reduced by 22% ($P_{\text{oxygen}}=0.0127$) and 38% ($P_{\text{oxygen}}=0.0349$) respectively in hypoxic pregnancy with no treatment. MitoQ treatment alone in pregnancy significantly decreased CYP1A2 activity by 12% ($P_{\text{treatment}}=0.0011$) and CYP3A activity increased by 22% ($P_{\text{treatment}}=0.0167$). Conversely, in late-gestation fetuses, no significant alterations in CYP activity were observed in any group.

Conclusions: The fetal data are consistent with many CYPs not becoming active until after birth. Hypoxaemia in late pregnancy programmed reduced CYP activity in adolescent offspring, although unchanged CYP3A activity was unexpected and contrasts previous work potentially

due differences in the timing and duration of the hypoxic insult. Despite MitoQ treatment offering protective benefits for hypertension in FGR offspring, our data suggests MitoQ programs increased CYP3A activity and decreased CYP1A2 activity. Programmed changes to hepatic CYP activity in adolescent offspring may alter the efficacy and safety of commonly used therapeutics throughout the life-course.

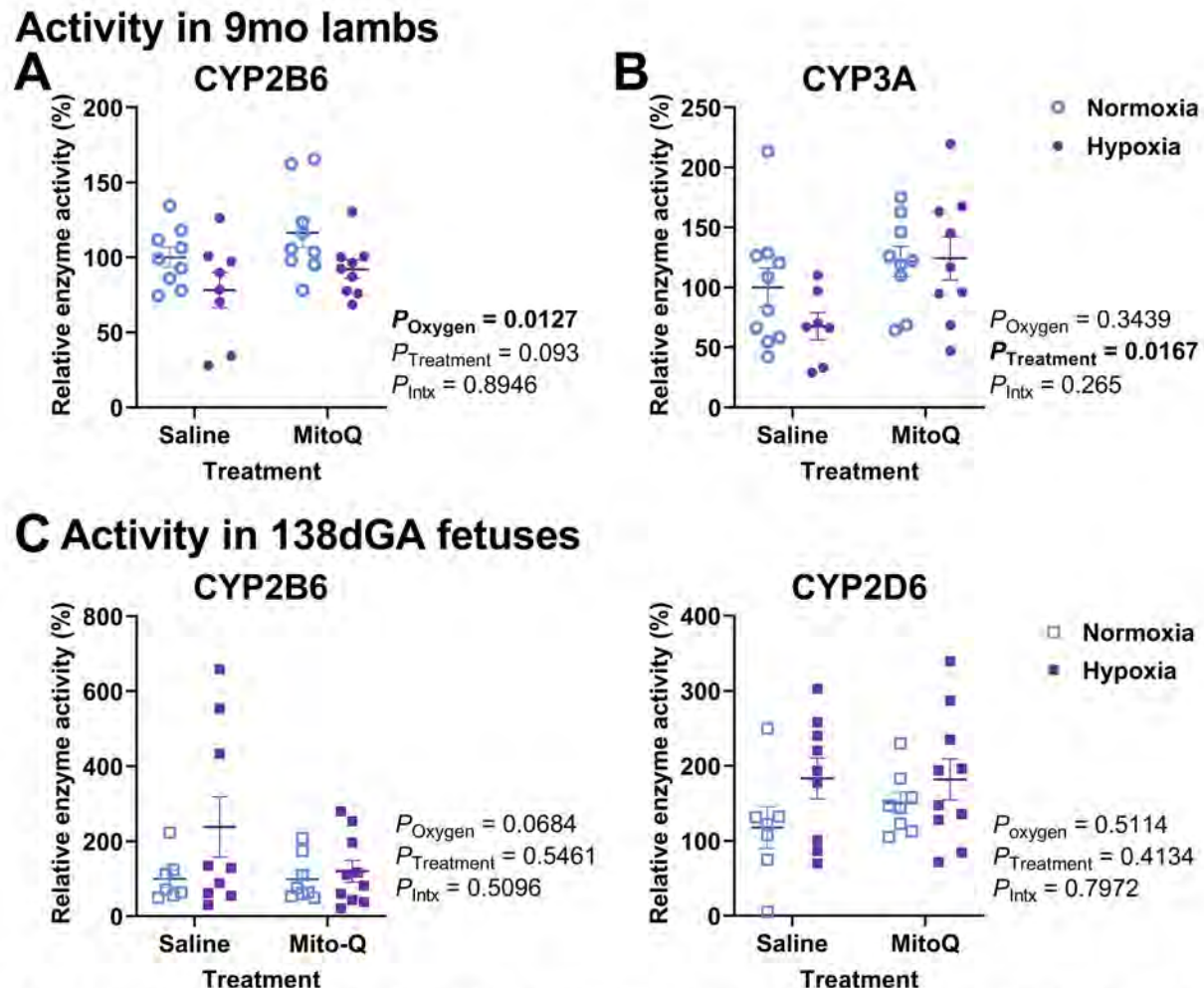


Figure 1: Activity of (A) CYP2B6 and (B) CYP3A in offspring at 9 months of age. (C) Fetal hepatic activity of CYP2B6 and CYP2D6.

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PCA069

Physiological Changes, Prevention and Management of Musculoskeletal Disorders Across the Lifespan: a Mini-Review

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Musculoskeletal disorders (MSDs) are increasingly common among older adults, and they are often associated with physiological ageing processes, such as muscle loss, decreased bone density, and changes in joint structure and function. This natural ageing process can contribute to MSDs, namely osteoporosis, osteoarthritis, and Sarcopenia. An analysis of the Global Burden of Disease (GBD) in 2019 estimated 1.71 billion people have developed MSDs worldwide. Accordingly, it is compulsory to highlight MSDs as they have critical adverse outcomes, including the risk of fractures, functional decline, frailty, and mortality.

For example, Osteoporosis is a quantitative metabolic bone disease that, on a cellular level, results in an imbalance between osteoclastic bone resorption and osteoblastic bone formation. Traditional pathophysiological concepts include low dietary intake of Calcium or Vitamin D, mainly focusing on endocrine mechanisms; however, recent research goes far beyond this. Mechanisms such as interactions between bone and immunity and cellular senescence attracted a growing area of research interest in this field.

In addition, Sarcopenia, or age-related muscle loss, is a frequent physiological change that contributes to developing MSDs. It leads to a decline in the size and mass of the muscle due to the decrease in the cross-sectional area of the fibres. Even though nearly 25% of 65+ year-olds develop sarcopenia, there is still yet no confirmatory diagnosis tool for it. This has attracted many researchers in the field to conduct meta-analysis using cohort and cross-sectional studies to assess the efficacy of different ways of diagnosis.

Osteoarthritis(OA), or “wear and tear arthritis” is a degenerative joint disease. OA was classified as a leading musculoskeletal cause of impaired mobility of the elderly. Taking this into consideration, accurate molecular and chemical causes or mechanisms that degrade cartilage are vague.

This review aims to i) highlight discovered molecular mechanisms that contribute to osteoporosis and how these mechanisms contribute to its pathophysiology, ii) accentuate possible ways of diagnosing sarcopenia and which is most accurate and reliable according to meta-analyses, and iii) summarise the precise molecular mechanisms involved in OA pathogenesis

PCA070

Isotope-specific effects of lithium on mitochondrial calcium phosphate cluster size distribution and calcium capacity

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Introduction and Aims

Lithium salts are established medications in a variety of mental health conditions. The molecular mode of action if lithium is incompletely understood. Many cellular effects associated with lithium treatment are linked to mitochondrial function. We investigated the effects of lithium on mitochondrial calcium handling due to its obvious role in neuronal signaling.

Methods

We investigated the calcium capacity of mitochondria from murine mouse liver in the presence and absence of lithium, both in its natural composition and as pure 6-lithium and 7-lithium. To achieve this, we utilized high-resolution respirometry and fluorometry techniques. Additionally, we examined the subcellular distribution of lithium isotopes using inductively coupled plasma mass spectrometry and nanoscale secondary ion mass spectrometry. Furthermore, we analyzed the formation of amorphous calcium phosphate, both in the presence and absence of lithium isotopes, using 31-phosphorus nuclear magnetic resonance and dynamic light scattering.

Results

Lithium protected against calcium-induced permeability transition (32.84±11.2 min to onset vs 15.69±7.3 min to onset, errors: SD, n=8, p>0.001) and decreased calcium capacity of liver mitochondria (589.8±99.1 nmol/mg vs 635.7±100.3 nmol/mg, errors: SD, n=10, p<0.01) at clinically relevant concentrations. Interestingly, brain mitochondrial calcium capacity was increased, not decreased, by lithium (601.3±64.8 nmol/mg vs 463.2±34.5 nmol/mg, errors: SD, n=5, p<0.01). Further analyses revealed that 7-lithium was more effective than 6-lithium in altering calcium capacity, whereas 6-lithium was more effective in delaying permeability transition. Interestingly, these effects were not attributed to differences in lithium isotope distribution within cells or subcellular compartments. Instead, our in vitro experiments demonstrated that lithium isotopes had distinct effects on the size distribution of amorphous calcium phosphate colloids, which is a plausible mechanism underlying the isotope-specific mitochondrial calcium capacities observed in our study.

Conclusion

We identified mitochondrial calcium management as a plausible component of clinical lithium effects and found evidence for a direct interaction of lithium with amorphous calcium phosphate aggregation. The isotope-specificity of lithium effects provide a promising avenue for the development of more effective lithium-based drugs.

PCA071

Inhibitors of focal adhesion complex formation disrupt anabolic responses to amino acid and growth factor provision in immortalised human primary myotubes

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Focal adhesion complexes (FACs) are large multi-protein structures anchored at the plasma membrane of cells to connect the extracellular matrix to the cytoskeleton. Due to this membrane-spanning location, FACs have predominantly been associated with mechanosensing signalling pathways, particularly in skeletal muscle where FAC-related proteins are loading-responsive(1). Recently, however, FACs have also been implicated in the regulation of mechanistic target of rapamycin complex 1 (mTORC1) activity, a primary regulator of cell anabolism, in response to amino acids (AAs) and/or growth factors (GFs)(2). Importantly, however, these investigations were conducted in non-muscle cell types and therefore FACs role in AA and/or GF-mediated mTORC1 activation in skeletal muscle is yet to be elucidated. Our recent data displayed that mTORC1 activation occurs in close proximity to FACs following anabolic stimuli in human skeletal muscle(3) suggesting signalling responses to elevated AA/GF availability may centre at these complexes *in vivo*. While this displayed the localisation of mTORC1 activity at FACs, further studies specifically assessing the importance of FACs in the regulation of anabolic responses to AAs and/or GFs are required. Therefore, in this investigation we utilised two pharmacological compounds known to disrupt FACs (Y-27632 dihydrochloride – Rho-associated protein kinase inhibitor (50µM), Cilengitide – integrin antagonist (2.5µM)) and studied their effects on anabolic signalling responses to elevated AA (2x minimal essential medium (MEM) concentrations) or GF (10ng/mL IGF-1) concentrations. Immortalised human primary myotubes (C25 cell line) were proliferated and differentiated before being starved of nutrients (EBSS) for 4h in the presence of each compound, followed by a 1h period of AA/GF stimulation. Myotubes were then collected, lysed and prepared for immunoblotting (n=8 for each condition) for a variety of mTORC1-related signalling targets. Independent t-tests were used to test for statistical significance between each compound and an untreated (control) condition (significance set at p<0.05). In response to elevated AAs, Y-27632 reduced RPS6^{Ser240/244} and RPS6^{Ser235/236} phosphorylation compared to control (~36% & ~67% respectively, p<0.01) whilst cilengitide reduced RPS6^{Ser235/236} phosphorylation (~34%, p=0.015) and elevated eEF2^{Thr56} phosphorylation (~50%, p=0.012), all indicative of impaired mTORC1 activity. In response to IGF-1, phosphorylation at both sites on RPS6 was reduced by Y-27632 (RPS6^{Ser240/244} - ~39%, RPS6^{Ser235/236} - 68%, p<0.01), whereas cilengitide had no effect. Further markers of mTORC1 activity (p-4EBP1^{Thr37/46}), GF signalling (p-AKT^{Ser473}) and autophagy (LC3b II/I ratio) were unaltered by the FAC inhibitors in both conditions. Importantly, abundance of a prominent FAC protein (Talin1) and mTOR itself were also unaltered suggesting the effects of Y-27632 and cilengitide occurred independently of changes to FAC or mTOR content. These results show that intact FACs are required for some, but not all, mTORC1-related signalling responses to elevated AAs and GFs in human skeletal muscle cells, which builds on our current understanding of anabolic regulation in this tissue. Further work will employ immunofluorescent staining to confirm FAC disruption and the SUNSET technique to determine effects of global protein synthesis.

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PCA072

Amylin aggregation and suppression of mitochondrial respiratory capacity in the diabetic heart

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Introduction: The diabetic heart is metabolically inflexible, displaying maintained fatty acid oxidation (FAO) but impaired glucose uptake. Amylin is co-released with insulin by pancreatic β -cells, forming deposits locally and in hearts of patients with Type II diabetes mellitus (T2DM). This contrasts with rodent amylin which has a reduced propensity to aggregate. In HIP rats expressing human amylin, deposits occur in the pancreas, and heart, and T2DM ensues. This is not observed in hyperglycaemic (UCD) rats expressing rodent amylin.

Objective: To investigate if cardiac amylin aggregates exacerbate mitochondrial and metabolic changes in diabetic cardiomyopathy.

Methods: All animal experiments conform to the NIH guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at University of Kentucky. Cardiac mitochondrial respiration was studied in male, wild-type (WT), HIP, and UCD rats (14-16 months) using a protocol optimised for frozen samples. Enzyme activities were measured by spectrophotometric assays, and gene expression by qPCR. Respiratory oxygen fluxes (JO_2) were normalised to cardiac tissue wet weight and analysed by One-way ANOVA (n=10).

Results: In HIP rats, cardiac respiratory capacity was lower than in WTs and UCDs. Complex I (CI), Complex I and II (CI&II), and Complex IV-supported (CIV) respiration rates were 57.3% ($P<0.01$), 49.0% ($P<0.01$), and 34.7% ($P<0.05$) lower, respectively, than in WTs. CI&II and CII-supported rates were 39.7% and 56.5% lower than UCDs ($P<0.05$). In comparison with WTs, β -hydroxyacyl CoA dehydrogenase (HOAD) activity was unchanged in UCDs, with a trend towards decreased activity in HIP rats, alongside a trend towards lower expression of other components important for fatty acid oxidation: *Ppara*, *Cpt1b*, *Ucp3*.

Conclusion: Cardiac amylin aggregation in HIP rats suppresses mitochondrial respiratory capacity, and potentially fatty acid oxidation, compared with UCDs and WTs.

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PCA073

Gestational diabetic myometrium and its responsiveness to an anti-diabetic plant, *Thunbergia laurifolia* L., in in vitro and in vivo study.

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Gestational diabetes mellitus (GDM) has a significantly increased risk of spontaneous preterm delivery (SPD) (Boriboonhirunsarn & Tanpong, 2023). Certain medications used in patients without diabetes to control premature contractions should be used with extreme caution in pregnant women with diabetes because they can significantly affect blood sugar control by causing increases in blood glucose concentrations (e.g., terbutaline/brethine) (Peterson et al., 1993). Therefore, there is a need to find complementary therapies. Our main aim was to examine the impact of *Thunbergia laurifolia* L. (TL), a well-established anti-diabetic plant (Kosai et al., 2015), on GDM rat myometrium contractility (*in vitro*) and ultrastructure (*in vivo*). TL leaves were ethanolic extracted, and the effect was tested. The animal care followed the guidelines of the committee of Care and Use of Laboratory Animal Resources, National Research Council of Thailand. The experiment procedures were approved by the Institutional Animal Care and Use Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand (Approval no. 13/2560). A single dose of streptozotocin (STZ) 60 mg/kg BW was given to induce GDM in pregnant rats on day 5 of gestation. Blood samples were obtained from a tail vein puncture, and glucose levels were monitored two days after STZ to confirm diabetic induction by a glucometer. Diabetes was defined as hyperglycemia exceeding 200-300 mg/dL. For *in vitro* study, GDM rats were humanely killed at term, and myometrial strips were isolated for isometric force measurements. For *in vivo* study, TL was orally and daily administrated at high (500 mg/kg BW) and low doses (50 mg/kg BW) from day 7 of gestation until term, and its effects on blood glucose and myometrial ultrastructure were investigated. The results showed that TL extract significantly inhibited spontaneous uterine contractility in a concentration-dependent manner with IC₅₀ of 1.19 mg/ml (n = 5). The spontaneous force was significantly reduced to 76±8% when compared with 100% control (n = 5). The significant reduction was still active, continuing in combination with KCl depolarization and oxytocin-mediated contractility in both groups. Thus, the force was reduced to 79±7% and 74±7% in the presence of KCL and oxytocin when compared with 100% control (n = 5). TL significantly decreased blood glucose levels in GDM (n = 5). Both high and low doses of TL significantly decreased blood glucose levels to 418 ± 29 and 431 ± 11 mg/dL when compared with GDM non-treated control 588 ± 13 mg/dL. Histological study revealed that the muscular layer significantly increased in thickness in both high and low doses of TL compared with GDM control (67.35 ± 2.89%, 62.30 ± 2.26%, and 52.66 ± 2.36%, respectively). Taken together, TL produced an inhibitory effect on GDM myometrium, irrespective of the type of contractility. Along with the effect of reducing blood glucose levels, TL also restores deleterious GDM myometrium by increasing its thickness. Therefore, TL is worth further investigation in human myometrium and has developed as a tocolytic agent to prevent SPD in GDM.

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PCA074

Garlic Oil Improves Small Intestinal Motility in Experimentally Induced Type II Diabetes Mellitus in Female Wistar Rats

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Diabetes mellitus impairs small intestinal motility; however, little studies have demonstrated the effect of garlic oil on small intestinal motility, so .his study evaluated the beneficial effects of garlic oil in type 2 diabetes mellitus. Thirty-six adult female Wistar rats were allocated into: control (C), garlic oil supplemented non-diabetic, diabetic, and garlic oil-treated diabetic groups. Rats were anesthetized with pentobarbitone (40 mg/kg BW), then small intestinal segments were studied for motility parameters and oxidative markers. Nasoanal length, waist circumference, fasting blood glucose level (FBG) and plasma insulin level were determined. Compared to control group, diabetic rats had reduced average force of contraction and motility index in all small intestinal segments, accompanied by decreased average duration of contraction only in jejunum, in addition to hyperglycemia, insulin resistance, prominent oxidative stress and obesity denoted by motility parameters, fasting blood glucose, HOMA-IR, intestinal MDA and waist circumference. Garlic Oil non-diabetic rats had reduced average force of contraction and motility index in all small intestinal segments, despite persistent higher Lee index and waist circumference. However, Garlic oil treated-diabetic rats had improved effects on small intestinal motility in almost all small intestinal segments and controlled the oxidative stress. In conclusion, decreased small intestinal motility was present in DM, mostly by oxidative stress, and in normal rats supplemented with garlic oil. However, garlic oil treatment in diabetic rats resulted in an improvement in small intestinal motility and in a remarkable anti-hyperglycemic effect, mostly due to its antioxidant effect.

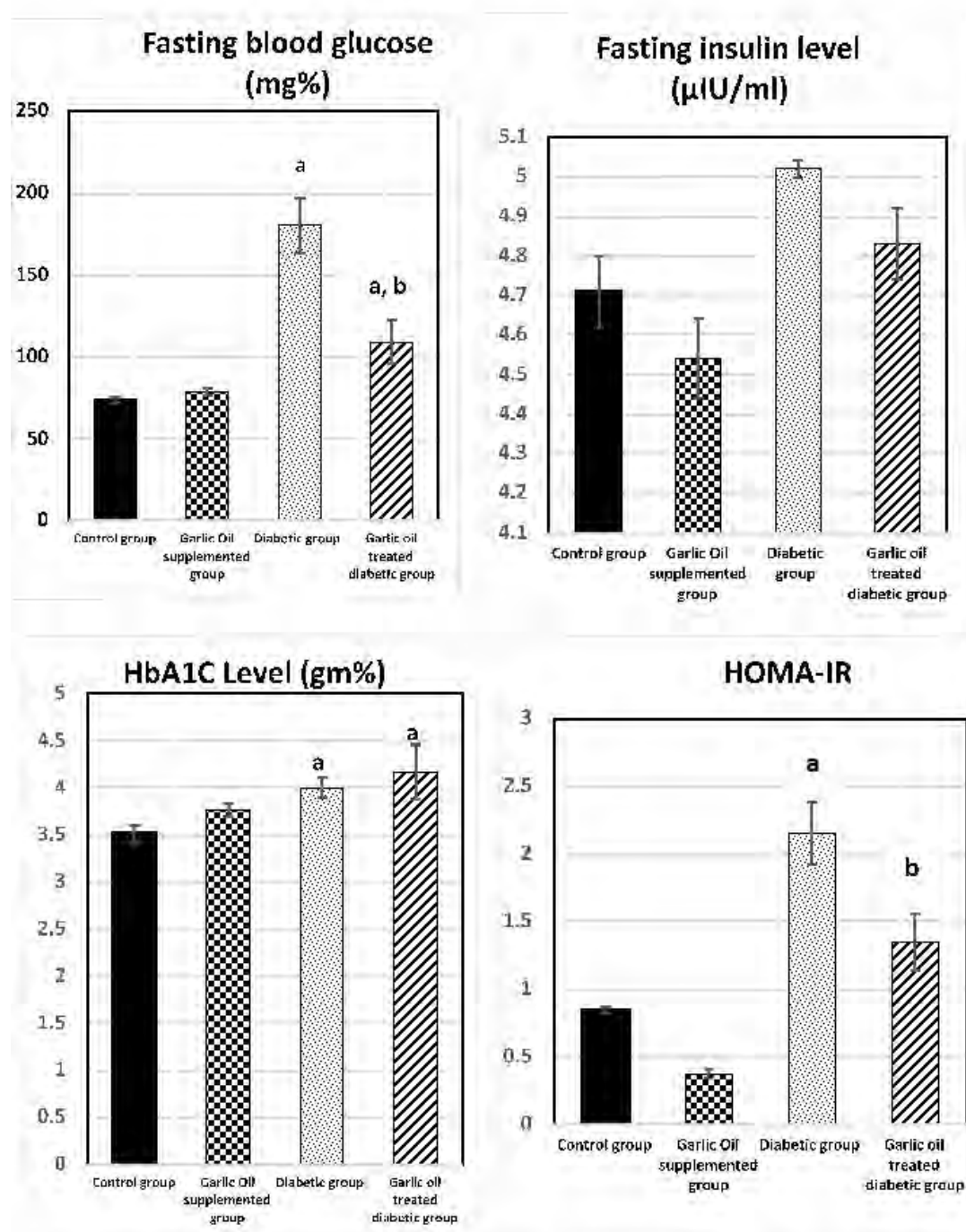


Figure (7): Changes in fasting blood glucose (mg%), fasting insulin level (μIU/ml), HOMA-IR and HbA1c (gm%) in the different studied groups.

a: significance by LSD < 0.05 from the control group

b : significance by LSD < 0.05 from the diabetic group

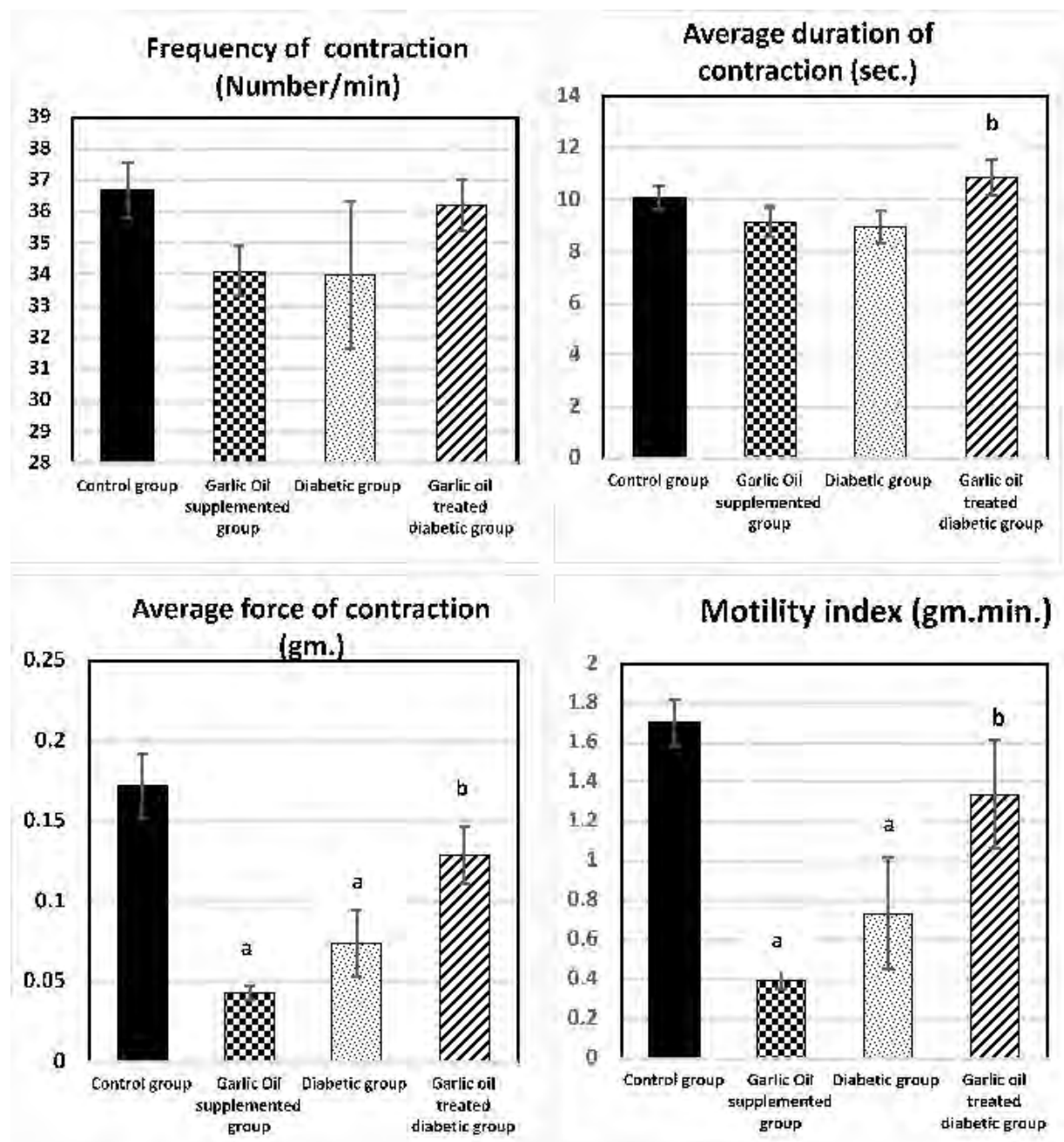


Figure (1): Changes in duodenal motility parameters [frequency of contraction (number of contractions / min.), average duration of contraction (sec.), average force of contraction (gm.) and motility index (gm.min.)] in the different studied groups.

a : significance by LSD < 0.05 from the control group

b : significance by LSD < 0.05 from the diabetic group

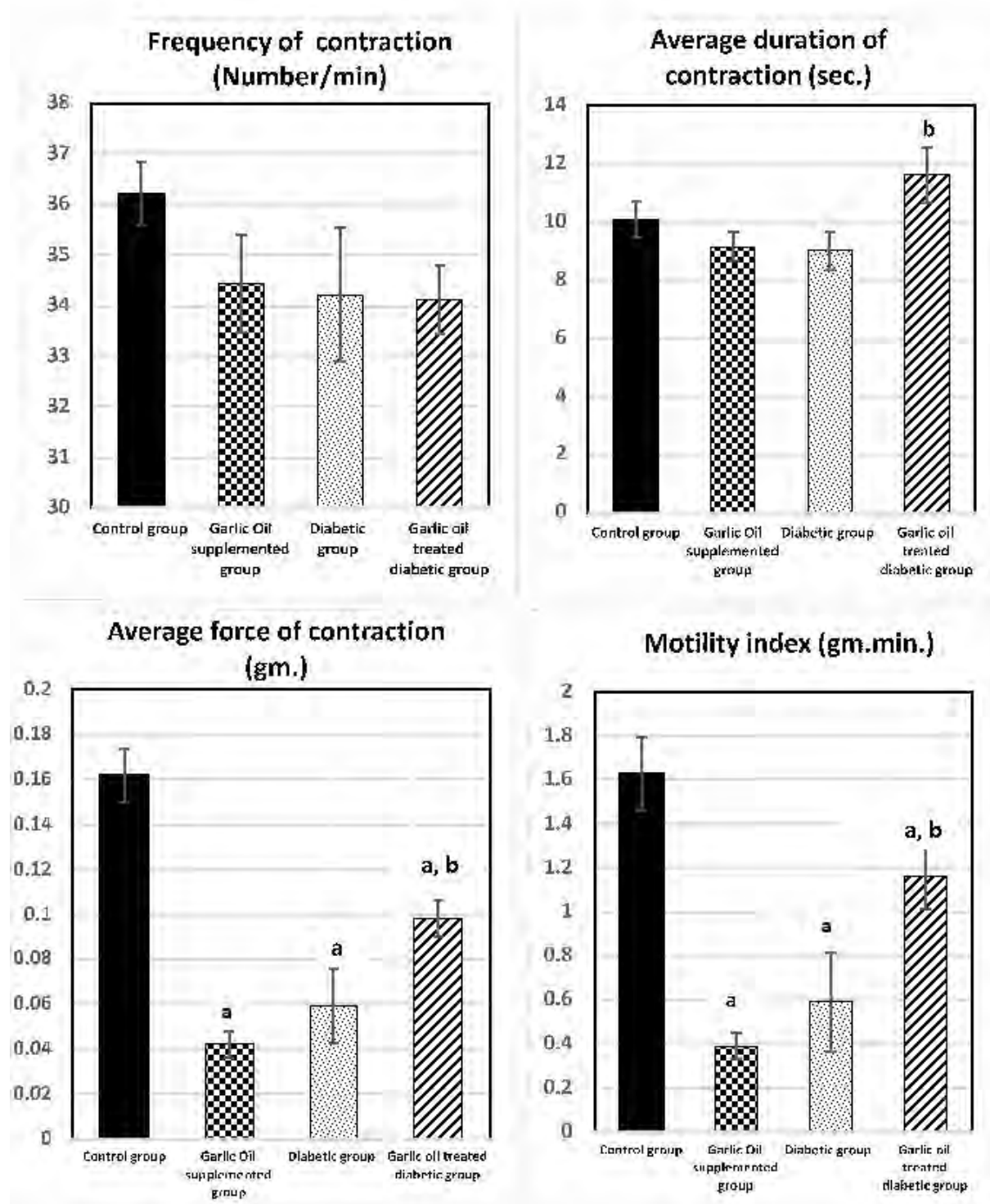


Figure (5): Changes in ileal motility parameters [frequency of contraction (number of contractions / min.), average duration of contraction (sec.), average force of contraction (gm.) and motility index (gm.min.)] in the different studied groups.

a : significance by LSD < 0.05 from the control group

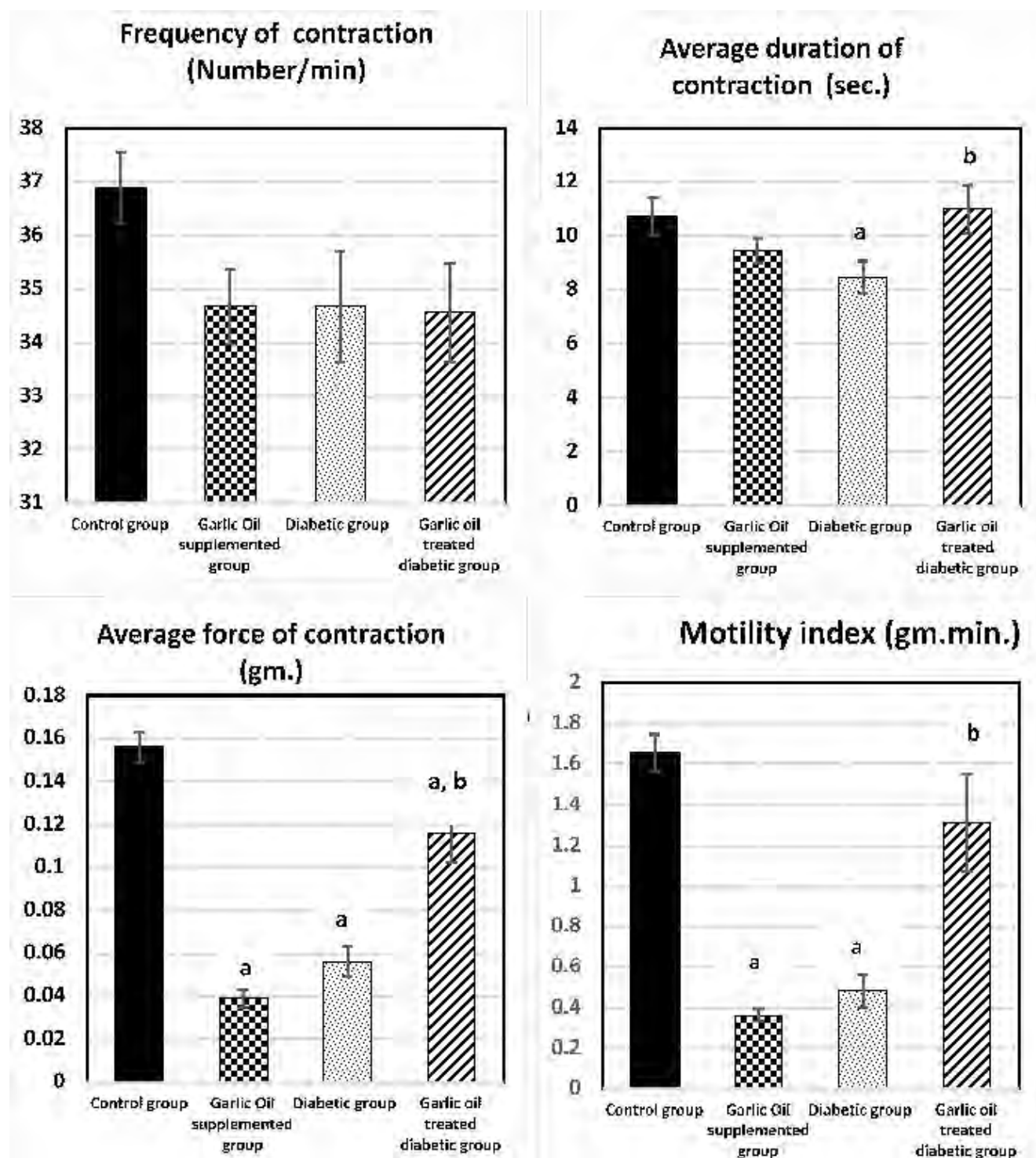


Figure (3): Changes in jejunal motility parameters [frequency of contraction (number of contractions / min.), average duration of contraction (sec.), average force of contraction (gm.) and motility index (gm.min.)] in the different studied groups.

a : significance by LSD < 0.05 from the control group

b : significance by LSD < 0.05 from the diabetic group

PCA075

The effects of pharmacological modulation of pancreatic cell death on acute/chronic pancreatitis and pancreatic fibrosis

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Acute pancreatitis (AP) is a serious pancreatic disease, characterised by sudden onset, severe pain, and overall mortality rates of up to 10%. Unlike its acute form, which is potentially reversible, chronic pancreatitis (CP) worsens over time, leading to atrophy of the pancreatic parenchyma and fibrosis mediated by activated pancreatic stellate cells (PSCs). This often results in impaired digestion, diabetes, and increases the risk of pancreatic cancer. Currently, there is no effective and specific treatment for AP or CP.

The hallmark of pancreatitis is premature activation of digestive enzymes stored in pancreatic acinar cells (PACs). Active enzymes damage neighbouring PACs causing necrotic death and triggering the release and activation of other enzymes, thus promoting a self-perpetuating chain reaction of autodigestion and inflammation.

A potential strategy to break the vicious cycle of pancreatic necrosis and mitigate the disease is to direct PACs towards apoptotic death, which is not normally associated with the release of cellular content. Therefore the aim of this study was to test the therapeutic benefit of pharmacological modulation of cell death by Bcl-2 inhibitors in mouse models of acute and chronic pancreatitis.

All animal experiments on C57BL6J mice were approved by our Local Ethics Committee, approval numbers: 106/2020, 312/2021. In both models, the mice were divided into equal groups (n=6). AP was induced by seven hourly intraperitoneal injections of caerulein (50 µg/kg), while control mice were only given saline. In the CP model, mice received caerulein administrations twice a week for eight weeks. In addition, mice received one of the tested Bcl-2 inhibitors or an appropriate vehicle. The efficacy of therapy was evaluated by histological scoring (H/E or Sirius Red staining) and by comparing the extent of necrosis and apoptosis (IHF for cleaved caspase-3) in the pancreata of the experimental animals.

Our results show that 1 h treatment with caerulein induced 20.24±2.43% of necrosis and 9.67±2.35% of apoptosis in freshly isolated mouse PACs; and two Bcl-2 inhibitors reduced caerulein-elicited necrosis to 6.4±1.67% (p<0.001) or 7.44±3.84% (p<0.001), while increasing apoptosis to 15.06±2.67% (p<0.001) or 17.13±1.44% (p<0.001).

Bcl-2 inhibition significantly improved histological score in the AP mouse model. The overall histological severity score for the AP group was 14.25±2.04 points, and Bcl-2 inhibition reduced it to 6.67±2.14 points (p<0.001) and 10.25±2.25 points (p=0.005). The inhibitors not only

decreased necrosis from $29.22 \pm 11.21\%$ (of the tissue area) in the AP group to $1.75 \pm 2.06\%$ ($p < 0.001$) and $10.81 \pm 4.24\%$ ($p < 0.001$) in the treatment groups, but also increased apoptosis from 0.7 ± 0.69 (apoptotic cells per mm^2 of the tissue) in AP to 26.87 ± 9.63 ($p = 0.002$) and 58.16 ± 41.83 ($p = 0.0027$). In contrast, long-term therapy did not show efficacy in the CP model, and one of the inhibitors exacerbated caerulein-induced fibrosis in the organ.

Short-term Bcl-2 inhibition has the potential to shift acinar cell death from necrosis to apoptosis and thus could find its application as a therapeutic strategy in severe cases of AP. Since one of the Bcl-2 inhibitors is currently approved for the treatment of leukaemia, its use should be carefully considered in patients with concomitant CP.

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PCA076

Exploring the link between pro-inflammatory cytokines and carotid body dysfunction in metabolic diseases

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Introduction and aim: Metabolic diseases are regarded as a leading cause of mortality and morbidity worldwide. Carotid bodies (CBs), which were traditionally considered oxygen-sensitive organs, are also metabolic sensors that play a role in the development of metabolic disorders [1-3]. In metabolic disease states the CB activity is increased and the abolishment of its activity improves metabolic function [1-3]. Inflammatory cytokines are among the factors that can contribute to CB dysfunction in dysmetabolic states [4]. Indeed, the presence of inflammatory cytokines and their corresponding receptors in the CB have been reported [1,4]. In this study, we investigated the contribution of CB to the ventilatory responses induced by pro-inflammatory cytokines, as well as the effect of pro-inflammatory cytokines on CB function.

Methods: Two groups of Wistar rats were used: a control group fed with a standard diet (CTL) and a high-fat (HF) group fed with a diet rich in lipids (60% energy from fat) for 3 weeks. The animals were anesthetized with pentobarbital sodium (60mg/kg i.p.) and at the end of the experimental protocol were killed with an overdose of the same anesthetic. The effect of IL-6 on ventilation was tested by administering IL-6 (0.5 and 5 ng/ml) into the femoral vein in CTL animals with and without carotid sinus nerve (CSN) resection and on the release of adenosine from isolated CBs [5]. To quantify adenosine release, CBs were incubated in normoxia (20% O₂ + 5% CO₂, balanced N₂) in the presence of erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA; an inhibitor of adenosine deaminase, 2.5 µM) for 10 min, followed by incubation during 30 min with IL-6 (1ng/ml) in normoxic conditions. Adenosine levels were quantified by HPLC with UV detection. TNF-α levels and the receptors for IL-1β and IL-6 of CTL and HF animals were evaluated by immunohistochemistry. One-way ANOVA with Dunnett's comparison test and Student's t-test was used. Experiments followed the 2010/63/EU European Union Directive and were approved by NMS Ethics Committee and the Portuguese Authority for Animal Health.

Results: In control animals, IL-6 enhanced basal ventilation, an effect abolished by CSN resection. Moreover, in CTL animals, IL-6 increased by 87% (p<0.05) the adenosine release from the CB. HF diet intake for 3 weeks, increased by 80% (p<0.01) and 46% (p<0.01), respectively, the TNF-α immunoreactivity and IL-1β receptor in CB, compared to CTL animals. Additionally, the immunoreactivity for the IL-6 receptor remained unaltered.

Conclusion: We conclude that the CB plays a crucial role mediating IL-6's effect on ventilation, an effect that can involve the stimulation of adenosine release from the CB. Additionally, HF diet intake promotes inflammation within the CB. Taken together, these results suggest that pro-inflammatory cytokines may contribute to CB dysfunction in metabolic diseases.

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PCA077

Evaluation of Risk Factors for Obesity among HIV Positive Adults on Antiretroviral Therapy in Delta State, Nigeria

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Abstract

A substantial shift in the causes of morbidity and mortality among HIV-infected humans has been reported in recent times as a result of increasing access to antiretroviral medications [1]. This paradigm shift has transformed the once fatal illness into a chronic medical condition [1-2]. Literature reports on the biomarkers of immunity and nutritional health status have been reported more frequently, especially in nations most severely hit by the HIV epidemic [3-5]. Information on the health indicators of obesity and HIV, and their associated risk factors is nonetheless scarce. This study therefore evaluated the risk factors for obesity in HIV positive adults on antiretroviral therapy (ART) in Central Hospital, Warri Nigeria. This study analyzed retrospectively collected data on HIV positive adults (18-50years) on ART in Central Hospital Warri Nigeria. A total of 500 records from 2018 to 2022 were included in this study. Obtained data includes clinical (CD₄ count in cells/mm³) and anthropometric parameters (body weight in kg, height in meters, body mass index in kg/m²). A multivariate analysis of obtained data was used to determine the risk factors for obesity among patients included in this study. The number of adults whose body mass index (BMI) is more than 30 increases progressively from commencement of this study. The observed differences were statistically significant ($p < 0.01$). Female gender (OR 2.09; 95% CI: 1.75 - 2.50, low baseline BMI less than 18.5 (OR 2.4; 95% CI: 1.5 - 2.9), age 31-50years (OR 1.33; 95% CI: 1.13 - 1.56), and baseline CD4 count less than 200cells/mm³ (OR 1.44; 95% CI: 1.18 - 1.76) were associated with the development of obesity at multivariate analysis. The patients' marital status, level of education, identifiable risk for HIV transmission, social class, and opportunistic infections were not associated with obesity after controlling for confounding variables. Hence, programs aimed at preventing obesity in HIV-positive adults on ART should be incorporated into the national guideline, with more focus on women, and other patients with the identified risk factors.

Keywords: Obesity, HIV Positive Adults, Antiretroviral Therapy

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PCA078

Effects of glyphosate-based herbicide exposure on adiposity and liver histopathology in female mice at reproductive age submitted or not to bilateral ovariectomy

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Women display augmented risk to obesity and hepatic steatosis, after bilateral oophorectomy. Some environmental chemicals (EC) can worsen these conditions. The glyphosate-based herbicides (GBHs) are an EC and studies have showed that may predispose to various chronic diseases. Here we evaluated the effects of GBH exposure on glucose tolerance, adiposity, and liver histopathology in female mice, at reproductive age, submitted or not to bilateral ovariectomy. For this, 80-days old C57Bl/6 female mice received a subcutaneous injection of 2 mg/kg meloxicam and an intraperitoneal (IP) injection of 3 mg/kg acepromazine. After 30 minutes, females were anesthetized via IP (10 mg/kg xylazine plus 100 mg/kg ketamine), and randomly ovariectomized (OVX) or sham-operated. After 30 days, sham and OVX females were underwent to a daily gavage of distilled water [vehicle; SHAM0 (n=9) and OVX0 (n=7) groups, respectively], containing or not, 0.5 [SHAM0.5 (n=9) and OVX0.5 (n=9) groups, respectively] or 50 mg/kg GBH [SHAM50 (n=9) and OVX50 (n=9) groups, respectively] for 60 days. Afterward, glucose tolerance, body weight (BW) and plasma lipids, and aspartate (AST) and alanine (ALT) aminotransferases were evaluated. Retroperitoneal fat pads and the liver were weighted and processed for histology. Results were analyzed using two-way ANOVA followed by Tukey test ($p < 0.05$). Experimental procedures were in accordance with brazilian's ethical standards and approved by the animal use committee (Process: 0477634/2021). OVX mice exhibited increased retroperitoneal fat stores (4.6 ± 0.6 mg/gBW) with hypertrophic adipocytes (53.1 ± 3.5 μ m), but similar BW (22.6 ± 0.6 g) of SHAM0 (2.5 ± 0.2 mg/gBW, 34.5 ± 1.2 μ m and 20.8 ± 0.6 g, respectively). GBH exposure did not change these parameters in OVX0.5, OVX50, SHAM0.5 or SHAM50 female mice. OVX0 and SHAM0 females displayed similar fasting glycemia (101.1 ± 4.4 and 87.7 ± 3.4 mg/dL), triglyceridemia (34.9 ± 1.3 and 42.4 ± 2.8 mg/dL), cholesterolemia (94.0 ± 3.1 and 100.3 ± 6.2 mg/dL), AST (36.8 ± 4.6 and 27.5 ± 3.4 U/mL) and ALT plasma levels (19.7 ± 2.7 and 15.3 ± 1.2 U/mL), respectively. Glucose tolerance did not differ among OVX0 (9834 ± 344 mg/dL.min⁻¹) and SHAM0 (8702 ± 287 mg/dL.min⁻¹). GBH exposure at 0.5 or 50 mg/kg/day did not modify all these plasma biochemical parameters in OVX and SHAM mice. Liver weight was not altered in OVX0 (39.7 ± 0.9 mg/g BW) and SHAM0 females (42.3 ± 1.2 mg/g BW), or by the exposure of these mice to 0.5 (OVX0.5: 40.7 ± 0.9 and SHAM0.5: 39.6 ± 1.3 mg/g BW) and 50 mg/kg GBH (OVX50: 41.6 ± 0.6 and SHAM50: 41.9 ± 1.2 mg/g BW). But liver samples of OVX0 mice exhibited mild hepatic microvesicular steatosis. GBH increased hepatic steatosis only in SHAM0.5 females, since 44.4% and 11.11% of their liver samples exhibited moderate and mild steatosis, respectively. Therefore, GBH exposure, at the doses and time used here, not modified adiposity or plasma biochemical parameters in OVX or SHAM females. But at the dose of 0.5 mg/kg/day, GBH may change hepatic lipid metabolism in females with regular ovarian cycle.

PCA079

Effect of nutraceutical combination as an integrative approach on the kidney function in the high-fat diet-induced obese Wistar rats

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Introduction: Globally, Obesity is found to be a separate risk factor for kidney damage, according to research using rat models. Nutraceuticals have garnered considerable interest in obesity research due to their role in etiopathogenesis. In addition to their potential nutritional, safety, and therapeutic effects, they provide various health benefits, including the prevention and treatment of diseases. Nutrition is also recommended as a therapy for managing CKD by KDOQI guidelines.

Aim: The study aims to investigate whether the developed nutraceutical combination (NuTC) is effective in preventing high fat diet (HFD) -induced chronic kidney disease in obese Wistar rats, by assessing physiological, biochemical, and histopathological indices.

Methodology: The protocol was approved by the Institutional Animal ethical committee (IAEC/KMC/66/2019). Obesity was induced in male Wistar rats by feeding with a high-fat diet for 11 months and studied the effect of change in nutrition on kidney functions. Rats were randomly assigned to one of three diets containing regular chow diet (Standard control- NC), HFD infused with lard (Disease control- DC), and NuTC(intervention group) (6 animals in each group). The diet used for intervention was made using locally available barley, fish oil, moong bean, flax seed, and foxtail millet. Blood samples were collected for renal functional measurements (urea and creatinine), and kidneys were examined for histological evaluation to study the variation across 0-90 days. Jamovi 2.3.21 is used to analyze the data statistically.

Results: After 90 days, the rats in the HFD group had significantly higher body weight compared to NC ($p < 0.001$) and HFD+NuTC ($p < 0.001$) groups. Similarly, the rats in the HFD group had significantly higher U (77.26 ± 4.89) and Cr (1.34 ± 0.38) in mg/dL compared to NC and HFD+ NuTC (42.41 ± 2.87 , 0.488 ± 0.04) respectively. Thus, compared to the HFD group, the intervention group had a significant decrease in body weight and renal functions ($p < 0.001$). The NC group showed normal renal articulator (Figure 1a); the HFD diet group showed renal cortex with cellular glomeruli with endocapillary congestion and adjoining tubular system showing luminal hyaline cast with congestion of stromal arterioles (Figure 1b); and HFD+NuTC group showed renal cortex with cellular glomeruli with endocapillary congestion and adjoining tubular system appearing unremarkable with congestion of the stromal arterioles (Figure 1c).

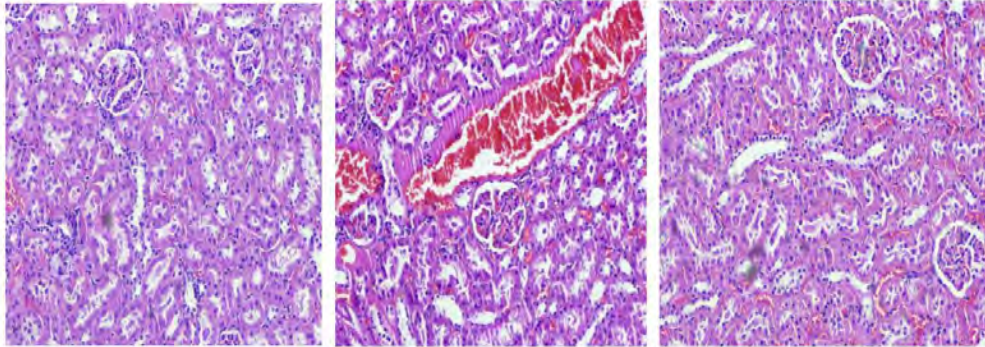
Conclusion:

Pharmaceutical therapy is the most common modality to reduce the CVD burden in CKD. Although pharmaceuticals aim to treat oxidative stress, inflammation, and nephropathies in CKD, they have inherent limitations, as they exhibit adverse side effects. Components of the

nutraceutical prepared in our study also contain omega-3 fatty acids, protein, fiber, vitamins, and minerals that help recover from kidney damage due to obesity. Overall our study observed a statistically significant ($p < 0.05$) reduction in body weight and an improvement in renal function tests in NuTC group compared with the DC group.

To conclude, the combination of nutraceuticals developed shows evidence of improvement in both obesity and renal functions in high-fat diet-induced obese Wistar rats.

Figure 01: Histopathology of Renal Cortex : a: NC; b: HFD; c: HFD+NuTC



PCA080

The effect of L-type amino acid transporter 1 overexpression in response to leucine administration in C2C12 muscle cells

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Introduction:

Skeletal muscle mass is regulated by the net balance between muscle protein synthesis and breakdown. Essential amino acids are the substrates of muscle protein and activate muscle anabolism strongly (1). Leucine, one of the essential amino acids, has the strongest anabolic capacity and is known to exert this after being transported into muscle cells through L-type amino acid transporter 1 (LAT1) (1-3). However, the role of LAT1 in skeletal muscle protein synthesis and hypertrophy is not fully understood.

Aims:

Here, we made murine C2C12 myoblasts overexpressing the LAT1 and investigated the role of LAT1 in muscle growth and muscle protein synthesis response after leucine administration.

Methods:

LAT1 overexpressing cells (LAT1-OE) and control cells were made by transferring the LAT1 gene or vehicle into C2C12 myoblasts using in vitro electroporation (n = 6 for each group). One day after the electroporation, the cells were cultured in DMEM containing 2% horse serum and induced differentiation into myotubes for 6 days. After the differentiation, the myotubes were administered with 5 mM leucine. Data were analyzed using t-test or two-way ANOVA (LAT1-OE × Leucine). If an interaction was observed, Sidak multiple-comparison test was performed.

Results:

The LAT1-OE myotubes showed a lower fusion index and embryonic myosin heavy chain expression compared to the control myotubes ($P < 0.05$). The LAT1-OE myotubes showed lower mTORC1 activity (indicated by the expression of phosphorylated p70S6K^{Thr389}) compared to the control myotubes (main effect of LAT1-OE, $P < 0.05$), but mTORC1 activation induced by leucine administration was not influenced (main effect of leucine, $P < 0.05$).

Conclusion:

These results suggest that the expression of LAT1 negatively regulates muscle growth but does not have a negative effect on the response to leucine administration.

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PCA081

31P-NMR reveals muscle metabolic abnormalities after exercise-induced muscle damage

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Introduction. Exercise-induced muscle damage (EIMD) is particularly prevalent after unaccustomed eccentric contractions and is characterised by muscle weakness and soreness lasting several days (Armstrong, 1984). Previous studies using ³¹P magnetic resonance spectroscopy (MRS) found metabolic muscle abnormalities at rest with increased inorganic phosphate (Pi) and phospho-diester (PDE) contents alongside reduced phosphocreatine (PCr) and adenosine triphosphate concentrations (ATP) (McCully *et al.*, 1988). However, it is unclear if muscle metabolism differs during and after recovery from exercise of damaged muscles, and if changes are associated with perceived effort during exercise. Therefore, the aim of the present study was to investigate whether phosphorous metabolism changes with EIMD and whether changes are related to perceived effort during exercise.

Methods. Twenty physically active, healthy volunteers (age 23.4±4.0 years; BMI 23.6±2.4 Kg/m²; training hours 5.4±3.3 (mean ±SD)) were eligible and provided written, informed consent to take part in the study. Participants visited the laboratory for a familiarization session, the first (baseline) experimental session and the follow-up session 48h after baseline (48h EIMD). EIMD was induced by repetitive high-intensity eccentric single-leg extension contractions. Assessments at baseline and 48h after EIMD were implemented in both legs. A visual analog scale (VAS_{SQ}) assessed knee-extensors soreness and strength was assessed by single-leg maximal voluntary knee extension isometric contractions (MVC). Both thighs were imaged for quadriceps cross-sectional area (Qcsa) and ³¹P MRS at rest, during 3-min sustained isometric contractions and for 2-mins of resting recovery (Sleigh *et al.*, 2016). Data between control and EIMD leg were analysed using two-way repeated measures ANOVA. The area under the curve (AUC) and differences for the kinetic changes (slopes) were determined in EIMD leg for Pi/PCr and PCr and were compared with a paired t-test.

Results. EIMD was evident at 48h from the significant time, leg, and time x leg interactions for MVC, Qcsa, and VAS_{SQ} (all p<0.002; Table 1). EIMD leg showed 18±4% reduction of MVC, 2.80 ± 0.04% increase of Qcsa, 20.8±0.1% increase in RPE, and 8267±1734% increase of VAS_{SQ} at 48h compared with baseline (all p<0.001). Resting PCr, Pi, Pi/PCr and ATP_γ all showed significant effects of Time (all p≤0.010). Resting PCr values were marginally (-2.3%) lower at 48h compared with baseline for both the EIMD and control legs (effect of time p=0.010; time x leg interaction p=0.446). Significant time x leg interaction for Pi, Pi/PCr and ATP_γ (all p≤0.002) was found, as values for Pi and Pi/PCr were higher and ATP_γ was lower in the EIMD leg at 48 h compared with baseline, but control leg values for all these measurements remained

unchanged over 48h (Table 1). Changes in RPE from baseline to 48h were significantly associated with changes to resting Pi, Pi/PCr and ATP γ only in the EIMD leg. Increased slopes from resting to exercise were found for Pi/PCr for EIMD leg, giving greater area under the curve (all $p < 0.01$; Figure 1) but no changes were found for exercise-to-recovery slopes.

Conclusion: Our results suggest that EIMD changes muscle metabolism at 48h, affecting both resting and exercising muscle as well as perceptions of effort.

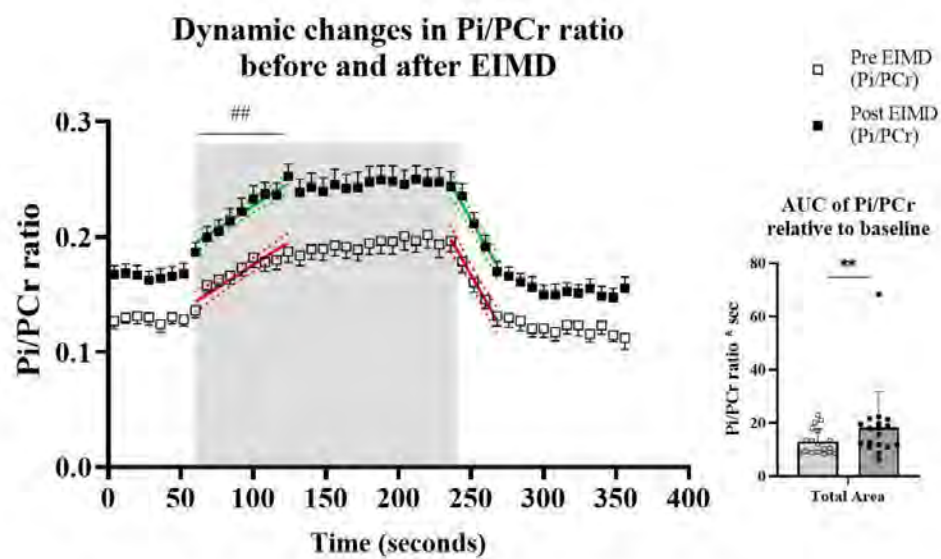


Figure 1. Dynamic changes in Pi/PCr ratio before and after EIMD. ## = $p < 0.01$; ** $p < 0.05$.

Table 1. Indices of skeletal muscle damage and metabolic perturbation following EIMD.

Outcome measured	EIMD Leg		Control Leg		Effects (p-values)	
	Pre	48 h post	Pre	48 h post	Time	Group x time
MVC (N)	634.6 ± 229.3	504.0 ± 160.5	607.2 ± 207.6	611.6 ± 219.50	0.001	<0.001
Qcsa (cm2)	78.2 ± 5.4	82.6 ± 5.7	77.4 ± 5.1	76.8 ± 5.9	0.003	<0.001
RPE	12.0 ± 2.2	14.3 ± 2.1	12.3 ± 2.2	12.5 ± 2.1	<0.001	0.002
Pi (a.u)	118.2 ± 24.5	155.6 ± 45.3	120.1 ± 23.7	117.2 ± 19.3	0.005	0.002
PCr (a.u)	946.5 ± 15.1	925.3 ± 16.7	951.5 ± 15.1	942.5 ± 15.3	0.010	0.446
Pi/PCr (a.u)	12.7 ± 2.6	16.9 ± 3.8	12.7 ± 2.4	12.5 ± 1.6	0.001	<0.001
PDE (a.u)	47.8 ± 22.3	40.6 ± 22.0	51.1 ± 21.5	50.7 ± 23.4	0.123	0.048
ATPγ (a.u)	255.9 ± 46.2	229.5 ± 44.5	25.8 ± 49.3	26.7 ± 42.9	<0.001	<0.001

Data are mean ± SD. Main effects are shown for time (Pre- and 48h post); group (EIMD vs Control leg) and the interaction of the two. Abbreviations: MVC: maximum voluntary contraction from isometric knee extension; CSA: cross sectional area of knee extensors; RPE: rate of perceived exertion; Pi: inorganic phosphate; PCr: phosphocreatine.

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PCA082

Quantifying the superabundance of mitochondria in the sensory terminals of mammalian muscle spindles.

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Mitochondria are unusually abundant in sensory terminals of mammalian muscle spindles; e.g., Landon (1966). We present the first quantitative morphological studies on these mitochondria, aiming to better understand their role in mechanosensory function. 3 adult C57/Bl6SJ mice were killed by Schedule 1 methods (Animals (Scientific Procedures) Act 1986 incorporating European Directive 2010/63/EU). Muscle-spindle-rich portions of deep masseter were removed in physiological saline, fixed (cacodylate buffer, 2.5 % glutaraldehyde/4 % paraformaldehyde; 4°C) for 24 hrs, postfixed (3 % potassium ferrocyanide and 4 % OsO₄), liganded with thiocarbohydrazide and 2 % OsO₄, block stained (1 % uranyl acetate and lead aspartate), and embedded in hard Epon. Sections (90 nm thick, transmission EM) were used to estimate the proportion of sensory-terminal volume occupied by mitochondria (V_v) and the areas of mitochondrial membranes per unit volume of mitochondria (S_v). Digital images (15000x magnification) were overlain with a 0.5mm sampling grid (ImageJ), for stereological analysis (Howard and Reed, 1998). Additional blocks were examined with serial block-face scanning EM (SBF-SEM) for 3-D reconstruction (Reconstruct; Fiala, 2005).

Mean V_v (% $\pm$ s.e.) for sensory terminals from the 3 mice were: 53.03 ± 4.38 ; 63.90 ± 7.37 ; and 47.88 ± 5.66 ; giving an overall average of $54.94 \pm 3.49\%$, when estimated by stereology. To cross-validate this estimate, virtual reconstruction of 575 mitochondria in a sensory-terminal loop within a 15mm-long segment of a bag₂ fibre was made using SBF-SEM, revealing the mitochondria to be unbranched, more or less ovoid, with mean volume $0.358\text{mm}^3 \pm 0.015$ s.e. (range, 0.015-3.57), highly skewed towards the smallest values (median = 0.260). Total mitochondrial volume was 209.41mm^3 and terminal-loop volume was 376.79mm^3 , giving V_v of 55.58%, remarkably close to overall average V_v estimated by stereology. Mean surface area of the mitochondria was $2.14\text{mm}^2 \pm 0.06$ s.e., also skewed towards the smallest values (median = 1.80). Surface area: volume ratio was much less skewed, with mean $7.80\text{mm}^{-1} \pm 0.11$ s.e. and median = 7.04. Overall values of S_v for the sensory-terminal mitochondria were: cristae, $13.67\text{mm}^{-1} \pm 0.58$ s.e.; outer membrane, $4.47\text{mm}^{-1} \pm 0.22$ s.e.; and inner membrane, $4.17\text{mm}^{-1} \pm 0.23$ s.e. These values correspond to absolute areas per mean mitochondrial volume of: 4.90mm^2 ; 1.60mm^2 ; and 1.49mm^2 , respectively.

These data show that volume proportion of mitochondria in the sensory terminals is extraordinarily high, at about 55%. By comparison a 21mm-long sarcomeric segment of a chain fibre, also reconstructed using SBF-SEM, contained 13.1% mitochondria by volume. These mitochondria were elongate, often branched, with mean volume $0.444\text{mm}^3 \pm 0.086$ s.e. and mean surface area $4.557\text{mm}^2 \pm 0.763$ s.e. Using a similar reconstruction technique, Bleck *et al.*, (2018) reported that small (approximately 1000mm^3) volumes of glycolytic and oxidative extrafusil muscle fibres contained about 4 and 10% mitochondria by volume, and even the highly energetic cardiac muscle contained just about 34% in mouse. The functional importance of this superabundance in sensory terminals has yet to be determined, but it may underlie the

observation that sensory ataxia is a commonly listed symptom in many mitochondrial genetic diseases (Ghaoui and Sue, 2018).

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PCA083

Quantifying the predictive value of brain oxygenation and neurometabolism for dementia and cognitive impairment: a study protocol and preliminary data

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Introduction

Dementia is associated with vascular and metabolic abnormalities, several of which may arise prior to prodromal stages (Toth et al., 2017). Detecting these abnormalities may thus be vital for early intervention. Unfortunately, current detection tools are inadequate: there are no objective, accessible, and effective biomarkers for dementia. Near-infrared spectroscopy (NIRS) is a non-invasive neuroimaging method which uses near-infrared light to quantify brain oxygenation and neurometabolism (the latter via broadband NIRS). Anatomical information can be incorporated with high-density NIRS to produce volumetric reconstructions of brain oxygenation, termed 'high-density diffuse optical tomography' (HD-DOT). Metabolic and structurally accurate oxygenation information, provided by broadband NIRS and HD-DOT, may be crucial to understanding dementia, but this has not been explored. A study was thus developed to evaluate the diagnostic value of measures of brain oxygenation and metabolism by interrogating resting state and functional differences in dementia.

Methods

This observational study will aim to recruit twenty participants with Alzheimer's Disease, Dementia with Lewy Bodies, Mild-Cognitive Impairment, and healthy controls (total $n = 80$). Clinicaltrials.gov ID: NCT05460143. Participants will have two NIRS scans. The first will use HD-DOT (Lumo; Gowerlabs Ltd.) with 34 sources and 48 detectors. The second will use broadband NIRS (miniCYRIL; Bale et al., 2014) using 1 channel. Cortical atrophy can lead to the misattribution of changes in optical absorption to functional activity. Therefore, AD/DLB patients will have a structural Magnetic Resonance Imaging scan for accurate image reconstruction. During the HD-DOT scan, participants will undergo data collection in resting state, and during activation in the following: Boston naming task (BNT; Kaplan et al. 1983), implicit memory task, mismatched negativity task, visual stimulation paradigm (inverting checkerboard), and a naturalistic motor task. During the broadband NIRS scan, participants will undergo data collection in resting state and in a hyper/hypocapnia paradigm. Statistical models integrating signal and functional connectivity metrics from broadband NIRS and HD-DOT, behavioural and clinical data, and structural data will be created to evaluate the diagnostic value of these metrics. The results from piloting a subsection of these paradigms ($n=2$) are presented here (one dataset for visual stimulation was removed due to low optical coupling), with further data to follow when the study begins in April.

Results

Visual stimulation elicited a clear, delayed rise in oxygenated haemoglobin (HbO; Figure 1; peak ~13.2s from stimulus onset) with a concurrent decrease in deoxygenated haemoglobin (HbR) in the contralateral hemisphere to the stimulated hemifield. The BNT yielded an immediate rise in HbO (Figure 2) only evident in longer source-detector separations, more likely to sample the brain.

Conclusion

A robust response to visual stimulation was observed, whereas the BNT may be insufficiently demanding. These results show promise that NIRS can detect differences between people with dementia and controls. Future work in these populations will test this hypothesis.

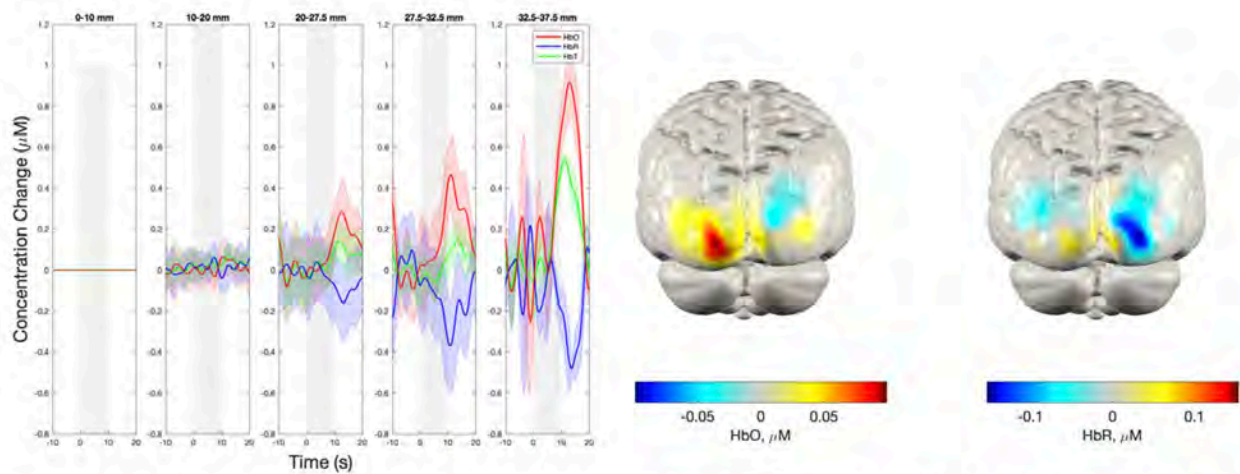


Figure 1. (left) Haemodynamic response functions ($\pm$ SEM) at several source-detector separations and (right) surface reconstructions averaged across 0-10s post-stimulus onset during 7.5 Hz right hemifield stimulation. $n=1$.

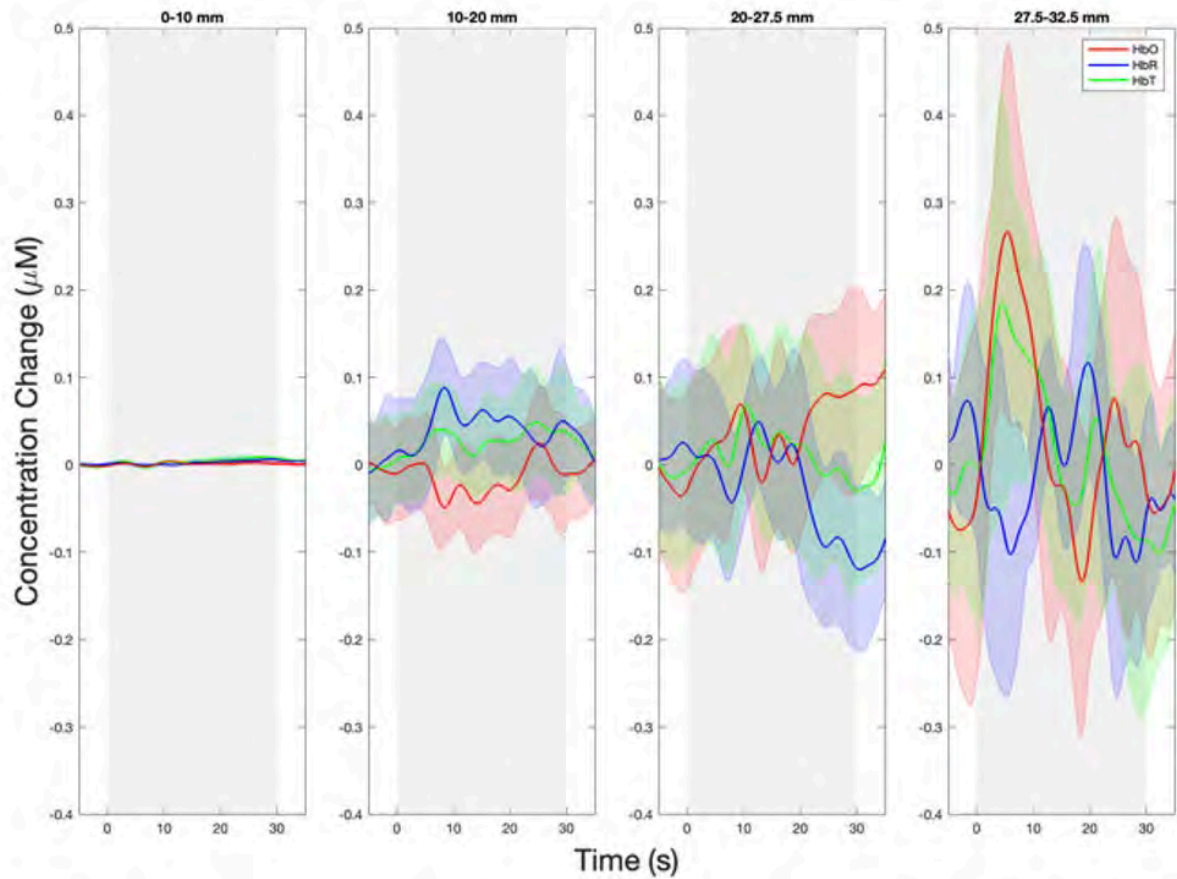


Figure 2. Haemodynamic response functions ($\pm$ SEM) during the Boston Naming Task. $n=2$.

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PCA084

Carotid sinus nerve resection prevents cognitive dysfunction and increased LTP levels in the hippocampus of prediabetic rats

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undefined

Introduction: Type 2 diabetes (T2D) is an established risk factor for the development of neurodegenerative diseases as Alzheimer's (AD) and Parkinson's Disease (PD)(1). The carotid bodies (CBs), peripheral chemoreceptors classically defined as O₂ sensors, have been recently described to have a role in energy and glucose homeostasis and its dysfunction associated with the development of dysmetabolic states (2). In agreement with the role of CB in dysmetabolic states, the abolishment of CBs activity, via the resection or neuromodulation of its sensitive nerve, the carotid sinus nerve (CSN), prevented and reverted dysmetabolic features of prediabetic and T2D animal models (3). Herein, we evaluated if the modulation of CB through the CSN resection may prevent the impact of metabolic dysregulation on cognition and synaptic plasticity.

Methods: Male wistar rats (8-10 weeks of age) fed a high fat-high sucrose (HFHSu) (60% lipid rich diet plus 35% sucrose in drinking water), or a standard (CTL) diet for fifteen weeks. After this period animals were randomly assigned to CSN resection or sham surgery. Metabolic profile and behavior were evaluated at 14 (before surgery) and 20 weeks of diet (5 weeks post-surgery). After final behavioral assessment, electrophysiological recordings in hippocampal brain slices were performed to evaluate synaptic function and plasticity, by recording fEPSPs in hippocampus CA1 area, evoked by Schaffer-colateral stimulation. We also assessed whether the induction or the maintenance of long-term potentiation (LTP) is altered between experimental conditions. Experiments followed the 2010/63/EU Directive and were approved by the NMS Ethics Committee and the Portuguese Authority for Animal Health. Significance between the means was calculated by one-way ANOVA with multiple comparison tests. Differences were considered significant at $p < 0.05$.

Results: HFHSu animals exhibited insulin resistance and glucose intolerance, and CSN resection reversed these phenotypes ($p < 0.05$). Behaviorally, HFHSu-animals: 1) spent 62% less time interacting with the novel/own object in the novel object recognition (NOR) test ($p < 0.05$); 2) sniff 91% less time novel/own scent and take 80% more time to identify the novel block in block test ($p < 0.05$) and 3) exhibit 43% less alternative behavior in the y-maze test in comparison with CTLs. ($p < 0.05$). All these effects were prevented by CSN resection ($p < 0.05$), except for the Y-maze test. Electrophysiological recordings showed no alterations in baseline synaptic transmission nor in PPF (pair pulse facilitation) but showed that in HFHSu sham rats, LTP expression is increased ($p < 0.05$). This effect was not observed in CTL animals and was completely reversed in HFHSu-CSN denervated rats ($p < 0.05$).

Conclusions: Altogether, we showed that HFHSu diet promoted peripheral dysmetabolism leading to cognitive functions impairment, and that CSN resection was able to restore cognitive performance and synaptic plasticity function. These results show that the modulation of CB

activity could be used as a therapeutic approach to prevent neurodegenerative diseases associated with T2D.

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PCA085

Differentiated neurons are damaged by organophosphate and carbamate pesticides by non-cholinergic mechanisms

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Organophosphate (OP) and carbamate compounds are widely employed pesticides that are toxic to target species through targeted inhibition of acetylcholinesterase (AChE). However, their toxicity to off-target species including humans remains a health and environmental concern. The neurotoxicity of a 24-hour exposure to the OP pesticides chlorpyrifos-oxon (CPO) and azamethiphos-oxon (AZO), and the carbamate pesticide, aldicarb, were investigated using undifferentiated and differentiated SH-SY5Y neuroblastoma cells. Pesticide concentration-response curves for cell viability were undertaken using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays. Concentration-response curves for pesticide inhibition of cellular AChE activity were also generated and the production of reactive oxygen species (ROS) was monitored using a 2',7'-dichlorofluorescein diacetate (DCFDA) assay. Pesticides reduced cell viability and neurite outgrowth in a concentration-dependent fashion, from a threshold pesticide concentration of $\geq 10 \mu\text{M}$. Neurotoxic potency was in the order $\text{AZO} > \text{CPO} > \text{aldicarb}$ for undifferentiated cells but $\text{CPO} > \text{AZO} > \text{aldicarb}$ for differentiated cells, and this toxic potency of CPO reflected its more extensive induction of ROS and generation of carbonylated proteins that were characterized by Western blotting. Hence, the relative neurotoxicity of OP pesticides and aldicarb in part reflects non-cholinergic mechanisms that likely contribute to tissue injury.

PCA086

The effect of low grade insular glioma on air hunger

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Background: Functional brain imaging studies of dyspnoea consistently report activation of insular cortex (Evans *et al.*, 2002; Banzett *et al.*, 2000). The precise role and importance of the insula in dyspnoea perception cannot be discerned from brain imaging studies alone, alternative approaches are needed to establish a structure-function relationship. We had the opportunity to study patients with insular gliomas and hypothesised that sensitivity to experimentally induced air hunger would be diminished.

Method: Three patients with low grade insular glioma and three healthy age matched controls underwent incremental hypercapnic air hunger (AH) tests. This involved 1min increments of 1.3% in inspired CO₂ raising end tidal PCO₂ up to 55mmHg while ventilation was constrained to their baseline level (mean±sd 10.7±2.3 and 9.7±0.5 L/min in patients and controls, respectively). Participants rated their air hunger on a 10cm visual analogue scale (VAS) every 15 seconds throughout the test.

Results: The three glioma patients (aged 38-45 years, one female) produced a substantially lower hypercapnic air hunger stimulus response slope compared to age matched healthy controls (aged 34-41 years, one female); mean±sd slopes were 2.27±2.6 vs 23.1±11mmVAS/mmHg PCO₂. This difference approached significance (p=0.08). The lowest slope in the healthy control group was 14.3mm/mmHg PCO₂. The mean±sd AH threshold was the same for both patient and control group (43±4.5 versus 43±3.6mmHg PCO₂). During debrief, patients comments suggested that it was specifically the affective component of breathlessness that was diminished. The one patient who has had surgery to remove the glioma continued to show a reduced sensitivity when re-tested post surgery(6.7mm/mmHg) with a slight rightward shift in threshold for AH.

Conclusion: This data suggests that low grade insular glioma generates insensitivity to hypercapnic air hunger, particularly the affective component. If a fully powered study verifies these findings, this will add support for targeting this region for symptomatic relief of intractable dyspnoea in patients with incurable cardiopulmonary conditions.

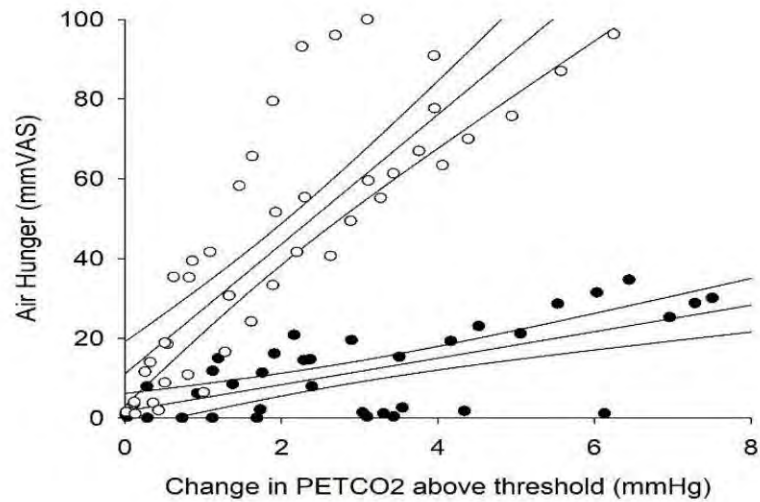


Figure 1 Reduced hypercapnic air hunger sensitivity in insular-glioma patients.

Pooled data from 3 insular glioma patients (closed circles) and 3 matched healthy control subjects (open circles). Linear regression (solid lines) with 95% confidence intervals indicate that in the glioma patients the air hunger response to increasing end tidal PCO₂ (PETCO₂) is significantly diminished.

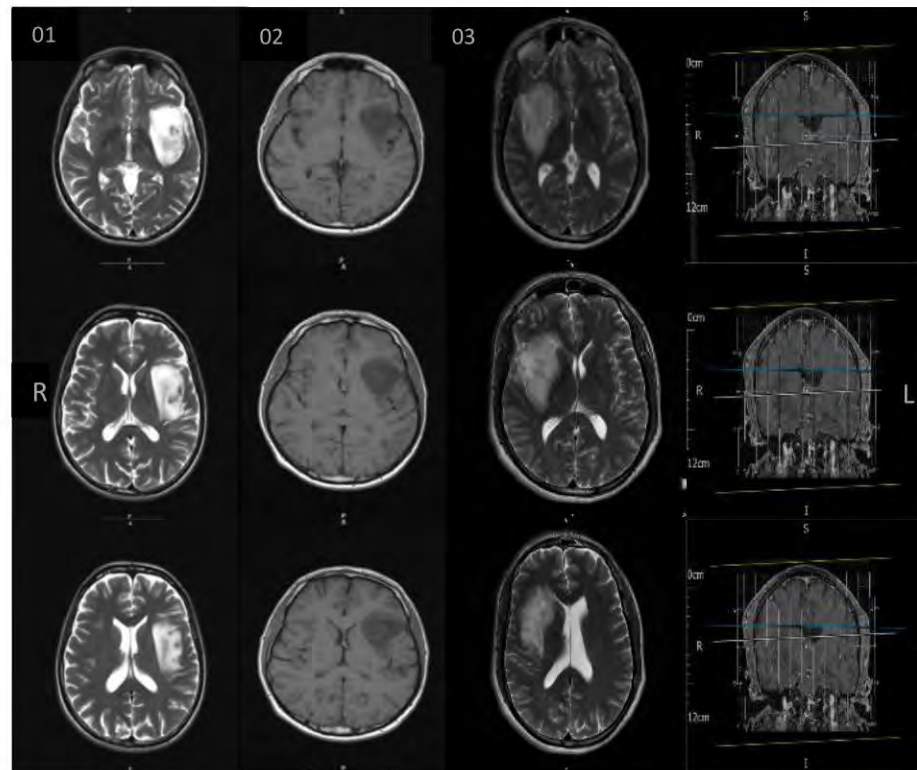


Figure 2 Pre-operative MRI scans of three individuals with Insular LLG. Top panel through to bottom panel represent slices at levels more inferiorly. Right vertical panel displays slice dimensions. Left panel; patient 01, middle left panel; patient 02, middle right panel; patient 0, right panel; slice dimensions.

Banzett RB, Mulnier HE, Murphy K, Rosen SD, Wise RJ and Adams L (2000) 'Breathlessness in humans activates insular cortex', *Neuroreport*, 11(10):2117-20. Evans KC, Banzett RB, Adams L, McKay L, Frackowiak, RSJ. and Corfield, DR. (2002) 'BOLD fMRI Identified Limbic, Paralimbic and Cerebellar Activation During Air Hunger', *J Neurophysiol*, 88(3):1500-11.

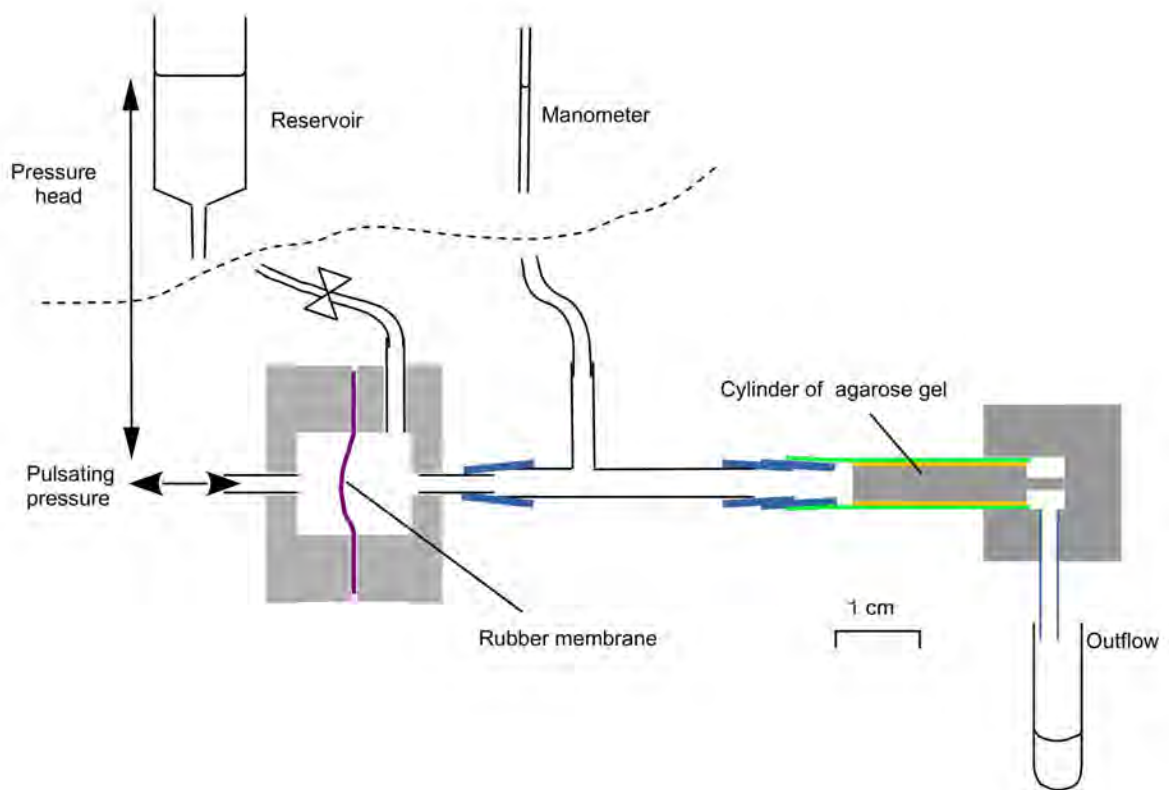
PCA087

Pulsating pressure enhances transport of fluid through a macromolecular matrix: support for a flow of cerebrospinal fluid along the basement membranes of brain capillaries.

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Cerebrospinal fluid (CSF) delivers molecules to the vicinity of brain cells and may carry away others, such as amyloid-beta. In the cortex, CSF flows down peri-arteriolar space: its subsequent pathway is uncertain (see Hladky & Barrand, 2022). Protein marker molecules in CSF are observed to accumulate in the pericapillary basement membranes of brain capillaries (e.g., Rennels et al. 1985; Fig. 3C in Liff et al. 2012) but it is improbable that a constant physiological pressure gradient could drive adequate CSF along a pericapillary pathway. The accumulation is slower if blood pulsation is reduced (e.g., Rennels et al. 1985). We have found only one publication on the effect of experimental pressure pulsation on transport through a porous medium of any kind: McMaster and Parsons (1938) on the movement of Evans Blue through rabbit ear tissue. We asked if a pulsating pressure could substantially increase fluid flow through a hydrogel mimicking basement membrane. We constructed a chamber with a central rod 20 mm long and 4 mm in diameter inside a silicone rubber tube i.d. 5 mm, wall thickness 0.5 mm (Fig. 1). This was filled with agarose gel 1% in NaCl 0.15M. A raised reservoir of 0.15M NaCl provided a static pressure of 20, 30 or 60 cm H₂O. Flow was measured by weighing the efflux and care was taken to avoid menisci in the circuit. As described for other porous media, the flow was not proportional to the pressure gradient: when pressure was increased from 30 to 60 cm H₂O, flow increased by a factor of 2.92 (SEM = 0.13, n = 17 tests), significantly more than 2 (P<0.0001, two-tailed t-test). The pressure could be modulated (without changing the mean pressure) by changing the air pressure applied to the external face of a rubber membrane bounding the vestibule upstream of the gel. The flow Q during a period of pulsation (lasting typically 15 - 40 min) was divided by the mean of the flows before and after to give the ratio Q(pulsation)/Q(no pulsation). With pressure heads of 20, 30 or 60 cm H₂O, pulsation at 3 Hz with an amplitude of no more than 5.5% increased the flow (two-tailed t-test, P< 0.001, n > 30). With a pressure head of 20 cm and pulsation of 5.5% the increase (P = 0.018) was by a factor of 1.39, SEM = 0.10, n = 5. This increase is much smaller than that described by Hale & Coles (2022): we suggest that in those experiments the main effect of pulsation occurred at the menisci of the bubble used to measure flow along a polyethylene microburette, and not in the gel itself. The effect now reported seems too small to account for physiological transport of CSF along capillary basement membrane. However, if the dimensions were scaled down to those of a real capillary, and an optimum hydrogel were used, there might be a bigger effect.



Hale MG & Coles JA (2022) Biorxiv doi: <https://doi.org/10.1101/2021.12.30.474515> Hladky SB & Barrand MA (2022). Fluids Barriers CNS 19.9. Iliff JJ et al. (2012). Sci Transl Med 4,147ra111 McMaster PD & Parsons RJ (1938). J Exp Med 68, 377-400. Rennels ML et al. (1985). Brain Res 326, 47-63

PCA088

From dendrites to nuclear envelope invaginations and beyond: A cell-wide web of cytoplasmic nanocourse coordinates calcium signalling in primary cortical neurons

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It has been proposed that cellular calcium signalling is coordinated through an intracellular network of cytoplasmic nanocourses, namely the cell-wide web, demarcated by sarco/endoplasmic reticulum (S/ER) nanojunctions that span from outer nuclear membrane to the plasma membrane¹. At the centre of the cell-wide web are cytoplasmic nanocourses demarcated by nuclear envelope invaginations (NEIs). Although their function remains open to question, evidence indicates that the nuclear envelope lumen holds a calcium store that is released into cytoplasmic nanocourses demarcated by NEIs rather than into the nucleoplasm, and that modulating calcium flux across the outer nuclear membrane correlates with changes in gene expression in pulmonary arterial myocytes¹. Nuclear invaginations are evolutionarily conserved and are found in other mammalian cells², including neurons³. Our aim was, therefore, to determine whether the cell-wide web coordinates calcium signalling in primary mouse cortical neurons isolated from mouse embryos (E17.5) after 7 days of culture. Fixed neurons were labelled by ER-kit (Abcam), Lamin A and Lamin B1 antibodies following immunolabelling methods adapted from those used previously¹. 3D reconstruction of deconvolved confocal images strongly suggested that both NEIs and the cell-wide web were present in primary mouse cortical neurons. There were ~55 (given mean $\pm$ SEM) NEIs per cell, of which 32.4 ± 3.0 ($n = 5$) were both lamin A and lamin B1 positive, 21.8 ± 4.9 ($n = 5$) were lamin A positive and lamin B1 negative, and 1.8 ± 0.7 ($n = 5$) were lamin B positive and lamin A negative. Lamin B positive NEIs were found to co-localise with the histone mark H3K9me2, consistent with their proposed role in gene expression regulation¹. Live-cell confocal imaging was then carried out on cortical neurons loaded with the calcium indicator Fluo-4 (Life Technologies), ER-tracker (Thermo Fisher) and DraQ5 (Thermo Fisher). This revealed a cell-wide web of Fluo-4 positive cytoplasmic nanocourses that colocalised with ER-Tracker. Within different cytoplasmic nanocourses asynchronous calcium signals were observed in the absence of applied stimuli. More strikingly still, pre-incubation (1 min) of Bicuculine (50 mM) and 4-Aminopyridine (250 mM), which have been shown to induce action potential firing by synaptic release of glutamate⁴, evoked calcium transients in primary cortical neurons that arose at dendrites ($F_{\max}/F_0 = 2.20 \pm 0.15$, $n = 5$) and propagated through cytoplasmic nanocourse into the soma and NEIs ($F_{\max}/F_0 = 2.46 \pm 0.19$, $n = 5$) but not the nucleoplasm, and finally through the axon. Notably the calcium signal within cytoplasmic nanocourse demarcated by NEIs declined more slowly (time to 50% of max = $4.27s \pm 0.61s$, $n = 5$) than in extraperinuclear nanocourses across the wider cell (time to 50% of max = $2.53s \pm 0.40s$, $n = 5$). We conclude that in cortical neurons calcium signalling is coordinated through the cell-wide web at NEIs and beyond.

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PCA089

Laryngeal effects of stimulation of the dorsolateral Periaqueductal Grey Matter in spontaneously breathing anaesthetized rats.

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Background

The stimulation of the Periaqueductal Gray matter (PAG) produces an increase in sympathetic tone including an increase in blood pressure, heart rate and respiratory frequency¹. PAG and nucleus retroambiguus (nRA) are necessary to produce vocalization². The nRA is the perfect target to turn passive into active expiration modifying the activity of laryngeal motoneurons located in the nucleus ambiguus (nA)³. We have shown that rostral and ventral pontine structures are involved in changes of laryngeal caliber⁴. A high expression of FOXP2 protein (transcription factor closely related to vocalization) at mesencephalic (PAG) and pontine regions (Parabrachial complex and A5 Region) involved in cardiorespiratory control has been described⁵.

Objectives

The aim of this study was to characterize the possible role of the dIPAG in modulating laryngeal activity and their effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=27), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/ kg, i.v., as necessary).

Neuromorphological study (n=6)

The pattern of staining for c-Fos and FOXP2 protein immunoreactivity (c-Fos-ir) were examined throughout the rostrocaudal extent of the nRa/nA region during electrical stimulation of the dIPAG.

Neuropharmacological study (n=21)

A double tracheal cannulation was used to obtain an “isolated glottis in situ” and to record respiratory airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, ±0,1 psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70ml/min with a thermal mass digital air flow meter

controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Thus, at constant air flow, changes in pressure indicate changes in laryngeal resistance. Bilateral parietostomy allowed access to the dIPAG. Electrical stimulations (n=7) of this region using concentric bipolar electrodes (1ms pulses, 20-40 μ A, 100Hz for 5s) were performed. Microinjections of PBS-Evans Blue (250nl, pH 7.4 $\pm$ 0.1, 5-s duration) (n=7) or glutamate (0,25M, 250nl) (n=7) were performed. Respiratory flow, pleural pressure, blood pressure and heart rate were also recorded.

Only data from animals in which the histology showed that the microelectrodes were positioned within the dIPAG and the A5 region were used for statistical procedures.

Results

Activation of the dIPAG elicited a selective increase in c-Fos-ir with an ipsilateral predominance in nRA/nA somatas (p<0.01) and confirm the expression of FOXP2 bilaterally in both nuclei. dIPAG PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. dIPAG electrical and chemical (glutamate) stimulations evoked a decrease of laryngeal resistance (subglottal pressure) (p<0,001) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate (p<0,001), together with a pressor (p<0,001) and tachycardic response (p<0,001).

Conclusions

Our study contributes with new data on the role of the mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Ethical approval

All experimental protocols were performed in accordance with the recommendations of the European Union directive (2010/63/EU) for animal care and experimental procedures. The experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía.

Keywords

Subglottic Pressure, Laryngeal Motoneurons, dIPAG, Nucleus Ambiguus

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PCA090

The effect of the indoor environment quality (IEQ) on cognition and health

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Background.

The indoor environment quality (IEQ) encompasses temperature, humidity, light, sound and air quality. Previous studies have shown that there was an increase in detrimental effect on cognitive performance and memory at high relative humidity (RH) levels >70% than low RH levels of <30% (Tian et al., 2021; Wu et al., 2020). However, these studies only examined short term exposure (<3 hours) to high humidity levels, outdoor environmental exposure, or field studies. Moreover, increase in thermal discomfort and humid sensitivity has been observed with higher humidity levels when temperatures were >30°C (Jing et al., 2013).

The effects of heat on cognitive performance depend on multiple personal factors – such as the level of motivation, expertise, sex, hydration status, and heat acclimation (HA) (Gaoua, 2010; Schmit et al., 2017). Many studies thus far have investigated the effects of higher temperatures (>35°C) on cognition have been conducted in hotter and more humid geolocations, but little have focused on the effects of higher humidity in more temperate landscapes (<35°C) temperate geolocations.

Aims.

Before the effects of heat and humidity is investigated in the field, it is important to study the acceptance and comfort of hot and humid environment first in a simulated lab study, where we can also measure the most relevant physiological outcomes in a controlled manner. Thus, the main aim of this study is to determine the effect of a humid and warm climate on cognition, physiological responses and environmental perception when compared to a more temperate condition. The primary objective of this study is to investigate the isolated effect of a warm and humid indoor environment for the duration of 8 hours on cognitive function compared with a lower humidity level at the same ambient temperature.

Methods.

Study population: 25 healthy participants - aged between 20 and 40 years with a between BMI >18.5 and <26 kg/m² will be included.

Study design: Each participant will be exposed to the four conditions in randomized order with at minimum of 1-2 day washout period between sessions. The four conditions differ only in the exposure level of indoor relative humidity (HIGH/LOW) and temperature (NEUTRAL/WARM). The difference between the conditions is the level of humidity in the chamber, which is either 30% of RH (LOW) or 70% of RH (HIGH) and temperature, which is either 25°C (NEUTRAL) or 32°C (WARM). Throughout the test day the participant will be conducting cognition tests,

stepping activities, and questionnaires. The main study parameters and endpoints are cognitive performance (cognitive tasks and subjective workload and motivation). While the secondary parameters are physiological measures including core temperature, skin temperature, skin blood flow, local sweat rate, heart rate, blood pressure, urine hydration, and salivary cortisol); along with perceptual evaluations of the environment (thermal, air quality and wetness sensation, comfort, acceptance, preference, pleasure).

Results.

Data collection is ongoing at the moment.

Conclusions.

Conclusions will be made when full results can be determined.

Keywords. Temperature, Humidity, Cognition, Metabolism, Performance

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PCA091

Relief of experimentally-induced breathlessness in healthy individuals, using repetitive Transcranial Magnetic Stimulation of Dorsolateral Prefrontal Cortex

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Background: Use of non-invasive brain stimulation to unravel the brain mechanisms of breathlessness is a novel application that may reveal new targets for relief of intractable breathlessness thereby addressing an urgent clinical need. Similarities in brain mechanisms of pain and breathlessness are evident; both are multidimensional with physical and emotional domains. Brighina *et al.* (2011) showed that capsaicin-induced pain was reduced by repetitive Transcranial Magnetic Stimulation (rTMS) over the left Dorsolateral Prefrontal Cortex (L-DLPFC). We hypothesized that L-DLPFC rTMS will also relieve 'Air Hunger' (AH), an unpleasant component of breathlessness.

Methods: Healthy volunteers underwent breathing tests of AH (hypercapnia with constrained ventilation) before and after 5Hz rTMS over L-DLPFC, before and after 5Hz rTMS over R-DLPFC, and before and after SHAM stimulation, on three separate days in random order. Subjects rated AH on a 100mm Visual Analogue Scale (VAS) during, and completed the D-12 multidimensional dyspnoea questionnaire after, each AH test.

Results: Preliminary data from 10 healthy individuals (aged 21-53yrs; 3 female) showed significant reduction in steady-state AH after L-DLPFC rTMS (mean±sd of -17±15mmVAS; p=0.006), and after R-DLPFC rTMS (-18±22 mmVAS; p=0.04) with smaller non-significant reduction after SHAM (-9 mmVAS). Treatment effects (rTMS minus SHAM responses) for left and right DLPFC rTMS averaged -7.9 and -9.6mmVAS which are below the minimal clinically important difference of 10mmVAS. However, the treatment effect met the minimal clinically important difference for VAS ratings of AH (Ries *et al.*, 2005) in 50% of individuals for both L and R rTMS (Figure 1). The data is currently underpowered and skewed by 2 individuals with disproportionately high SHAM responses. The physical domain of D-12 scores fell by -13, -6 and -2% full-scale for the R-DLPFC, L-DLPFC and SHAM stimulations, approaching significance only for R-DLPFC (p=0.06).

Conclusion: Unlike for pain, 5Hz rTMS of both L-DLPFC and R-DLPFC relieved AH. Attenuation of the physical domain of breathlessness appeared to be linked more to the rTMS of the R-DLPFC. Furthermore, low frequency stimulation of the R-DLPFC is known to relieve anxiety and depression. This suggests that the modulation of dyspnoea from the rTMS of the R-DLPFC and L-DLPFC may operate via separate mechanisms. Reconciling these findings with those of brain imaging studies may help unravel the cerebral network for breathlessness and reveal new targets for relief of clinical dyspnoea.

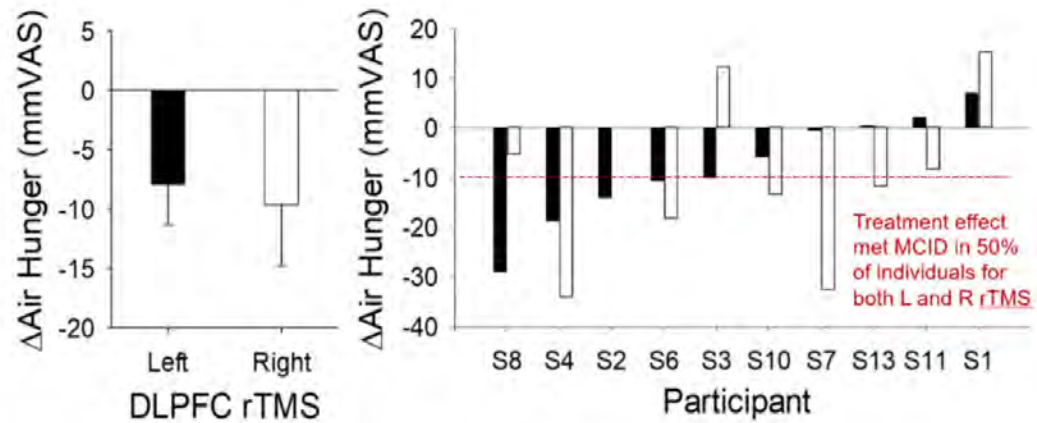


Figure 1: Mean and individual treatment effects.

Mean±sem difference in air hunger (Δ AH) between rTMS and SHAM for the L-DLPFC (black bars) and R-DLPFC (white bars) in 10 healthy volunteers. Individual treatment effects are shown in the right panel. Dashed horizontal line indicates the Minimal Clinically Important Difference for AH ratings on VAS (Ries et al., 2005)

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PCA092

Blood-Brain and Blood-CSF barriers in a Genetic Model of Hydrocephalus

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Hydrocephalus is a result of cerebrospinal fluid (CSF) accumulating in the brain's ventricles caused by imbalances in CSF secretion, flow, and/or absorption. Treatments for hydrocephalus include invasive procedures such as surgical shunt placement, which commonly fail and are revised throughout a patient's life¹. Taking a cell and molecular approach to characterize changes occurring during hydrocephalic development may lead to novel targets for pharmacological treatment.

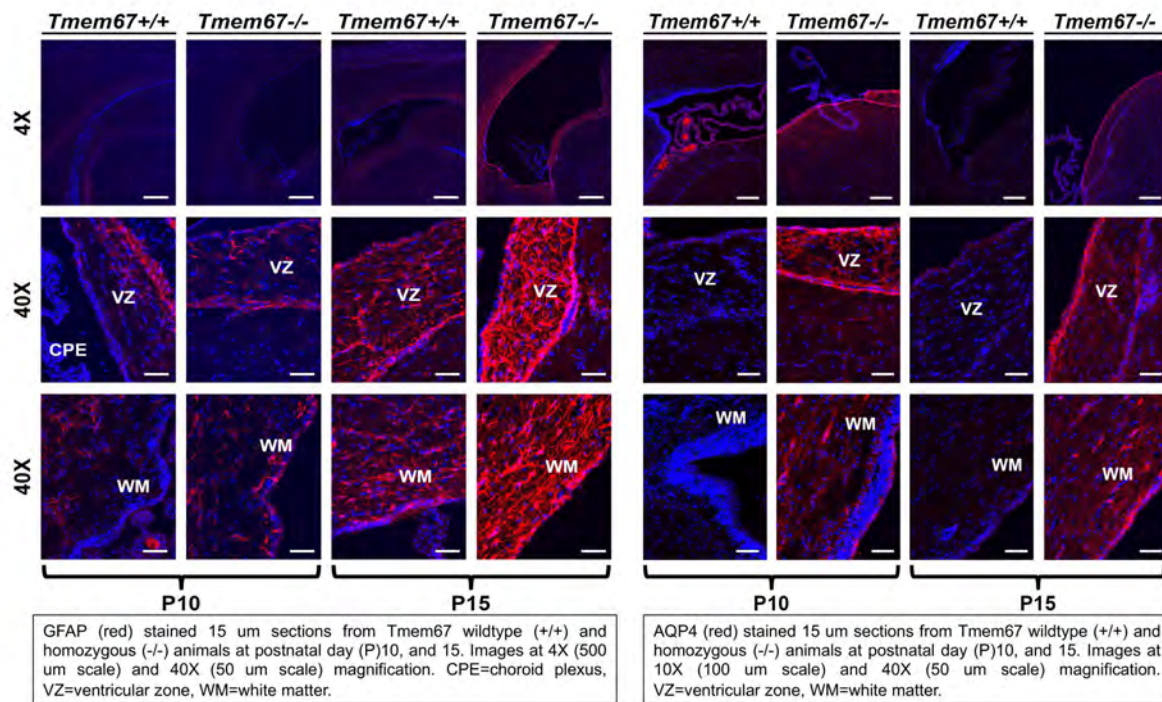
Cell types associated with the blood-brain, brain-CSF, and blood-CSF barriers may be altered in hydrocephalus. This study focuses on choroid plexus epithelial (CPE) cells, ependymal cells, and astrocytes. The choroid plexus is a contiguous layer of tightly regulated epithelial cells surrounding a fenestrated capillary network that is responsible for the production of CSF. The ependymal cells are ciliated neuroglial cells that line the ventricles of the brain and play roles in CSF maintenance and waste clearance. Astrocytes are glial cells that perform various functions in the brain, one of them being blood-brain barrier regulation². These cells serve in brain fluid/electrolyte regulation, an important component of barrier integrity. These cells contain aquaporins (AQP), AQP1 in the CP, and AQP4 in ependymal cells and astrocytes, that are important in regulating CSF and brain interstitial fluid. Aquaporins are implicated in various diseases associated with brain fluid regulation³.

In Wistar rats, a missense point mutation in the Transmembrane 67 (TMEM67) protein, causes a ciliopathy resulting in hydrocephalus⁴. Using this model, changes in barrier integrity and aquaporins were evaluated using immunohistochemistry, real-time quantitative polymerase chain reaction (RTqPCR), and western blot. Animals were euthanized using 1ml/kg body weight intraperitoneal injection of Euthasol (pentobarbital sodium 390mg/ml, phenytoin sodium 50mg/ml) or carbon dioxide gas according to the IUPUI IACUC protocol, then decapitated. Brains were harvested and processed for their respective experiments. Ventriculomegaly appears in TMEM67 homozygous animals before postnatal day (P)10 by quantitative magnetic resonance imaging⁵ and visualized with Nissl (n=3). Barrier integrity was investigated by examining glial activation and tight junction expression and appearance. Increased glial fibrillary acidic protein, a marker of astrocytes, appeared in hydrocephalic animals by immunohistochemistry as early as P10 (n=3). Fluorescent intensity of the tight junction proteins expressed in CPE; claudin-1 and 2, and adherens junction protein; E-cadherin, increased in P15 hydrocephalic animals compared to wildtype (n=3). Interestingly, P10 wildtype animals appeared to have more claudin-1 labeling than hydrocephalic animals (n=3). Expression will be confirmed using western blot (n=3).

Aquaporin localization was examined in hydrocephalic animals at P15, 10, and 5 (n=3). Increased fluorescent intensity of AQP4 in the subventricular zone of hydrocephalic and AQP1 apical localization in the CP was observed in hydrocephalic animals at P15 and P10. RTqPCR showed increased AQP1 in CPE⁵ (*p=0.0101) and no change in AQP4 mRNA in the

periventricular cortex ($p=0.4359$) of P15 hydrocephalic animals compared to wildtype. Post-transcriptional changes will be evaluated using western blot ($n=3$).

These results provide further characterization of the role of the brain barriers and aquaporins in the pathophysiology of hydrocephalus and elucidate how brain fluid regulation may be altered in hydrocephalus. They also produce targets for pharmacological development.



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PCA093

Investigation of the role of PKC δ in an in vitro model of neuropathic pain

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Background. Neuropathic pain (NP) is caused by lesion or disease of somatosensory system. It impacts quality of life of 8% of UK population and only 33% of these patients have effective NP management with existing medications¹. Protein kinase C delta (PKC δ) is calcium-independent novel PKC isozyme and is involved in numerous cellular functions, including neurotoxicity, neurodegeneration and apoptosis. PKC δ expression correlates with augmented sensitivity and intensity of NP^{2,3}. Rottlerin, a compound isolated from *Mallotus Philippensis* plant, was reported to act as PKC δ inhibitor⁴.

Hypothesis. Inhibition of PKC δ activity by rottlerin may represent a novel therapeutic strategy for effective NP management.

Methods. *Cell culture.* HD33n1 human induced pluripotent stem cells (hiPSC) were used to generate fully functional sensory-like neurons according to previously developed protocol⁵. To verify the data obtained on hiPSC-derived sensory-like neurons, we used trigeminal ganglia (TG) neurons isolated from 10-week-old Sprague-Dawley rats (n=3); Schedule 1 for tissue harvesting.

Electrophysiology. Patch-clamp conventional whole-cell electrophysiology technique was used to investigate electrical properties of neurons. Electrophysiological parameters were recorded with Axopatch 200A amplifier, Digidata 1440A digitizer and pCLAMP 10.2 software (Molecular Devices) at room temperature. Bath solution was prepared with 144.8mM NaCl, 2.5mM KCl, 0.5mM MgCl₂, 1.2mM CaCl₂, 10mM glucose, and 5mM HEPES, pH 7.4 with 1M NaOH. Pipette solution contained 140mM KCl, 6mM NaCl, 4mM Na₂-ATP, 4.2mM Na-GTP, 3mM MgCl₂, 1mM CaCl₂, 5mM HEPES, pH 7.2 with 1M KOH.

qPCR. RNA was extracted from HD33n1 hiPSCs-derived sensory-like neurons treated with rottlerin in the presence and absence of “pain cocktail” (10 μ M adenosine triphosphate, 1 μ M noradrenaline, 1 μ M bradykinin and 1 μ M substance P). The Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit was used for cDNA synthesis following standard procedure. Changes in relative gene expression of *PKCD* in the presence and absence of “pain cocktail” in control and rottlerin treated samples were calculated using the $2^{-\Delta\Delta CT}$ method, and the data were normalised to *GAPDH*.

Statistical analysis. All data are expressed as mean $\pm$ S.E.M. Statistical comparisons were performed using Student's t-tests; differences were considered significant at $p < 0.05$.

Results. Acute application of rottlerin hyperpolarised HD33n1 hiPSCs-derived sensory-like neurons and rat TG neurons in concentration-dependent manner. 10 μ M rottlerin significantly hyperpolarised HD33n1 hiPSC-derived sensory-like neurons from -45.6 ± 0.1 mV (n=3) to -54.2 ± 1.8 mV (n=3); $p < 0.01$. Similarly, acute application of 10 μ M rottlerin significantly hyperpolarised rat TG neurons from -57.7 ± 4.0 mV (n=5) to -66.4 ± 5.1 mV (n=5); $p < 0.05$.

Synergetic application of all components of the “pain cocktail” was ineffective to cause depolarisation in rat TG neurons in the presence of rottlerin, thus suggesting its anti-excitatory effect.

Chronic administration of HD33n1 hiPSC-derived sensory-like neurons with 10µM rottlerin decreased PKCδ expression, whereas administration with “pain cocktail” increased it. Combined chronic administration of rottlerin and “pain cocktail” reduced PKCδ expression compare to “pain cocktail” alone.

Summary. These data show for the first-time the ability of PKCδ inhibitor rottlerin to attenuate excitability of HD33n1 hiPSC-derived sensory-like and rat TG neuronal *in vitro* NP model. This may suggest a novel strategy to improve pharmacological management of NP as well as associated co-morbidities.

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PCA094

Ultrastructural and molecular features of rodent brain Calyx-of-Held-like synaptogenesis: neuroendocrine contributions to the molecular logic

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The hindbrain parabrachial complex (PBc) is a sensory relay in the CNS. The forebrain extended amygdala (EA) orchestrates adaptive responses to emotional events. While PBc-EA connection has been extensively studied, cell type specificity and types of synapses remained unclear. In order to address these questions we used a systematic anatomical analysis (immunohistochemistry IHC and double in situ hybridization, DISH) of neuropeptides PACAP, CGRP and neurotensin-NT distribution in rat N=20 and mice N=20 brains, we observed perisomatic ring-like structures containing the three peptides as well as VGLUT1/VGLUT2 in the EA. The origin of those structures was studied using in vivo juxtacellular labeling and tracing study using *Adcyap1-Cre* for Cre-dependent expression of fluorescent marker fused with artificial channelrhodopsin. PACAP/CGRP/NT/ VGLUT1/VGLUT2 expression was a molecular signature of the pre-synaptic neurons from the posterior-ventral division of PBc. PKCdelta-GABAergic neurons in the EA, co-expressing *Adcyap1r1* and *Vipr1* mRNA were identified as the post-synaptic cells. This signature, except co-expressing PKCdelta is characteristic of the Calyx-of-Held, a rare perisomatic large glutamatergic synapse in brainstem' medial nucleus of the trapezoid body (MNTB), which receive axons from globular bushy neurons in the ventral cochlear nucleus. Using transmission electron microscopy (TEM) and focused ion beam scanning electron microscopy (FIBSEM), in combination with immunohistochemistry, we demonstrated that the ring-structures in EA are highly similar to the Calyx-of-Held observed in the MNTB. Taken together, our results suggest a common molecular basis for calyceal synaptogenesis both in hindbrain and forebrain. The PBc-->EA Calyx-of-Held synapse may represent a previously unappreciated morphological substrate for high fidelity sensory alert to the forebrain center for adaptive response. This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experiments were approved by the NIMH Institutional Animal Care and Use Committee (ACUC, LCMR-08) and the Research and Ethics Committee of the Faculty of Medicine, Universidad Nacional Autónoma de México (CIEFM 062/2016).

PCA095

Effects of stimulation of Cuneiform nucleus and the dorsomedial Hypothalamic nucleus and Perifornical area on the mechanisms involved in the control of laryngeal activity and subglottic pressure in spontaneously breathing anaesthetized rats

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Background

The dorsomedial Hypothalamic nucleus and Perifornical area (DMH-PeF) and the mesencephalic Cuneiform nucleus (CnF) have been involved in sympathetic activity due their connectivity with several nuclei involved in cardiorespiratory control, e.g. dorsolateral Periaqueductal Gray Matter (dIPAG), the Parabrachial/Kölliker-Fuse complex (PBc/KF), the Solitary Tract Nucleus (NTS) and the Rostral Ventrolateral Medulla (RVLM) (1). In previous studies we have demonstrated a functional interaction between hypothalamic and mesencephalic structures (DMH-PeF, dIPAG) with several pontine regions (PBc, A5) (2, 3). We have also shown that rostral and ventral pontine structures are involved in the changes of laryngeal caliber (4).

Objectives

The aim of this study was to characterize the relations between hypothalamic and mesencephalic regions involved in cardiorespiratory control and their possible role in modulating laryngeal activity and their possible effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=42), SPF, Sprague-Dawley (250-300g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/kg, i.v., as necessary). A double tracheal cannulation was used to obtain an “isolated glottis in situ” and to record respiratory airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, $\pm 0,1$ psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Thus, at constant air flow, changes in pressure indicate changes in laryngeal resistance.

Bilateral parietostomy allowed access to the DMH-PeF and CnF. Electrical (n=14) and chemical (n=14) stimulations of these regions using concentric bipolar electrodes (1ms pulses, 20-40 μ A, 100Hz for 5s) or glutamate (0,25M, 250nl) was performed. Microinjections (n=14) of PBS-Evans Blue (250nl, pH 7.4 $\pm$ 0.1, 5-s duration) served as control purpose. Respiratory flow, pleural pressure, blood pressure and heart rate were also recorded.

Only data from animals in which the histology showed that the microelectrodes were positioned within the CnF and the DMH-PeF region were used for statistical procedures.

Results

DMH-PeF and CnF PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. However, electrical stimulations in both regions evoked a decrease of laryngeal resistance (subglottal pressure) ($p < 0,001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p < 0,001$), together with a pressor ($p < 0,001$) and tachycardic response ($p < 0,001$).

Glutamate microinjections within the DMH-PeF and CnF evoked a decrease of laryngeal resistance (subglottal pressure) ($p < 0,01$ and $p < 0,001$ respectively) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p < 0,001$ in both cases), together with a pressor ($p < 0,001$ and $p < 0,01$ respectively) and tachycardic response ($p < 0,001$ in both cases).

Conclusions

The results of our study contribute with new data on the role of the hypothalamic-mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Ethical approval

All experimental protocols were performed in accordance with the recommendations of the European Union directive (2010/63/EU) for animal care and experimental procedures. The experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía.

Keywords

Subglottic Pressure, Laryngeal Motoneurons, DMH-PeF, CnF, Nucleus Ambiguus

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PCB001

Comparative Microarray Profiling of Exosomal miRNAs in the Serum of Acute Ischemic Stroke Patients and Healthy Controls

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Circulating exosomal miRNAs have demonstrated potential as both biomarkers and therapeutics for various diseases. However, their expression in acute ischemic stroke (AIS) patients has not been extensively investigated (Xu et al., 2022). Therefore, this study sought to compare the circulating exosomal miRNA profiles of AIS patients to those of healthy controls to identify differentially expressed miRNAs. The study adhered to the guidelines set by the Declaration of Helsinki and was approved by Hamad Medical Corporation Institutional Review Board.

Five AIS patients were recruited within 24 hours of the attacks' onset and matched with five healthy controls. Exosomal RNA was extracted using the exoRNeasy kit (Qiagen), and miRNA expression profiles were analyzed using the Affymetrix GeneChip miRNA arrays. Signals were then normalized using the robust multi-array average method. To identify present or absent genes the Detection Above BackGround algorithm was applied. An empirical Bayes moderated t-test (limma package), was applied to detect differences in gene expression between patients and controls (Ritchie et al., 2015). *P*-values were adjusted for multiple testing using the method of Benjamini and Hochberg, and a statistical significance level of $p < 0.05$ and log2 fold-change of 1 were employed (Benjamini & Hochberg, 1995). Targets of the differentially expressed miRNAs were identified using miRTargetLink 2.0 (Kern et al., 2021). ClusterProfiler package in R was used with different functional annotation databases such as KEGG and GO to perform enrichment analysis (Wu et al., 2021). Protein-protein interaction (PPI) networks were generated using STRING and hub genes were identified.

We observed differential expression of five miRNAs in patients with AIS compared to controls. Specifically, we found upregulation of hsa-let-7b-5p, hsa-miR-16-5p, and hsa-miR-320c, and downregulation of hsa-miR-548a-3p and hsa-miR-6808-3p. To gain insight into the potential biological implications of these miRNAs, we performed functional and pathway enrichment analyses of their target genes. Our findings suggest that the PI3K/AKT signaling pathway, as well as miRNAs involved in cancer, were the most enriched KEGG pathways. The top enriched GO biological process terms were the regulation of transcription by RNA polymerase II, negative regulation of apoptosis, and regulation of proliferation and protein phosphorylation. A PPI network was constructed to investigate the interactions between the proteins encoded by the target genes of these miRNAs. Our analysis revealed a significant network of protein associations ($P = 1.0 \times 10^{-16}$) with 241 edges between 101 nodes, compared to the expected 86 edges if the associations were random. Furthermore, we identified hub proteins based on the network's connectivity degree, which included TP53, HRAS, KRAS, NRAS, IGF1-R, VEGF-A, MTOR, CCND1, CCNA2, and CDC25A.

This study is the first investigation of the expression profile of exosomal miRNAs in AIS in the Middle East. However, additional research and validation in a larger cohort of patients are

necessary to further elucidate the specific roles of these exosomal miRNAs in AIS, including their potential as predictive, diagnostic, or prognostic biomarkers. Ultimately, this research has the potential to identify novel prognosis markers for AIS, which could significantly impact the diagnosis, treatment, and management of this disease.

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PCB002

Electrophysiological effects of extracellular histones in isolated cardiomyocytes

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Histones are highly basic proteins present in eukaryotic cell nuclei. DNA wraps around histones creating structural units called nucleosomes, the building blocks of chromatin¹. Previous work² has shown that following sepsis or blunt trauma, circulating histones released from damaged cells cause secondary damage to organs, including the heart. Here we investigated the mechanism of H3 histone toxicity in isolated ventricular myocytes. Wistar rats (150–200 g) were sacrificed in accordance with the UK Home Office Guidance on the Operation of Animals (Scientific Procedures) Act of 1986. H3 histone (5 µg/ml) induced membrane damage and cell necrosis. Following 10 min exposure, topographical images obtained using scanning ion conductance microscopy (SICM) showed fine dark lines consistent with 'micro-tears'. The effects of H3 on membrane potential were assessed using whole cell current clamp recording. Under control conditions, the mean ($\pm$ SEM) resting membrane potential (E_m) was -79.1 ± 0.65 (n=87). H3 (µg/ml) application caused a dose and time dependent depolarisation of E_m , spontaneous action potentials and the development of early afterdepolarisations (EADs, **Table 1**). Low levels of H3 (0.1-0.01 µg/ml) increased the action potential duration measured 90% of maximal amplitude ($APD_{0.9}$; **Table 1**). These data show that H3 induces membrane damage, leading to myocyte depolarisation and pro-arrhythmic changes in the action potential. Such effects may contribute to cardiac dysfunction in pathological conditions that result in a rise in circulating histones.

% Cells developing EADs	#cells	#hearts	H3 ($\mu\text{g ml}^{-1}$)	Start (S) End (E)	Membrane potential (E_m)		Pvalue	time for E_m loss (sec)		APD _{0.9} (ms)			Pvalue
					mean	$\pm$ SEM		mean	$\pm$ SEM		mean	$\pm$ SEM	
78.57	14	9	1	S	-78.23	1.13	****	127.15	27.52	Control	53.96	7.67	*
				E	-42.36	2.88				H3	68.03	11.62	
66.66	9	7	0.1	S	-79.05	1.84	****	218	64.06	Control	66.55	13.71	*
				E	-38.51	4.98				H3	85.2	14.91	
85.71	8	4	0.01	S	-80.86	1.41	***	236.57	68.89	Control	77.78	17.56	*
				E	-43.84	5.33				H3	102.83	24.98	
None	10	4	0.001	S	-79.36	0.43	ns	NO Loss		Control	64.90	11.58	ns
				E	-80.32	1.02				H3	63.74	10.93	

Table 1 Summary data showing the effects of H3 on the electrophysiological properties of rat ventricular myocytes. Calculated mean values for membrane potential (E_m) and APD_{0.9} before (E_m start) and after (E_m end) H3 application. Also shown calculated time until loss of E_m following H3 application. Paired t-test with $P \leq 0.05$ considered significant (* $P \leq 0.05$, *** $P \leq 0.001$, **** $P \leq 0.0001$).

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PCB003

In silico Investigation of Pro-arrhythmogenic Effects of KCNE2 Mutations in Human Atrial Fibrillation

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Functional analysis has shown that gain-of-function mutations (I57T and M23L) in the slow delayed rectifier potassium current (I_{Ks}), carried by the *KCNE2* channel, are associated with early-onset lone atrial fibrillation (AF) (Nielsen et al., 2014). These mutations affect not only I_{Ks} but also the rapid delayed rectifier current (I_{Kr}) and the transient outward potassium current (I_{to}). Using biophysically detailed computer models, this study aimed to investigate the underlying mechanisms by which the two mutations (I57T and M23L) facilitate and promote AF. In the simulations, the MCZ (Colman et al., 2013) model of the human atrial cell was modified to incorporate experimental data on changes of I_{Ks} , I_{Kr} , and I_{to} induced by *KCNE2* I57T and M23L mutations. The cell models were then incorporated into homogeneous multicellular one- (1D) and two-dimensional (2D) models of atrial tissue, as well as a 3D realistic model of the human atria. Functional effects of the two mutations on atrial electrical activities were quantified on the action potential (AP) profile and the AP duration (APD) restitution at the single-cell level; and on the conduction velocity (CV), the effective refractory period (ERP), and the wavelength (WL) restitutions at the tissue level. The widths of the temporal vulnerability window (VW) to re-entry were measured. Dynamical behaviours of re-entrant excitation waves (lifespan (LS), tip trajectory patterns, and dominant frequency (DF)) in 2D and 3D models were investigated. It was shown that both mutations shortened APD and flattened its restitution curve. At the 1D level, they abbreviated both ERP and WL restitutions and displaced the CV curve to the left, facilitating the conduction of atrial excitations at high rates. Although they reduced the temporal VW widths, *KCNE2* I57T and M23L mutations increased the lifespan and stabilization of the re-entrant excitation waves at the 2D level, which was corroborated by 3D simulations. Collectively, these simulation results revealed the pro-arrhythmic effects of the *KCNE2* I57T and M23L mutations, which are attributable to the shortened APD, ERP, and WL, and altered CV, which, in combination, facilitate the maintenance of re-entrant excitation waves leading to AF.

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PCB004

Machine-learning analysis of near-infrared spectroscopy to improve clinical decision making for hypoxic-ischemic encephalopathy in term infants

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Background: Hypoxic-ischemic encephalopathy (HIE) is a severe brain injury that occurs in neonates due to perinatal oxygen deprivation, often leading to adverse neurodevelopmental outcomes or death. The therapeutic window for HIE is within the first 6 hours of life but implementing therapeutic hypothermia up to 12 hours after birth has been shown to be effective in reducing the severity of brain injury. To ensure the timely and effective recognition of HIE during this critical therapeutic window, it is essential to use objective methods for diagnosis. Near-infrared spectroscopy (NIRS) provides a non-invasive continuous regional measurement of cerebral oxygenation.

Objective: We have sought to assess the potential clinical utility of NIRS as an additional tool in the diagnosis of HIE.

Methods: We analysed 53 infants with all grades of HIE (>36 weeks GA) enrolled in the Multimodal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy (Monitor) trial. All infants had continuous cerebral oxygenation monitoring for at least 2 hours in their first 12 hours after birth. HIE was graded (mild, moderate, severe) based on assessment using the modified Sarnat score at 1 hour of life. The NIRS signals recorded in the first 12 hours of life were pre-processed, and quantitative features were extracted. Furthermore, prolonged relative desaturations (PRDs; data-driven desaturations lasting 2-15 minutes) were identified and removed from NIRS signals, termed filtered NIRS. The quantitative features were combined in a machine-learning model using a leave-one-out cross-validation approach to determine the likelihood of requiring hypothermia treatment, distinguishing between mild vs moderate and severe HIE. We used logistic regression models to control for the potentially confounding effects of clinical features on the NIRS machine-learning model. We controlled for Apgar score (5min) and mode of delivery for the NIRS and filtered NIRS models for detecting mild HIE. In all models, the significance level was set at $p < 0.05$.

Results: Logistic regression analysis revealed that features extracted from NIRS were significant predictors of requiring hypothermia in this population ($\beta = 0.61$, $p = 0.01$). Furthermore, features extracted from filtered NIRS were found to be significant predictors of mild HIE ($\beta = 0.72$, $p < 0.001$). The predictability of the Apgar score when assessed independently was significant ($\beta = -0.11$, $p < 0.001$), while the mode of delivery did not demonstrate a significant impact. The regression model, which included filtered NIRS, Apgar score, and delivery mode, accounted for 50.4% of the variance in the outcome variable (R -squared = 0.504), with the root mean squared error (RMSE) of 0.36. This model performed better than both the logistic model based on filtered NIRS and Apgar score (R -squared = 0.48,

RMSE = 0.37) and the model based on Apgar score and delivery mode (R-squared = 0.41, RMSE = 0.40).

Conclusion: Utilizing machine-learning methods to analyse NIRS in the first 12 hours of life, allows for early objective identification of infants at risk of adverse short-term outcomes and may aid in the stratification of infants for intervention in the effective therapeutic window.

PCB005

The role of Sodium fluoride on electrolytes and blood pressure in male Wistar rats

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This study investigated the role of sodium fluoride on electrolytes and blood pressure in male Wistar rats. Sodium fluoride and the damage done to human health have been of public concern in recent years. Electrolyte imbalance poses threat and can predispose the body to life-threatening cardiovascular conditions including hypertension. Hypertension has been identified as one of the most significant risk factors for morbidity and mortality worldwide. Ten male adult Wistar rats (150-180g) divided into two groups (n=5) were used for this study. Group 1 was the control group and received distilled water orally for 21 days, group 2 received 10mg/kg sodium fluoride *p.o.* through oral gauge for 21 days. Twenty-four hours after the last treatment with sodium fluoride, blood pressure indices; systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were determined non-invasively in awake animals by tail plethysmography using an automated blood pressure monitor (CODA S1, Kent Scientific Corporation, CT). The average of no less than nine readings was recorded for each animal at rest during the blood pressure measurements after the acclimatization period. About 3 mL of blood was collected by retro-orbital venous puncture using plain capillary tubes into plain bottles and left to clot. The clotted blood was then centrifuged at 4,000 rpm for 10 min. Clear serum was separated using a Pasteur pipette into another plain tube and then stored at 4°C. Data collected were expressed as mean $\pm$ SEM. Statistical significance was set at $P < 0.05$ using Student's t-test analysis. The results from this study showed that sodium fluoride caused a significant increase $P < 0.001$ in systolic blood pressure, diastolic blood pressure, mean arterial pressure, and $P < 0.01$ in heart rate compared to the control group that received distilled water. Also, the administration of sodium fluoride caused a significant increase $P < 0.05$ in serum sodium and calcium when compared to the control group. The oral administration of sodium fluoride caused a significant decrease $P < 0.05$ in potassium and phosphorus when compared to control group. There was no significant difference in chloride level in sodium fluoride-treated rats compared to the control. This study showed that sodium fluoride has hypertensive effects on blood pressure as seen in the significant increase in systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate. The results from this study also showed that sodium fluoride elevate serum electrolytes that have the potency to lead to increasing blood pressure as seen in sodium and calcium while reducing the electrolytes that have beneficial effects on lowering blood pressure such as potassium and phosphates.

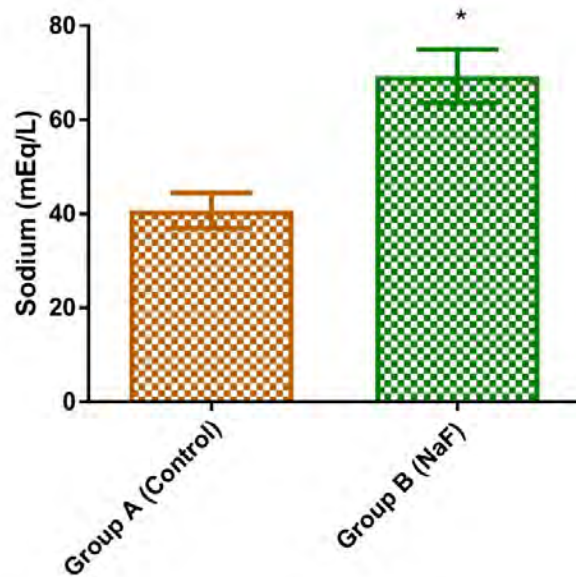


Figure 1 The effect of Sodium Fluoride on Sodium. Values are presented as Mean $\pm$ Standard error of mean. Group A (Control), Group B (Sodium Fluoride NaF at 10mg/kg body weight).

Superscript (*) indicates significant difference at $P < 0.05$ compared with Group A (Control).

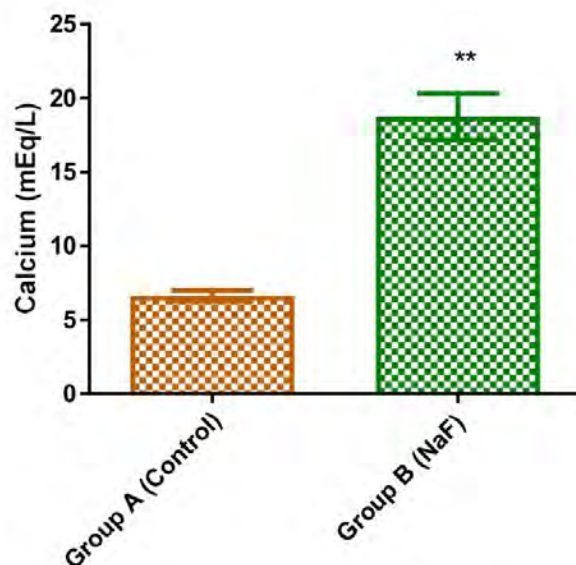


Figure 2 The effect of Sodium Fluoride on Calcium. Values are presented as Mean $\pm$ Standard error of mean. Group A (Control), Group B (Sodium Fluoride NaF at 10mg/kg body weight).

Superscript (*) indicates significant difference at $P < 0.05$ compared with Group A (Control).

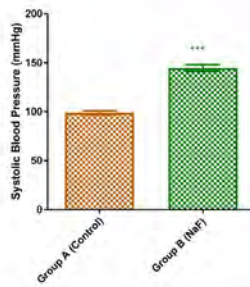


Figure 6. The effect of Sodium fluoride (NaF) on Systolic Blood Pressure. Values are presented as Mean $\pm$ Standard error of mean (SEM). Group A (Control), Group B (Sodium Fluoride, NaF at 10mg/kg body weight).

Superscript ("") indicates significant difference at $P < 0.001$ compared with Group A (Control).

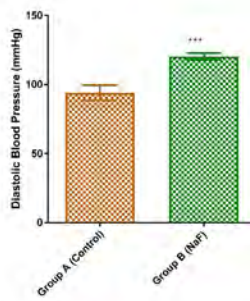


Figure 7. The effect of Sodium fluoride (NaF) on Diastolic Blood Pressure. Values are presented as Mean $\pm$ Standard error of mean (SEM). Group A (Control), Group B (Sodium Fluoride, NaF at 10mg/kg body weight).

Superscript ("") indicates significant difference at $P < 0.001$ compared with Group A (Control).

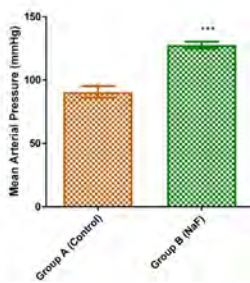


Figure 8. The effect of Sodium fluoride (NaF) on Mean Arterial Pressure. Values are presented as Mean $\pm$ Standard error of mean (SEM). Group A (Control), Group B (Sodium Fluoride, NaF at 10mg/kg body weight).

Superscript ("") indicates significant difference at $P < 0.001$ compared with Group A (Control).

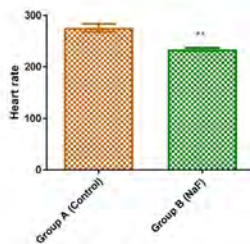


Figure 9. The effect of Sodium fluoride (NaF) on Heart rate. Values are presented as Mean $\pm$ Standard error of mean (SEM). Group A (Control), Group B (Sodium Fluoride, NaF at 10mg/kg body weight).

Superscript ("") indicates significant difference at $P < 0.01$ compared with Group A (Control).

PCB006

Functional and pharmacological differences between the contractility of iPSC-derived atrial and ventricular cardiomyocytes assessed on the FLEXcyte 96.

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Commercial human iPSC-derived ventricular cardiomyocytes have been available from multiple providers for over a decade, with extensive use in research, drug development and toxicology testing due to their appropriate modelling of primary human ventricular cardiomyocytes. Less work has been performed on human iPSC-derived atrial cardiomyocytes despite the clear phenotypic and pharmacological differences between atrial and ventricular cardiomyocytes, rendering the standard ventricular cardiomyocytes a poor model for atrial research. This is of particular concern as atrial fibrillation (AF) is the most common form of arrhythmia worldwide affecting over 33M people and rising, being a co-morbidity with obesity, stroke, dementia and congestive heart failure. Despite the clear clinical need for better AF treatments there are limited therapeutic options with poor success rates and the 1-year mortality rate for patients with AF remains around 25%. Therefore, there is a clear need for better, more human models of AF.

Axol Bioscience Ltd have developed their iPSC-derived atrial cardiomyocytes (ax2518) and have performed extensive validation studies showing differences in key markers, such as MLC2A, ANP and KCNA5 and electrophysiology between their isogenic atrial and ventricular cardiomyocytes (ax2508). There has been less investigation carried out on the contractility differences between the two cell-types which is of particular relevance to AF. Therefore, Axol have partnered with innoVitro GmbH to examine the phenotypic and pharmacological differences in contractility of Axol's two forms of cardiomyocytes on their FLEXcyte 96 platform.

innoVitro tested the baseline contractility and the effects of isoprenaline, S-Bay K8644 and the atrial-specific 4-AP and Carbachol, at a range of concentrations on both ax2508 and ax2518. Both sets of cardiomyocytes were grown for 6 days, according to Axol's protocols, on innoVitro's FLX-96 plates at a range of seeding densities. They displayed markedly different baseline contractility waveforms and contractility parameters on the FLEXcyte 96, consistent with the typical results seen with the corresponding primary atrial and ventricular cardiomyocytes. The FLEXcyte 96 measured multiple contractility parameters allowing the assessment of the effect of each of the tested compounds. Isoprenaline, Carbachol, 4-AP and S-Bay K8644 produced distinctly different effects on ax2508 and ax2518 in terms of waveform shape, beat rate and beat duration. The I_{KACH} agonist Carbachol for example marked effects on the atrial cells; increasing beat duration (500ms to 700ms) and downstroke duration (140%), but only minimal effects on the ventricular cardiomyocytes. In contrast, the β -adrenoceptor agonist isoprenaline produced only a small increase in amplitude (less than 20%) and reduced beat duration (500ms to 350ms) in atrials but caused a marked reduction in amplitude (to less than 5%) and beat duration (800ms to 400ms) with the ventriculans. All compounds and concentrations were tested at n=4 and assessed using the Wilcoxon rank-sum test. This confirmed Axol's previous comparison work focussed on the two cell-type's electrophysiology.

Therefore, Axol's human iPSC-derived Atrial and Ventricular Cardiomyocytes correctly reproduced the different phenotypes and pharmacology of primary cardiomyocyte sub-types as assessed using the FLEXcyte 96 and, as such, provide the starting point to develop more reliable, physiological-relevant models for research on atrial cardiomyocytes and AF.

PCB007

Screening for small molecules that rescue the defective trafficking of mutant KCNQ1 channels

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Introduction: The congenital long QT syndrome (cLQTS) increases the risk of arrhythmia and is an important cause of sudden death in the young. The ion channel subunits KCNQ1 (Kv7.1) and KCNE1 assemble in cardiac myocytes to produce the repolarising slow delayed rectifier potassium current I_{Ks} . Loss-of-function (LOF) mutations in the *KCNQ1* gene cause cLQTS type 1 (LQTS1) (Wang *et al.*, 1996; Sanguinetti *et al.*, 1996). Missense LQTS1 mutations cause LOF by altering channel gating (Class III), promoting defective channel trafficking (Class II) or through a combination of mechanisms. Recent studies highlight that Class II mechanisms underlie disease pathogenesis for a substantial proportion of LQTS1 mutations (Huang *et al.*, 2018) but current therapeutic managements for LQTS1 patients do not target this defect. Therefore, the aim of this study was to identify small molecules that rescue defective mutant KCNQ1 channel trafficking.

Methods: *Trafficking assays:* LI-COR-based On/In-Cell Western assays were used to quantify channel trafficking as described in Royal *et al.*, (2017). The 'On-Cell' assay quantifies cell surface expression (CSE). *Cell lines:* Two HEK-293 cell lines were generated which stably express the trafficking deficient KCNQ1 mutant channel G325R (VSV-KCNE1-G325R) and the wild-type channel (VSV-KCNE1-KCNQ1). *Compound Screening:* 26 compounds were tested in three phases. Compounds were applied for 24 hours at 37 °C unless otherwise indicated. DMSO was the vehicle control. Data are presented as fold-matched control (fold) or normalised arbitrary fluorescent units (NAFUs) $\pm$ SEM. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison test or multiple unpaired *t*-test.

Results: In Phase 1, clinically approved CFTR channel modulators were screened because they may exhibit cross-channel activity (Mehta *et al.*, 2018). Four modulators (including VX-809 and VX-661) were tested but none altered the CSE of VSV-KCNE1-G325R ($P>0.05$, $n=3$). In Phase 2, seven compounds that act as proteostasis regulators were screened. Of these, the proteasome inhibitor MG-132 (1 μ M) and Thapsigargin (10 μ M) promoted increases in VSV-KCNE1-G325R CSE (3.24 ± 0.26 fold ($P<0.0001$, $n=12$) and 1.42 ± 0.04 fold ($P<0.01$, $n=3$), respectively). However, both compounds exhibited cell toxicity. In Phase 3, 15 KCNQ1 channel modulators (blockers and activators) were screened. Of these, the activator R-L3 (L-364,373) (100 μ M) induced a small but significant increase in CSE (1.44 ± 0.15 fold, $P<0.05$, $n=3$) and the activator Docosahexaenoic acid (DHA) at 100 μ M significantly increased VSV-KCNE1-G325R CSE by 2.27 ± 0.26 fold ($P<0.01$, $n=3$). Furthermore, upon extended treatment (for 48 hours), 5 and 10 μ M DHA promoted large increases in VSV-KCNE1-G325R CSE (5.57 ± 0.94 and 8.24 ± 0.66 fold; NAFUs: 0.0768 ± 0.0152 and 0.1123 ± 0.0118 vs. DMSO 0.0135 ± 0.0006 , both $P<0.001$, $n=5$). By contrast, 10 μ M DHA for 48 hours did not alter wild-type (VSV-KCNE1-KCNQ1) channel trafficking ($P>0.05$, $n=5$).

Conclusions: These data highlight that the channel activators DHA and R-L3 may be able to promote trafficking rescue. However, the underlying mechanisms are unknown and whether these compounds can correct other Class II mutants within a cardiomyocyte cellular setting warrants investigation.

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PCB008

Embryonic origins of vascular dysfunction in developmental hypoxia: the role of miR-21-5p

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Background: Chronic fetal hypoxia is a common complication during pregnancy, which reduces fetal growth, promotes endothelial dysfunction and can trigger a developmental origin of cardiovascular disease (Giussani, D.A. (2021) *Circulation* 144(17):1429-1443). Underlying mechanisms remain unknown, preventing identification of targets for intervention. Epigenetics may underpin these alterations, however the role of microRNAs in affecting cardiovascular risk in the hypoxic fetus has not been investigated. MicroRNA-21-5p is of particular interest, as it is a modulator of endothelial function that is downregulated by hypoxia (Peñaloza et al. (2020) *Biochem. Pharmacol.* 182:114288). Therefore, this study tested the hypothesis that treatment with synthetic microRNA-21-5p (AgoMiR-21) rescues peripheral endothelial dysfunction in the hypoxic chicken embryo. This model system permits isolation of the direct effects of therapy on the developing vasculature independent of effects on the maternal and/or placental physiology.

Aims: To determine the effects of treatment of the chronically hypoxic chicken embryo with synthetic microRNA-21-5p (AgoMiR-21) on embryonic growth and endothelial function.

Methods: This research was carried out under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Board. Fertilised Bovans Brown eggs were incubated under normoxia (21% O₂) or chronic hypoxia (14% O₂) from day 1 to day 19 (hatching occurs at 21 days). Chicken embryos were treated topically onto the chorio-allantoic membrane via a small hole in the air cell with AgoMiR-21 (1µg in 100µl sterile H₂O/embryo/day) or vehicle (100µl sterile H₂O) on days 13, 15 and 17 of incubation. On day 19, following biometry, embryos were killed via cervical transection, and cranio-tibial arteries were isolated for analysis of vascular reactivity by *in vitro* wire myography.

Results: Chronic hypoxia promoted asymmetric growth restriction, shown by a reduction in embryo weight (Fig. 1A, n=13-29, P<0.0001) and an increase in relative brain weight (Fig. 1B, n=10-29, P=0.0077). Hypoxic embryos also showed an impairment in endothelium-dependent vasodilatation (Fig. 1C-E, n=9-13, P=0.0039). Treatment of hypoxic embryos with AgoMiR-21 had no effect on growth (Fig. 1A&B), but it partially rescued endothelium-dependent vasodilatation in hypoxic embryos (Fig. 1C-E, n=9-13, P=0.0051). Treatment of normoxic embryos with AgoMiR-21 had no effects.

Conclusions: AgoMiR-21 is a promising candidate for preventative therapy against developmental origins of vascular dysfunction in offspring of hypoxic pregnancy.

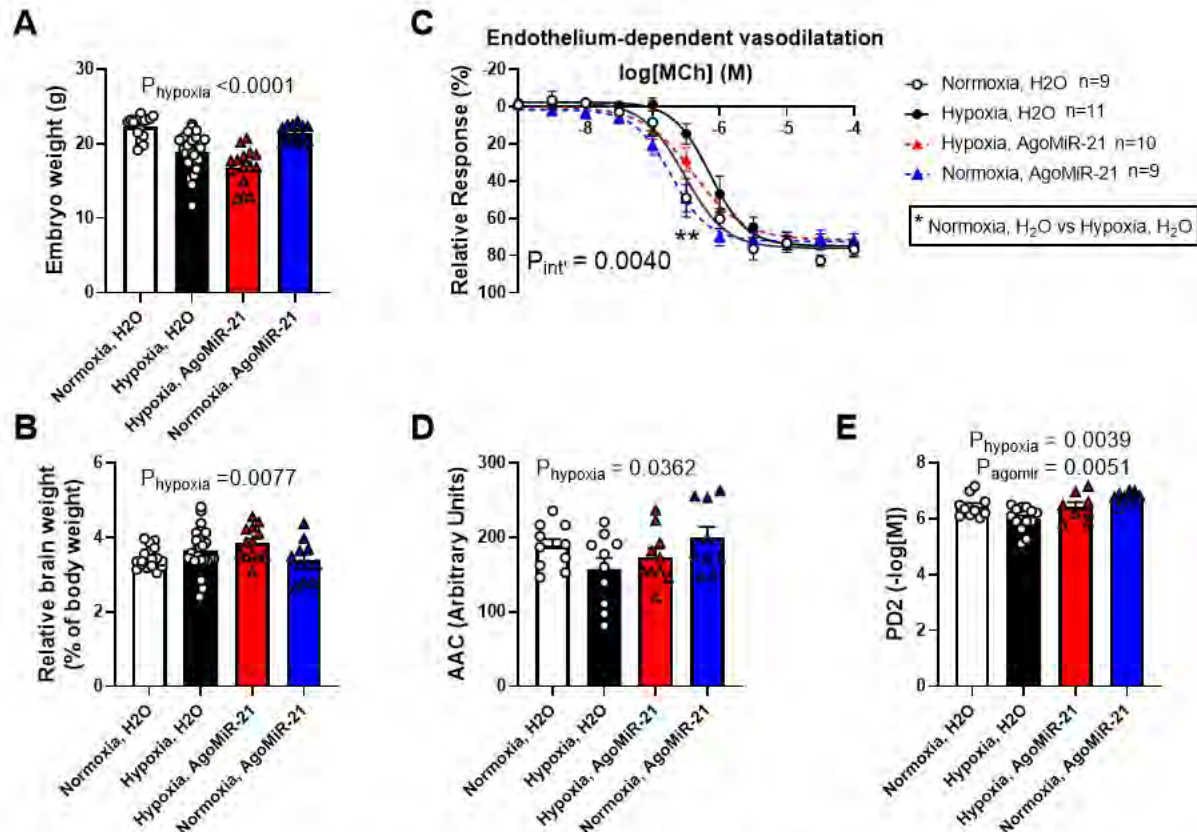


Figure 1. Growth and vascular function in the chicken embryo. Day 19 outcomes from chicken embryos incubated under normoxic or hypoxic conditions, with and without vehicle (H₂O) or AgoMiR-21 treatment. A, Embryo weight; B, Relative brain weight (as a % of body weight); C, Relative relaxation of pre-constricted cranio-tibial artery segments in response to increasing doses of cholinergic agonist methacholine (MCh) as a % of starting tension; D, Area above the curve (AAC) of the relative response to methacholine; E, Sensitivity pD2 (-logEC₅₀) of cranio-tibial arteries to methacholine. Data are presented as mean±SEM. Significant differences ($P < 0.05$) are: * for Normoxia, H₂O vs. Hypoxia, H₂O (Two-way ANOVA with Tukey's *post hoc* test).

PCB008

The influence of acute dietary nitrate supplementation on endothelial resistance to ischemia reperfusion injury in postmenopausal women

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With postmenopausal women expected to reach a population of 1.2 billion by 2030, there is a great need for low-risk, non-estrogen therapies for cardiovascular disease prevention. Evidence suggests that relative to the early follicular phase, women in the late follicular phase are protected against endothelial ischemia-reperfusion (IR) injury when estradiol concentrations are highest.^{1,2} This suggests that the concurrent loss of estrogen following menopause may impair recovery from endothelial ischemia-reperfusion injury (ex. heart attack, bypass surgery).³ Consumption of beetroot juice and other nitrate-rich foods (celery, spinach, lettuce, etc.) is an effective non-pharmaceutical intervention to increase systemic bioavailability of the vasoprotective molecule, nitric oxide, through the exogenous nitrate-nitrite-nitric oxide pathway. The purpose of this randomized, placebo-controlled, double-blind crossover clinical trial was to determine if a single dose of dietary nitrate supplementation, in the form of beetroot juice, can improve endothelial resistance to IR injury in postmenopausal women at two distinct stages of menopause. We hypothesized that a single dose of nitrate-rich beetroot juice would improve endothelial resistance to IR injury to a greater extent in early- compared to late-postmenopausal women. Early- (1-6 years following their final menstrual period (FMP), n=12) and late- (>6 years FMP, n=12) postmenopausal women consumed a single dose of nitrate-rich (600 mg/140 mL) and nitrate-depleted (placebo, 0 mg/140mL) beetroot juice. Study visits were separated by a washout period of at least two weeks. Whole arm endothelial IR injury was induced by inflating a pneumatic cuff (250 mmHg) for 20 minutes followed by 15 minutes of reperfusion. Brachial artery flow-mediated dilation (FMD, duplex ultrasound) was measured at baseline, acutely (90 minutes post-juice consumption), 15-, and 30 minutes after IR injury for each drink. Analyses with general linear models (SPSS) revealed a significant ($p<0.05$) time*treatment interaction effect for FMD. Pairwise comparisons revealed that FMD was significantly lower 15-minutes post-IR in comparison to all other time points with nitrate-depleted beetroot juice (Early-FMD_{placebo}=2.553.48%, Late-FMD_{placebo}=1.322.06) and was lower than post-IR with nitrate-rich beetroot juice (Early-FMD_{nitrate}=3.924.15%, Late-FMD_{nitrate}=3.242.67%, $p=0.014$). There was no significant interaction effect of menopausal stage. These results suggest that a single dose of dietary nitrate supplementation is sufficient to increase endothelial resistance to whole-arm IR injury to a similar extent in women at both stages of postmenopause. Our observations emphasize the endothelial protective benefits of dietary nitrate supplementation for postmenopausal vascular health.

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Gosalia J, Delgado JM, Flanagan M, Basu S, Kim-Shapiro DB, Murias JM, Proctor DN. A single dose of dietary nitrate supplementation protects against endothelial ischemia-reperfusion injury in early postmenopausal women. *Appl Physiol Nutr Metab*. 2022 Jul 1;47(7):749-761. doi: 10.1139/apnm-2021-0693. Epub 2022 Mar 31. PMID: 35358395.

PCB009

Effect of Vitamin D Supplementation on Doxorubicin-Induced Cardiotoxicity in Rats

Faten Diab, Noha Abdel-Aziz Nassef, Manal Abd-El Hamid, Yasmin Khaled

undefined

Background: Doxorubicin, a chemotherapeutic agent, that has great efficacy in treatment of many solid tumors, bone sarcomas, and cancers of the breast, ovary, and other tumors. Unfortunately, the clinical use of this valuable anticancer drug is limited due to its life-threatening cardiotoxic effect¹. Vitamin D is recently known for its cardioprotective effects². **The aim of this study:** To investigate role of vitamin D on the doxorubicin-induced cardiac dysfunction. **Materials and Methods:** 70 female Albino-rats were divided into 4 groups: control group (C: n=21); doxorubicin-treated group (Dox: n=18): given i.p. injection of 2.5 mg/kg twice per week (cumulative dose:15 mg/kg) over 3 weeks; vitamin D-supplemented group (Vit D: n=16): given vitamin D by oral gavage in a dose of 500 IU/kg daily, 5 days a week, also for 3 weeks; and combined Doxorubicin-treated+vitamin D-supplemented group (Dox+Vit D: n=15): rats received the same used doses for the same duration. After 3 weeks, all rats were subjected to EGG recording, determination of plasma levels of brain natriuretic peptide (BNP), cardiac troponin I (cTnI), vitamin D concentration (vit D) and total calcium level (Ca). Hearts were excised and perfused in Langendorff preparation to record intrinsic in vitro activity of the heart under basal conditions according to the technique of Langendorff. Malondialdehyde (MDA), total antioxidant capacity (TAC) and heat shock protein 20 (HSP 20) were assessed in the cardiac tissue. Also, cardiac tissue histopathological studies were performed.

Results: Dox-treated rats showed significant depression in peak tension (PT) and myocardial flow rate (MFR) together with significant prolongation in time to peak tension (TPT), half relaxation time (HRT) and contraction time (CT). These changes were accompanied by significant elevation of plasma brain natriuretic peptide (BNP), cardiac troponin I (cTnI) and in cardiac tissue malondialdehyde (MDA) and a significant decrease in plasma vit D, total calcium, and cardiac tissue total antioxidant capacity (TAC) and heat shock protein20. Histopathological examination of the Dox treated rats revealed markedly distorted muscle fibers with indistinct cell borders, bright eosinophilic cytoplasm, intra-cytoplasmic vacuoles and small pyknotic nuclei or absent nuclei, together with interstitial edema & aggregates of inflammatory cells and thick irregular collagen fibers in between the muscle fibers. This functional, biochemical, and histopathological data reflects development of doxorubicin-induced cardiomyopathy.

Concomitant supplementation of vitamin D to doxorubicin treated rats resulted in significant increases in PT, MFR, plasma vitamin D, total calcium as well as cardiac TAC and HSP20; while the MDA, plasma BNP and cTnI were significantly decreased, all compared to the Dox-treated rats. These findings were associated with regaining the normal collagen fiber distribution between cardiac muscle fibers with resolution of interstitial edema.

Conclusion: Vitamin D supplementation can partially mitigate cardiac dysfunction induced by chronic doxorubicin by improving the cardiac antioxidant state and heat shock protein 20 level.

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PCB010

GP1R increases expression of vascular α 2C-adrenoceptors and mediates cold-induced vasoconstriction

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Background: Raynaud's phenomenon (RP), which results from exaggerated cold-induced vasoconstriction, is more prevalent in females than males, implicating 17 β estradiol (estrogen; E2) in its etio-pathology. We previously reported that estrogen acts through the cAMP/Epac/Rap/JNK pathway to induce the expression of vascular α 2C-adrenoceptors (α 2C-AR), the sole mediator of cold-induced vasoconstriction. An impermeable form of E2, namely E2:BSA, mimics estrogen's effects, suggesting a role for the membrane estrogen receptor (GP1R) in E2-induced α 2C-AR expression.

Objective: Therefore, we hypothesized that GP1R mediates E2-induced upregulation of α 2C-AR through cAMP/Epac/JNK/AP-1 pathway.

Methods: In-cell ELISA, Luciferase assay, cAMP assays were used to measure kinase activity, transcription and intracellular accumulation of cAMP, respectively. Ethical approval was obtained for isolating cells from dermal arterioles (post-circumcision clinical "waste").

Results: Here, we show that G15, a selective GP1R antagonist (1 μ M), diminished E2 (10^{-10} M)-induced transcription of α 2C-AR in primary arteriolar smooth muscle cells that we extracted from human dermal arterioles (n=3; p<0.05). G-1, a selective GP1R agonist, (10 μ M) was sufficient to induce α 2C-AR transcription, increase cAMP levels and induce JNK activation (n=3; p<0.05 for all). Pretreatment with ESI09 (10 μ M; an Epac inhibitor) abolished both G-1-induced α 2C-AR upregulation (n=3; p<0.05), and JNK activation (n=3; p<0.01). Moreover, pretreatment with SP600125 (3 μ M; a JNK specific inhibitor) but not H89 (2 μ M; a PKA specific inhibitor) suppressed G-1-induced α 2C-AR upregulation (n=3 for both; p<0.05 or p>0.05, respectively). Similarly, overexpression of Epac dominant negative mutant (Epac-DN) attenuated G-1-induced expression of α 2C-AR (n=3; p<0.05). This inhibitory effect of Epac-DN was overridden by the co-transfection of constitutively active JNK mutant (n=3; p<0.05). Furthermore, G-1 caused a concentration-dependent increase in the transcriptional activity of AP-1-driven reporter construct (n=3; p<0.01). Mutation of this AP-1 site in the α 2C-AR promoter significantly reduced its G1-induced transcription (n=3; p<0.01).

Conclusion: Collectively, these results show that GP1R acts through the cAMP/EPAC/JNK/AP-1 signaling to induce expression of vascular α 2C-AR. These findings unravel a new mediator of cold-induced vasoconstriction, namely GP1R, and present it as a potential target in the management of RP in estrogen-replete females.

PCB011

Effect of home-based dynamic intermittent pneumatic compression therapy on vascular and functional health outcomes in chronic stroke: A randomized controlled trial

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Introduction: Stroke is the second leading cause of disability worldwide (Feigin et al., 2022). Although individuals undergo traditional physiotherapy, intermittent pneumatic compression (IPC) therapy may benefit stroke patients as increases in venous return may allow people to engage with more physical activity and more intensive training sessions (Shroeder et al., 2019) that may result in better health, mobility and ultimately quality of life (Park and Kim, 2019).

Aim: The purpose of this study was to assess the effect of using a home-based IPC device on vascular health and functional outcomes in individuals with chronic stroke using a randomized controlled study.

Methods: Research was conducted following institutional human ethics committee approval, while the study was registered with Clinical Trials.gov Protocol Registration and Results System (NCT05276453; <https://clinicaltrials.gov/ct2/show/NCT05276453>). Thirty-one stroke survivors (64.3 ± 14.3 y; 4.3 ± 2.7 y since stroke) took part in this study and completed pre- and post-intervention assessments which consisted of measures of vascular health (pulse wave analysis, carotid-femoral pulse wave velocity) and functional capacity (six-minute walk test, timed-up-and-go, 10m walk test). On completion of the initial (pre) assessment, individuals were randomly assigned to either a daily, 12-week, home-based IPC condition, or to a usual care control (CON) group. Outcomes were assessed using analysis of covariance, controlling for any baseline differences.

Results: A Time by Condition interaction was observed for peripheral systolic blood pressure ($p < 0.05$, $\eta^2 = 0.140$), with significantly greater reductions reported between pre- and post-intervention for IPC (147.4 ± 18.1 to 139.5 ± 15.6 mmHg, respectively) than CON (139.1 ± 17.5 to 137.7 ± 16.4 mmHg, respectively). Similar findings were observed for central systolic blood pressure and the six-minute walk test (both $p < 0.05$). For the six-minute walk test, participants significantly increased their walking distance between pre- and post-intervention assessments for IPC (158 ± 73 to 181 ± 109 m, respectively) but not CON (170 ± 87 to 174 ± 117 m, respectively) ($\eta^2 = 0.248$). Average weekly physical activity levels significantly increased, and time spent sitting significantly decreased for IPC compared to CON (both $p < 0.05$).

Conclusions: The observed improvements in blood pressure and six-minute walk test distance, in combination with an increase in physical activity and reduced sedentary behaviours, are important positive findings when considering the use of IPC training for “at home” rehabilitation therapy for chronic stroke survivors.

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PCB012

The effect of uninterrupted and interrupted sitting on vascular health and cognitive function in people with Long COVID

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Long COVID-19 is defined as signs, symptoms and conditions that develop after initial SARS-CoV-2 infection. Two million people in the UK in private households are currently experiencing self-reported long COVID, of which 61% of these people have been living with symptoms for more than a year. (Office for National Statistics, 2023).

There have been over fifty different symptoms attributed to long COVID. The most commonly presented symptom is fatigue (Aiyegbusi *et al.*, 2021). Fatigue can cause behavioural changes, resulting in increased sedentary time. This can have an adverse effect on vascular function, and increased risk of cardiovascular disease (Natelson, Brunjes and Mancini, 2021). Sitting for periods as little as an hour can worsen vascular function (Taylor *et al.*, 2022). Interrupting this period with short bouts of movement has been shown to mitigate the negative effect on vascular health (Paterson *et al.*, 2020). The mechanisms which cause Long COVID are still mainly undetermined, however, it is evident that physical exertion, cognitive effort, and stress can lead to relapses which result in worsened symptoms for hours up to months in Long COVID populations. This study used questionnaires (DePaul Symptom Questionnaire Post-Exertional Malaise) and telephone conversations to ensure that participants felt they were able to undertake the activity outlined and to ensure they were not suffering from irregular post-exertional malaise (PEM) following involvement. Ethical approval was obtained by Health and Care Research Wales (IRAS: 309606 22/SC/0120).

This study aimed to investigate whether interrupting continuous sitting in people with Long COVID with movement could prevent the decline in vascular health. Inclusion criteria required a clinical diagnosis of Long COVID. Activities chosen represent movements that would occur during daily living. Interruption includes three bouts of five sit-to-stands, five calf raises, and three minutes of self-paced walking. Measures of central and peripheral blood pressure, arterial stiffness (SphygmCor XCEL), executive function (TrailMaking Tasks), and cerebral oxygenation (Near Infrared Spectroscopy) were recorded at baseline, during, and following two hours of interrupted or uninterrupted sitting.

This study is currently still collecting data. To date, one participant with Long COVID has fully completed the testing procedure. Six additional participants have begun testing (59.25 ± 13.84 years, symptoms lasting >12 months), and three are scheduled to be familiarised and completed in the coming month, with more than 14 others expressing interest, with initial contact made. Preliminary results demonstrate an observed, yet insignificant increase in seated pulse wave velocity from baseline, $0.4 \pm 0.14\text{m/s}$ (uninterrupted) and $0.6 \pm 0.24\text{m/s}$ (interrupted).

This research could provide rationale for future policies at a government level and advice from a GP surgery to be centred towards reducing the length people with Long COVID are sitting for continuously, to mitigate any detrimental impact on their vascular health. Additionally, results with respect to cognition and cerebral oxygenation may provide a rationale for people with Long COVID, including those still having to work while symptomatic, engaging in cognitively stimulating tasks following light physical activity i.e. making a cup of tea.

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PCB013

The molecular basis of control of vascular tone by the therapeutic drug niclosamide

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TMEM16A Ca²⁺-gated Cl⁻ channels (CaCCs) are highly expressed in vascular smooth muscle cells and contractile pericytes, where they provide a key depolarising mechanism. Thus, the TMEM16A channel has been proposed as a drug target for diseases of altered vessel tone including stroke, vascular dementia and (systemic, pulmonary) hypertension (1, 2). However, no therapeutic drugs interacting with the channel have yet reached clinical practice. The FDA-approved anthelmintic drug niclosamide inhibits the TMEM16A channel (3), however the effect of niclosamide on vascular smooth muscle and contractile pericytes is not fully defined. Here, we examine the effect of niclosamide on the tone of isolated rat aorta and the diameter of cerebral cortical capillaries and examine the underlying mechanism.

Niclosamide (1 mM) led to a reduction in both phenylephrine- and KCl-induced contraction of rat aortic rings, assessed through wire myography. The force generated by aortic rings in response to phenylephrine (10 µM) or KCl (100 mM) was reduced in the presence of niclosamide by 82.0±5.5% (n = 7) and 94.8±3.6% (n = 5), respectively. Niclosamide also impaired the constriction of pericytes in rat cortical brain slices by 41.6±11.3% (n = 6) after exposure to endothelin-1 (10 nM), assessed through differential interference contrast imaging.

Heterologous TMEM16A current in HEK293T cells and native CaCC currents in isolated rat aortic smooth muscle cells (SMCs) were similarly modulated by niclosamide; when measured at +100 mV, the heterologous TMEM16A and native CaCC currents were reduced by 27.8±3.9% (n = 10) and 18.7±3.7% (n = 6) in the presence of niclosamide (1 mM). However, at negative potentials (ranging from -100 to -40 mV) niclosamide activated these currents by ~2.9 folds and 3.1-folds, respectively. The potentiation of the CaCC currents at negative potentials could not explain the niclosamide-mediated vasorelaxation, since CaCC currents are depolarising, and promote smooth muscle contraction. In SMCs, niclosamide (1 mM) significantly inhibited voltage gated Ca²⁺ currents and potentiated a hyperpolarising current; these effects are likely determinants of niclosamide-induced relaxation.

This study elucidated the effects of niclosamide on a range of ionic currents and excluded the TMEM16A channel as a mediator of niclosamide-induced relaxation in arterial smooth muscle and contractile pericytes. Since niclosamide has been proposed for drug repurposing for a variety of indications, knowledge of its molecular targets will increase our understanding of the therapeutic and possible side effects of this drug. This work also offers insight into the relative contribution of a range of ionic currents to the physiological control of vascular tone.

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PCB014

NATRIURESIS INDUCED BY ACUTE INTRAVENOUS SALINE INFUSION IN MICE IS MEDIATED BY TUMOR NECROSIS FACTOR-ALPHA (TNF α) - EVIDENCE FOR PHYSIOLOGICAL ROLE OF THIS CYTOKINE IN KIDNEY.

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Although TNF α is considered to play its role in many pathological conditions, the physiological role of this cytokine in regulating function of many organs including the kidney is increasingly recognized in recent days. Chronic high salt (HS) intake in diet induces an immune response that activates the mononuclear phagocyte system (MPS) cells to release TNF α that appears in the circulation in its soluble form (sTNF α). It has been shown that HS (4% NaCl) diet alone for 2 weeks increased MPS cell infiltration in the renal tissue in mice and this is associated with increases in the circulating sTNF α level in the plasma (Singh et al-2013). It was also demonstrated that 1% salt added to the drinking water for 3 days in mice increases the urinary excretion rate of sTNF α indicating that its release in the kidney from the infiltrated MPS cells occur even at the early stage of HS intake (Hao et al-2013). We have previously demonstrated that intravenous infusion of recombinant TNF α in mice induces natriuretic response by inhibiting tubular sodium reabsorption (Shahid et al-2008; Majid-2011). We hypothesize that the intravenous saline infusion can also induce the production of sTNF α from activated MPS cells due to an increase in salt content in immune tissues and such increase in sTNF influences saline induced natriuretic response in the kidney. To examine this hypothesis, we measured the changes in sTNF α levels in plasma and urinary excretion rate ($U_{\text{TNF}\alpha}V$) during intravenous infusion of isotonic saline (0.9% NaCl), first at euvoletic conditions (3 $\mu\text{L}/\text{min}$ for 60 min; Baseline period) and then at an enhanced infusion rate (12 $\mu\text{L}/\text{min}$ for 90 min; saline volume infusion period) in anesthetized mice ($n=5$). The concentration of sTNF α in plasma and urine samples were determined using ELISA kit (Ebioscience, Woburn, MA) for measuring this cytokine. Baseline level of plasma sTNF α was undetectable, however, the level was increased to 3.7 ± 1.3 pg/mL during saline volume infusion period. Baseline $U_{\text{TNF}\alpha}V$ level was 0.01 ± 0.002 pg/min/g of kidney wt, which was increased to 0.11 ± 0.03 pg/min/g ($P<0.05$) during volume infusion period. In another group of mice ($n=5$) pretreated with a TNF α inhibitor, etanercept (0.5 mg/kg intraperitoneally once daily for 3 days prior to the experiment day), it was observed that this increase in $U_{\text{TNF}\alpha}V$ during saline volume infusion period was markedly attenuated (0.003 ± 0.002 to 0.006 ± 0.004 pg/min/g; $P=n.s.$). Interestingly, the diuretic and natriuretic responses to enhanced saline infusion were markedly attenuated in these etanercept pretreated mice without any significant changes in renal blood flow or glomerular filtration rate. The usual natriuretic response (0.5 ± 0.2 to 4.8 ± 1.1 $\mu\text{mol}/\text{min}/\text{g}$; $P<0.05$) to enhanced saline infusion observed in control mice was seen markedly attenuated (0.4 ± 0.1 to 0.8 ± 0.3 $\mu\text{mol}/\text{min}/\text{g}$; $P=n.s.$) in etanercept pretreated mice. These findings demonstrate for the first time that an intravenous saline volume infusion resulted an increase in sTNF α level in plasma and in urine. These results strongly suggest a physiological natriuretic role for sTNF α in regulating renal excretory function during acute saline volume infusion.

PCB015

Back to the future – mathematical models to capture ion channel kinetics using short high-information voltage clamp protocols

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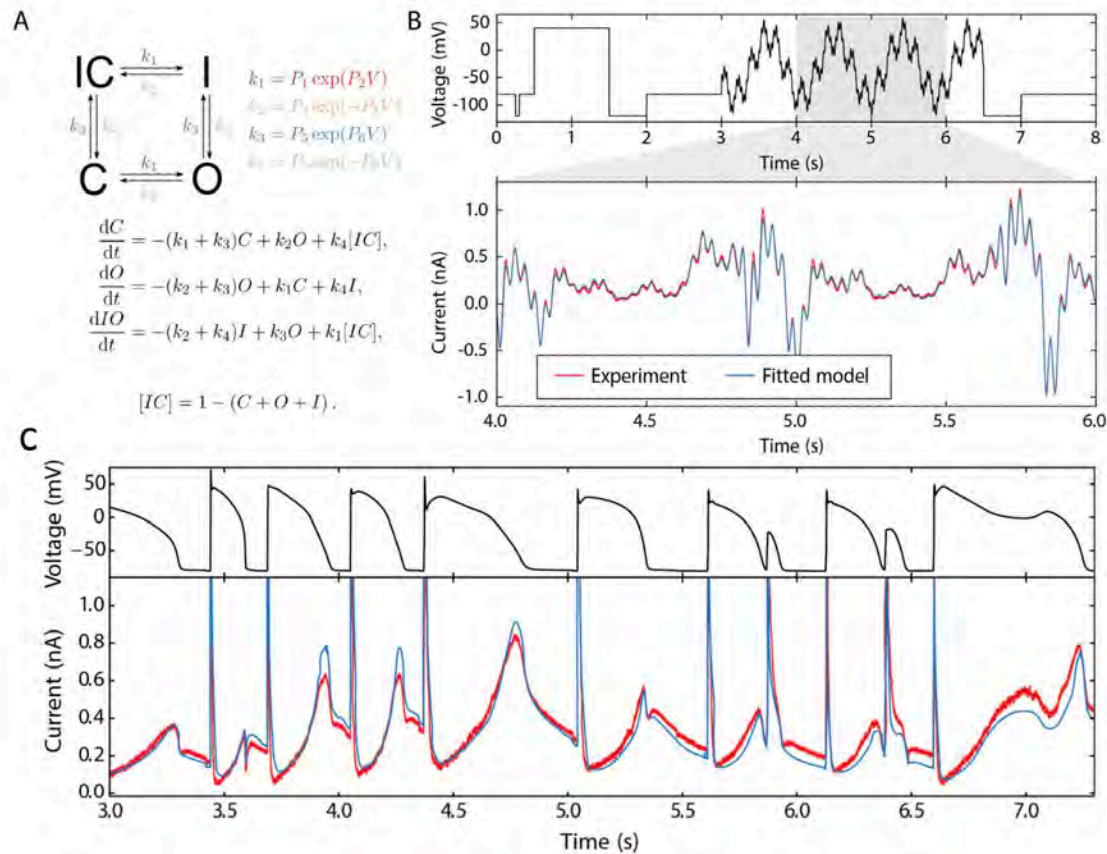
Introduction: since the work of Hodgkin & Huxley, mathematical models of ion channel gating have been used to understand and predict the effects of ion currents in action potential formation. Today we still tend to use the same approach to voltage-clamp protocol design that Hodgkin & Huxley used: designs that enable model parameter values to be estimated manually from graph paper.

Aims/Objectives: to use short high-information voltage clamp protocols in partnership with computational modelling to characterise ion currents. To allow a model (Figure 1A) to be fitted and tested, with this process repeated after performing multiple experimental interventions in the same cell. Here we show results of using short protocols to capture the kinetics of I_{Kr} / $K_{v11.1}$ / hERG currents in a range of settings.

Method: we apply either a short sinusoidal voltage clamp protocol (Figure 1B, [1]) or a square wave 'staircase' version [2] to CHO cells stably expressing hERG1a at room temperature or physiological temperature in manual and automated patch settings. We then use computational optimisation to fit a simple mathematical model for hERG to the resulting currents, and use it to predict the results of conventional voltage clamp protocols and physiological action potentials.

Results: the short protocols result in highly predictive mathematical models: Figure 1C shows a prediction from a model fitted to the sinusoidal protocol shown in Figure 1B against experimental data. The parameter values within these models then capture our knowledge of channel gating more accurately than a series of Current-Voltage or Time Constant-voltage curves, with the benefit they can be re-used to predict currents in new situations/voltage-clamp protocols that were not examined in the original experiment. There are also opportunities to use mathematical models to account for patch clamp artefacts [3] to consolidate information from different patch clamp recordings more reliably.

Conclusions: this approach offers the opportunity to intervene and reassess currents multiple times in one experiment, and to generate a mathematical model that quantitatively captures our understanding about channel gating. For instance, we can alter temperature to examine the temperature dependence at the level of individual rate and voltage-dependence parameters within a model, rather than at the level of processes such as 'activation' or 'recovery from inactivation' [4]. We can also build models of mutant channels, and/or characterise changes in currents in the presence of drug compounds that alter channel gating [5].



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PCB016

Glutaredoxin1-overexpression attenuates chronic angiotensin-II induced hypertension and changes cardiac dynamics

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Angiotensin-II (ANGII) induces hypertension and cardiac hypertrophy, leading to heart failure in humans. ANGII is a potent inductor of oxidative stress, which changes proteins function via oxidative post-translational modifications (oxPTM). Glutaredoxin-1 (Grx) catalyses the removal of an oxPTM, S-glutathionylation, and has been shown to be important in peripheral artery. However, the role of Grx in ANGII induced cardiac failure is unknown. This project aims to investigate the effect of ANGII infusion in mice that overexpress Grx.

Grx transgenic (TG) and wild-type (WT) littermates (C57Bl6/j, Male, 24.7-30.5g) were implanted with osmotic pumps (Alzet) containing either saline or ANGII (1.1mg/kg/day; s.c, n=4-8.). After 2 weeks, mice were anaesthetised with isoflurane (2%) and a pressure-volume catheter (Transonics, 1.2F, 4.5mm) was inserted retrogradely into the left ventricle (LV) via the aorta in a closed chest preparation. To obtain aortic blood pressure (BP) and LV pressure-volume loops. All values are mean±SEM, compared by 1-way ANOVA.

In WT mice (n=4-8), ANGII increased systolic BP(SBP) (Saline: 95.7±4.0 mmHg vs ANGII: 116.0±5.6mmHg, $p<0.05$) and diastolic BP(DBP) (Saline: 65.2±3.9mmHg vs ANGII: 82.9±4.0mmHg, $p<0.05$). However, no significant increase in SBP and DBP were observed in TG mice (SBP Saline: 96.8±2.3mmHg vs ANGII: 109.4±6.1mmHg, $p=ns$) and DBP (Saline: 64.26±2.1 mmHg vs ANGII: 74.58±4.9 mmHg, $p=ns$) remained constant. Heart rates were not significantly different between the four groups ranging from 560 to 588bpm.

Although there was an increase in BP in WT ANGII mice, left ventricle end-diastolic/systolic pressure (EDP/ESP) and end systolic volume(ESV) remained unchanged between the four groups. However, ANGII significantly lowered end diastolic volume (EDV) in TG compared to WT hearts (WT:42.2±2.7uL vs TG:22.4±2.0uL, $p<0.05$). Hence, stroke volume(SV) was also lowered in TG compared to WT (WT:24.4±3.0uL vs TG:16.4±1.6uL, $p<0.05$). Yet, these changes had no overall effect on LV cardiac output (ANGII, WT: 13947±1353 mL/min vs. TG: 2191.0±286.2mL/min, $p=ns$) or stroke work (ANGII, WT: 2191±286 mmHg*uL, vs. 2191.0±286.2mmHg*uL, $p=ns$). Interestingly, ANGII significantly lowered LV contractile state (Powermax) in response to ANGII in TG compared to WT mice (WT:343467±782502 mmHg*uL/s vs TG:161963±29044 mmHg*uL/s, $p<0.05$).

In the control groups, there was no difference in cardiac function between saline treated WT and TG mice. With stroke work (WT: 1719±109 mmHg*uL vs TG: 1341±402mmHg*uL, $p=ns$), cardiac output (WT: 12004±783 mL/min vs TG: 9227±2237mL/min, $p=ns$) and stroke volume (Saline: 20.5±0.8uL vs 16.4±1.6uL , $p=ns$) remained unchanged between WT and TG mice.

In summary chronic ANGII infusion did not increase blood pressure in mice overexpressing Grx, but lowered pre-load with subsequent lower stroke volume and cardiac contractility. Future studies will find which redox sensitive proteins undergo reversal oxPTM to elicit these functional changes, leading to identification of novel therapeutic targets.

PCB017

CARDIOPROTECTIVE EFFECT OF CO-ENZYME Q10 ON DOXORUBICIN INDUCED CARDIOTOXICITY IN ADULT MALE ALBINO WISTAR RAT

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Introduction: Doxorubicin (DOX) is a widely used chemotherapeutic agent but it is limited by its cardio-toxic side effect. Coenzyme Q10 (CoQ10) which could be considered as a vitamin is one of the most significant lipid antioxidants, which prevents the generation of free radicals and modifications of proteins, lipids, and DNA. However, it is not known whether CoQ10 is capable of preventing or ameliorating DOX induced cardiotoxicity.

Aim: The present study was thus designed to ascertain the cardioprotective effects of CoQ10 on DOX induced cardiotoxicity.

Method: All procedures were undertaken in accordance with regulations as set out by the Babcock University Research and Ethical Committee (BUREC). Adult male Wister rats weighing 180-200 g were randomly divided into five groups (n=7). Animal experimentation lasted for 14 days. Group 1 animals served as control and were untreated. Groups 3-5 animals were treated with dexamethasone (3 mg/Kg), CoQ10 (1 and 10 mg/kg) respectively, for 14 days. Group 2-5 animals were given a single dose of DOXO (15 mg/Kg) on day 11 of the study. Cardiovascular, biochemical, histological and molecular parameters were determined at the end of the study.

Results: DOXO altered cardiovascular function in rats evidenced by cardiac arrhythmia, hyperlipidemia and increased serum creatine kinase-myocardial band (CK-MB) levels which was associated with increased cardiac oxidative stress markers. CoQ10 especially at the higher dose ameliorated the DOXO induced cardiovascular dysfunction which was associated with decrease cardiac expressions of TNF- α and GSK3B genes.

Conclusion: CoQ10 especially at 10 mg/Kg mitigated DOXO induced cardiac dysfunction which involved the modulation of TNF- α / GSK3B signaling pathway.

PCB018

Analysis of seasonal changes in cardiac conduction system

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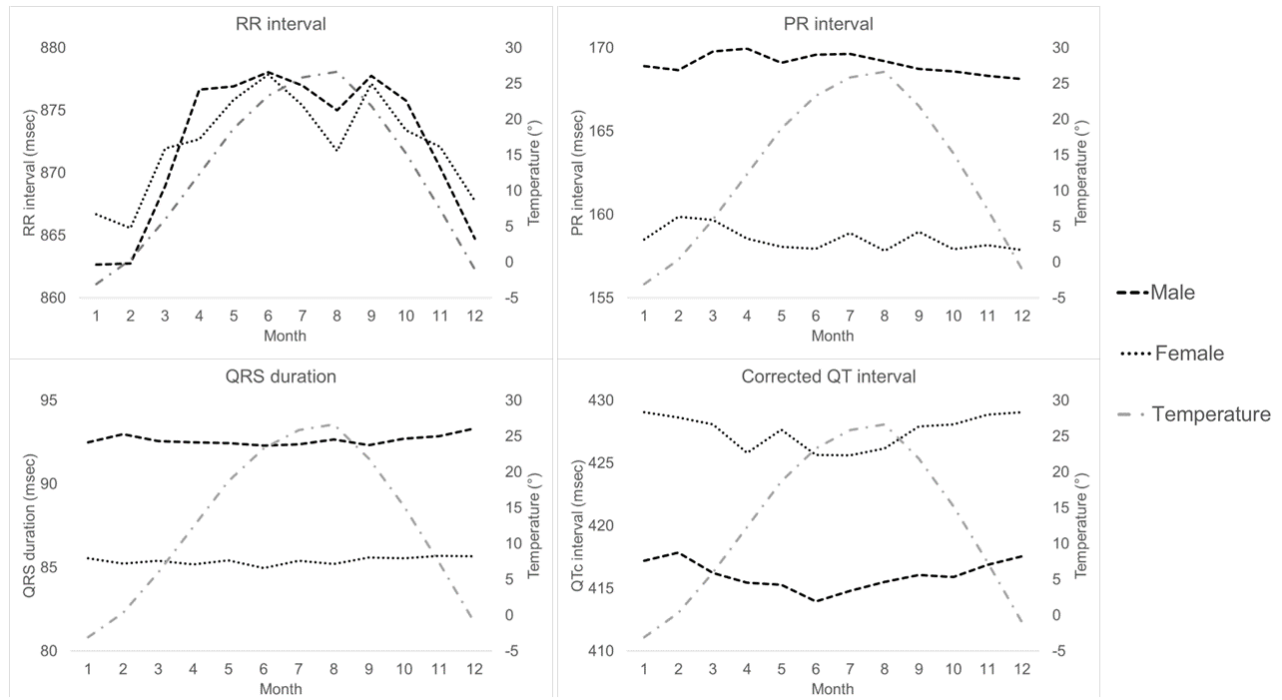
Most studies report there is a 'winter peaks' in cardiovascular-related events. It is known that temperature affects the velocity of electrical conduction in the heart. This study was aimed to investigate the change of intervals in an electrocardiogram (RR, PR, QTc interval and QRS duration) according to the season.

We analyzed 121,286 electrocardiograms from 60 to less than 100 beats per minute among people performed for health surveillance at Seoul National University Bundang Hospital for the past 10 years (2009-2018). The mean of each interval including QTc interval using Bazett's formula, were compared according to the month and sex. The mean temperatures in Seoul during the above period was obtained from the Korea Meteorological Administration database.

During the 10-year period during which ECGs were obtained, Seoul, located in the northern hemisphere, had the lowest average temperature in January and the highest in August. The RR interval changed similarly to the change of temperature, so heart rate (obtained by dividing 60000 by the RR interval) was the fastest in January and shortest in August. The PR interval and QRS duration were consistently observed even with temperature changes and maintained constant levels throughout the four seasons. The QTc interval showed an inverse correlation with temperature, being longest in January and shortest in August.

In winter, when the temperature was low, the heart rate was fast and the QTc interval was long, and in the summer when the temperature was high, the heart rate was slow and the QTc interval was short. This may be one of the reasons for the high frequency of cardiovascular diseases in winter.

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PCB019

Cardiac Energy Metabolism in Crotonaldehyde (Beta-methyl Acrolein) Exposed Male Wistar Rats

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Introduction: Alterations in cardiac energy metabolic pathways arising from exposure to environmental pollutants have been linked to the development of cardiac dysfunction. Crotonaldehyde (CRO), a hazardous environmental pollutant, has been reported to be cardio-toxic.

Aim: However, dearth of information exists on the effect of CRO exposure on cardiac energy metabolism with respect to its cardio-toxicity. This study was therefore designed to investigate cardiac energy metabolism in male Wistar rats exposed to CRO.

Method: 36 male Wistar rats (150-170g; n=9) were grouped into 4 (I-IV): Control (10mL/kg normal-saline), CRO (0.75, 1.5, and 2.5mg/kg, p.o) for 28 days. Blood samples were obtained and evaluated for plasma Creatinine Kinase-Myocardial band (CK-Mb), cardiac troponin-I (cTnI), glucose, triglyceride, Free Fatty Acid (FFA) and insulin levels by spectrophotometry. Cardiac triglyceride, FFA, pyruvate, glucose-6-phosphate, cardiac hexokinase, pyruvate dehydrogenase (PDH) and nuclear factor erythroid-2 related-factor (Nrf2) activities were determined using ELISA. Cardiac malondialdehyde (MDA), hydrogen peroxide (H₂O₂), reduced glutathione (GSH) level, superoxide dismutase (SOD), and catalase activities were measured by spectrophotometry. Cardiac glucose transporter-4 (GLUT4), Peroxisome Proliferator-activated Receptor-alpha (PPAR α), Carnitine Palmitoyl Transferase-1 β (CPT1 β) and AMP-activated Protein Kinase (AMPK) activities were determined immunohistochemically. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$. This study complied with ethical standard and was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/18/007).

Results: Levels of CK-Mb, cTnI, glucose increased significantly while plasma and cardiac triglyceride, FFA, and insulin reduced significantly in all CRO-treated groups compared with control. Cardiac Pyruvate level, hexokinase and PDH activities increased significantly in all CRO-treated groups compared with control. Cardiac levels of MDA and H₂O₂ increased significantly while GSH and Nrf2 reduced; SOD and CAT activities decreased following CRO exposure compared with control. Cardiac GLUT4 (15.31 and 28.86 %) and PPAR α (73.25, 102.79 %) expression increased in groups II and III, while CPT1 β expression decreased in group II (66.46 %) but increased in groups III and IV (52.80 and 33.45 %) compared with control.

Conclusion: Crotonaldehyde exposure exerts cardio-toxic effects by altering cardiac energy metabolism resulting in reduced free fatty acid, increased glucose uptake and utilization; down-regulation of nuclear factor erythroid-2 related-factor and up-regulation of peroxisome proliferator-activated receptor-alpha.

Keywords: Crotonaldehyde, energy metabolism, cardio-toxicity, redox imbalance

PCB020

Losartan treatment improves the cardiovascular profile in rats with right-sided insular experimental stroke

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Intro: Stroke, a cerebrovascular disease, is the second leading cause of death in the world. Patients who survive stroke present physiological alterations and prognostic depends on the affected area. In this regard, stroke in the insular cortex (IC) results in marked sympathetic-mediated increase in baseline heart rate, cardiac molecular changes and arrhythmias. Therefore, investigating strategies that can alleviate post-insular stroke consequences becomes extremely relevant. We have recently described an experimental model of right side insular hemorrhagic stroke in rats that reproduces some physiological alterations observed in humans, including marked increases in cardiac sympathetic output (Marins FR et al. 2020, 2021). Evidence indicates that the renin-angiotensin system (RAS) peptides interact with the sympathetic nervous system (Fontes MAP et al., 2016). **Aims:** Here, we evaluated the effect of angiotensin II (Ang II) AT₁ receptor blocker, losartan, on cardiovascular changes generated from experimental hemorrhagic stroke at the IC of rats. **Methods:** Experiments were conducted in accordance with the U.S. NIH Guide for the Care and Use of Laboratory Animals and approved by CEUA UFMG; protocol 112/2019). Wistar rats were: 1) anesthetized (ketamine 80mg/kg - xylazine 7 mg/kg); 2) submitted to unilateral nano-injection of blood into the IC (Stroke IC; 200 nL; n=6) or saline into the IC (Sal IC; NaCl 0.9%, 200 nL; n=6; control), and 3) prepared for recording of mean arterial pressure (MAP) and heart rate (HR). Just after experimental stroke, separated groups of rats were submitted to three days of treatment (single daily intraperitoneal dose) of losartan (Los i.p.; 10 mg/kg) or saline (Sal i.p.; NaCl 0.9%, 0.1ml /100g). **Results:** Stroke IC rats showed elevated baseline HR when compared with the Sal IC group (422 ± 10 bpm vs 365 ± 6 $P<0.01$). Baseline MAP was similar between groups. In Stroke IC rats, treatment with Los i.p. restored HR to baseline levels and decreased baseline blood pressure values when compared with Stroke IC rats treated with Sal i.p. (HR: Los i.p. 358 ± 7 vs Sal i.p. 428 ± 10 bpm $P<0.01$; MAP: Los i.p. 99 ± 2 vs Sal i.p. 111 ± 2 mmHg $P<0.01$). The effects produced by Los in Stroke IC rats were prevented by concomitant treatment with Ang-(1-7) antagonist, A-779 (200 µg/kg SC). In agreement with this observation losartan treatment also increased the cardiac expression of Ang-(1-7)/Mas receptor (0.93 a.u. Sal i.p. vs 1.6 a.u. Los i.p. $P=0.03$). **Conclusions:** The present data suggests that AT₁ receptors blockade may reduce the impact of cardiac sympathetic activity exacerbation observed after insular stroke as well as promote a protective reduction in baseline blood pressure values. At least part of these effects may involve activation of Ang-(1-7)/Mas receptors. The present study indicates that immediate treatment with losartan may help to minimize cardiovascular risk in the acute phase after insular stroke. Support: FAPEMIG APQ-01128-21; CNPq.

Marins FR, Limborço-Filho M, Iddings JA, Xavier CH, Biancardi VC, Stern JE, Ramiro Diaz J, Oppenheimer SM, Filosa JA, Peliky Fontes MA. Tachycardia evoked from insular stroke in rats is dependent on glutamatergic neurotransmission in the dorsomedial hypothalamus. *European Journal of Neurology*. 2021 Nov;28(11):3640-9. Fontes MA, Lima AM, dos Santos RA. Brain angiotensin-(1-7)/Mas axis: a new target to reduce the cardiovascular risk to emotional stress. *Neuropeptides*. 2016 Apr 1;56:9-17. Marins FR, Limborço-Filho M, D'Abreu BF, de Almeida PW,

Gavioli M, Xavier CH, Oppenheimer SM, Guatimosim S, Fontes MA. Autonomic and cardiovascular consequences resulting from experimental hemorrhagic stroke in the left or right intermediate insular cortex in rats. *Autonomic Neuroscience*. 2020 Sep 1;227:102695.

PCB021

Baroreflex dependent cardiovascular autonomic reactivity is deranged in COVID-19 survivors

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Abstract Body:

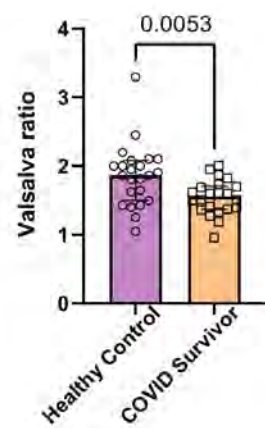
Introduction: COVID-19 has been reported to produce multi-system disorder during the acute as well as the post-acute 'long COVID' states. Emerging literature suggests long-term effects of COVID-19 on the autonomic nervous system manifesting as orthostatic intolerance or hypotension in COVID-19 survivors the mechanistic basis of which are currently not delineated.

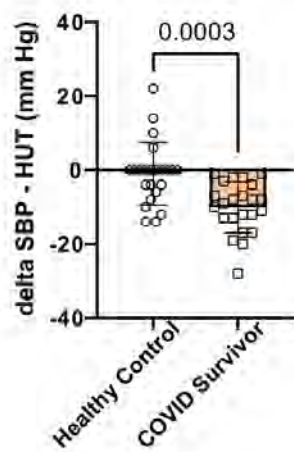
Aims & Objectives: The study aimed at assessing the cardiovascular autonomic functions in COVID-19 survivors and compare it with age and sex-matched data from a historic (pre-pandemic) healthy control group.

Materials and methods: Ewing battery of cardiovascular autonomic reactivity tests along with cold pressor test (CPT) was conducted at least one month after clinical recovery from acute COVID-19 on 23 survivors free of any comorbidities (Age: 28 ± 4 years; 9 females). The responses were compared with that of 23 age and sex-matched pre-covid era healthy historic controls. Ewing battery of tests included head-up tilt test (HUT), deep breathing test (DBT), Valsalva maneuver and hand grip test (HGT).

Results: Resting systolic and diastolic BP and heart rates were comparable between the COVID-19 survivor group and the control group. COVID-19 survivor group had significantly lower Valsalva ratio (1.55 ± 0.25 vs 1.86 ± 0.46 ; $p = 0.005$) and displayed greater fall in systolic blood pressure during head up tilt test (-10.1 ± 6.7 vs -1 ± 8.5 mmHg; $p = 0.0003$) in comparison to the control group. Three out of the 23 COVID-19 survivors were diagnosed with orthostatic hypotension. Autonomic reflex responses to DBT, HGT and CPT were found to be comparable between the two groups.

Conclusion: COVID-19 survivors show abnormal responses in baroreflex dependent cardiovascular autonomic reactivity tests. This possibly indicates COVID-19 associated baroreceptor reflex dysfunction as a medium to long term autonomic sequelae and might explain the clinical presentation of orthostatic intolerance and hypotension in COVID-19 survivors.





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PCB022

Chronic hypoxia increases noradrenaline transporter activity in the rat left atrium

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Atrial fibrillation (AF) is the most common cardiac arrhythmia with a prevalence of 2-4% in adults (Hindricks et al., 2021). Emerging evidence indicates that autonomic dysfunction is a key driver of atrial arrhythmogenesis. Conditions associated with chronic hypoxia (CH), such as chronic obstructive pulmonary disease and heart failure, are independent risk factors for AF (Grymonprez et al., 2018). In addition to sympathetic activation secondary to peripheral hypoxia sensing, it is possible that CH causes remodelling of left atrial sympathetic neurones, pre-disposing to arrhythmia. At sympathetic terminals, efficacy of noradrenergic outflow is governed by multiple regulatory processes, including the noradrenaline transporter (NAT). As yet, little is known about the regional differences in NAT function in the left atrium and whether this is modified by CH. Here, we have studied NAT kinetics in the left atrial appendage (LAA), left atrial posterior wall (LAPW) and pulmonary veins (PVs) from animals exposed to normoxia (N) and CH.

A recently developed method for dynamic analysis of single-terminal NAT rate (Cao et al. 2020) was employed in atrial tissue obtained from adult male Wistar rats (200-300g). All experiments and procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986. Hearts were excised under non-recovery, terminal inhalation isoflurane (3-5% in O₂, flow rate 1.5L min⁻¹, death by cervical dislocation) and immediately transferred to a superfusion chamber. Atria were dissected free and separated into regions of interest. Tissues were subsequently loaded with a low-concentration solution (1:100 dilution) of Noradrenaline Transporter Assay (NTUA, Molecular Devices), a fluorescent substrate for the NAT, and imaged with confocal microscopy to identify sympathetic terminals. Tissues were then superfused with a 1:20 solution of NTUA, and image stacks were recorded over 15 minutes to monitor uptake rates. Images were analysed using FIJI (v1.53t) to allow quantification of single-terminal NAT activity. Experiments were performed on N (n=6-11; F_iO₂=0.21) and CH (n=3-5; F_iO₂=0.12, 9-10 days) animals. Values are expressed as mean ± SEM. Significance was taken as p<0.05, two-way ANOVA with Tukey post-hoc analysis.

Analysis of fluorescence traces revealed a qualitatively similar uptake profile in all 3 regions regardless of CH exposure, demonstrating a linear 6-minute rising slope followed by a plateau phase. CH caused a marked increase in the magnitude of the rising slope in the LAA (N 6.52 ± 0.50% minute⁻¹ vs CH 10.39 ± 0.66% minute⁻¹, p=0.001) and LAPW (N 5.93 ± 0.47% minute⁻¹ vs CH 8.81 ± 0.90% minute⁻¹, p=0.02), alongside 15-minute plateau fluorescence peaks (LAA N 68.0 ± 4.7% vs CH 102.3 ± 6.7%, p=0.0004; LAPW N 51.3 ± 4.7% vs CH 87.9 ± 6.0%, p=0.003). NAT kinetics were unaffected by CH in the PV (p>0.05).

Our data demonstrate that exposure to CH augments NAT activity in the LAA and LAPW but not in the PV. This could be an adaptive response to a chronic rise in atrial sympathetic nerve firing

frequency. Further investigation is required to reveal the mechanism underpinning this upregulation and to what extent this may be of relevance to CH-related atrial arrhythmogenesis.

Grymonprez M et al. (2018). *Int J Cardiol* 276, 118-124. Hindricks G et al. (2021). *Eur Heart J* 42, 373-498.

PCB023

VEGF receptor roles in human cardiac progenitor cell contributions to new vascular formation

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Introduction. Human cardiac progenitor cells (CPCs) have been identified as an endogenous myocardial cell population, capable of aiding myocardial tissue maintenance, including the potential to differentiate into endothelial and vascular smooth muscle cells. Vascular endothelial growth factor (VEGF) ligands have been identified playing critical roles in angiogenesis. VEGF receptors have three major subtypes (VEGFRs 1, 2 and 3); previous data identified CPC VEGFR expression and pro-angiogenic growth factor secretion.

Aims. This study examined whether human CPCs utilise VEGFR signalling to potentiate angiogenesis, both directly by CPC differentiation and indirectly through pro-angiogenic paracrine signalling.

Methods. Human adult myocardial tissue was collected during cardiac surgery and c-Kit-positive (c-Kit⁺), CD45-negative (CD45⁻) CPCs were isolated by immunomagnetic bead sorting; five unique CPC lines were generated from individual donor samples. c-Kit⁺/CD45⁻ CPCs were then characterised *in vitro* by clonogenicity assays, immunocytochemistry and RT-qPCR. Human CPC lines were sorted by FACS into three lineages: endothelial (CD31⁺), smooth muscle (CD91⁺/CD140b⁺/CD31⁻) or uncommitted (CD91⁻/CD140b⁻/CD31⁻) groups. VEGFR and marker (SDF1; TGF- β) expression in CPC sub-populations were quantified (qPCR; Western blot; immunocytochemistry). VEGF-A stimulation and effects on signal transduction were examined (Western blot; immunocytochemistry). Statistics: ANOVA plus Tukey's test, significance: $p < 0.05$; data are mean $\pm$ SEM. This work was approved by the Faculty Research Ethics Committee at the University of Leeds and by the Wales Research Ethics Committee for NHS clinical tissue samples (NREC 17/WA/0314).

Results. Human CPC lines were isolated (n=5) and the stem cell phenotype confirmed by analyses of differentiation (immunocytochemistry) and self-renewal (clonogenicity: 50-90% of single-cell clones generated clonal colonies; RT-qPCR: 'stemness' genes confirmed in all 5 lines, n=3 technical replicates). CPCs from a representative line were then FACS-sorted into populations, separated by markers of: endothelial lineage CD31⁺ (1.99% of total cells), smooth muscle lineage CD91⁺/CD140b⁺/CD31⁻ (13.77%) and CD91⁻/CD140b⁻/CD31⁻ (31.28%) cells. Gene expression analyses identified mRNA for VEGFRs 1, 2 and 3 in all sub-populations (n=3). However only VEGFR1 protein expression was confirmed in all three sub-populations, not VEGFR2 or VEGFR3 (n=3). For growth factors previously identified as being secreted by CPCs (SDF, TGF- β , VEGFs, FGF-2), we identified high gene expression levels in human CPCs, with expression seen in all sub-populations (n=3). Application of CPC secretome, from each sub-population, to human endothelial cells on Matrigel *in vitro* did not show a clear increase in tube junction formation or tube segment length (n=6).

Conclusions. We isolated and analysed human CPCs, in bulk lines and sub-populations, identifying VEGFR1 gene and protein expression, but not VEGFR2 nor VEGFR3. We are building on this work to identify signalling pathways in human CPCs linked to VEGF-A stimulation, and further assessing impacts of VEGF-A stimulation on CPC secretome and associated potential to drive angiogenesis.

PCB024

Investigation of human tissue-specific proteome enrichment.

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Tissues throughout the human body exert finely-tuned specific functions even when there is considerable overlap in the cellular constituents. Even when the major cellular component of tissues is the same – for example in the case of smooth muscle-rich tissues - discrete phenotypes are evident. Yet, the molecular expression profiles underpinning such specialised functionality is often unclear. This presents a challenge for introducing new medicinal therapies for pathophysiologies: can one target what makes tissue functionality unique without producing unwanted effects on the features that are similar.

Such a scenario can be considered for the human uteroplacental unit. Ideally, one would like to target complications of pregnancy like pre-eclampsia (hypertension and proteinuria) or spontaneous preterm birth (early activation of parturition) by targeting particular types of smooth muscle tissues (e.g. arteries versus myometrium) without adversely affecting others. This situation is further complicated by the need to consider maternal and fetoplacental circumstances. Three smooth muscle-rich tissues of importance here are myometrium, maternal uterine (myometrial) arteries and placental arteries. Indeed, each display subtly distinct phenotypes suggesting that distinct molecular signatures may underlie these differences. As proteins determine cell/tissue structure and function, the aim of this work was to compare the proteome-wide expression profiles of these three tissues.

Paired uterine biopsies and placentas were obtained, following written informed consent (LREC 10/H0906/71), from healthy pregnant women undergoing elective Caesarean section at term (39-40 weeks gestation, n=9). Myometrial strips(M), myometrial arteries(MA) and placental chorionic plate arteries(PA) were each isolated, and cleaned of surrounding material, by careful microdissection, snap frozen in liquid N₂ and stored at -80°C until further use. Frozen samples were homogenised (5% SDS in 50mM TEAB, pH 8.5), digested (1:20(w:w) trypsin:protein ratio, 47°C for 2hours) and peptides analysed in triplicate via liquid chromatography mass spectrometry(LC-MS) using SWATH^[1].

5895 proteins were quantified (using ≥ 5 unique fragment ion intensities per peptide, $\log(2)$ transformed and median-corrected) and 2832 differentially expressed across the three tissue types (ANOVA with multiple corrections FDR<0.01). Hierarchical k-mean clustering indicated five main groupings of relative protein changes across the tissue types. Paired t-test (FDR<0.05) identified differentially expressed proteins between tissues as follows: M vs MA: 1277 proteins; M vs PA: 3376 proteins; MA vs PA: 2486 proteins. Multiple pathway enrichment analyses indicated that the three tissues possess distinct proteomic profiles with PA being the most distinguishable from the other two. Notable biological pathway differences across these tissues are proteins related to ribosomal structure, oxidative phosphorylation, cytoskeleton and extracellular matrix(ECM). One cluster was indicated by notably higher relative protein abundances from placental arteries than each of the other two tissue types. For example,

identified in this cluster ECM laminin isoforms LAMB2 and LAMC1 had higher abundances in PA (log(2) fold-change: 4.94 ± 0.57 ; 6.10 ± 0.48 respectively), followed by MA (3.81 ± 1.04 ; 5.33 ± 0.36) and M (2.13 ± 0.74 ; 4.81 ± 0.44).

In summary, distinctive proteomic profiles are evident between human placental arteries, myometrial arteries and myometrium. This opens the possibility of uncovering the molecular signatures, and biological pathways, that serve to furnish different smooth muscle-rich tissues with specialised physiological (and pathophysiological) phenotypes.

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PCB025

BIOMARKERS OF HEART FAILURE WITH A PRESERVED EJECTION FRACTION IN BLACK SOUTH AFRICAN PATIENTS

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BACKGROUND AND OBJECTIVES: Almost 50% of all heart failure (HF) cases have a preserved ejection fraction (EF), which is often observed in the elderly (≥ 75 years). However, the mean age of HF presentation is considerably lower (40-55 years) in sub-Saharan Africa. Heart failure with a preserved ejection fraction (HFpEF) is therefore unlikely to be due to arterial stiffness. The risk factors associated with HFpEF might also be different in a middle-age population group when compared to the elderly. The aim of this study is to investigate two biochemical markers, N-terminal pro b-type natriuretic peptide (NT-proBNP) and galectin-3, that predict HFpEF and specifically markers that predict or are associated with HFpEF in a black population in South Africa.

METHODS: The nature of the study was a case-control investigation. Sixty-six participants with HFpEF and 213 participants without HF from African descent and older than 18 years of age were enrolled. All participants gave informed consent and completed a standardised questionnaire. Echocardiographic, anthropometric, central haemodynamic measurements, pulse wave velocity (PWV) and biomarker analysis using commercially available enzyme-linked immunosorbent assay (ELISA) kits were done.

RESULTS: The mean age of HFpEF in black South African patients was 54.88 ± 13.51 years. PWV was significantly increased in participants with HFpEF (9.97 ± 2.78 m/s) when compared to participants without this pathology (6.11 ± 2.18 m/s) with a p-value of $p < 0.0001$, however there were no significant associations between central haemodynamic parameters, NT-proBNP ($p = 0.9746$) and galectin-3 ($p = 0.2166$). Lastly, NT-proBNP, but not galectin-3, was significantly associated with left ventricular hypertrophy (LVH) ($p = 0.0002$) and left atrial (LA) diameter ($p = 0.0005$).

CONCLUSION: HFpEF is more prevalent in a middle-aged black South African sample with increased arterial stiffness when compared to European and American populations. NT-proBNP, but not galectin-3, is independently associated with LVH and LA diameter and hence could be used for the diagnosis of HFpEF in this community sample.

PCB026

Technical skills for basic scientists- Hands on skills in basic science courses is appreciated and promotes employability/life skills which otherwise are difficult to demonstrate to employers

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Students studying physiology undergraduate programmes often report aspirations of careers in clinical practice and clinical investigation (Steele *et al.*, 2020), which are heavily skills-based and theoretically grounded. Previous evidence suggests that simulation-based learning improves perception of life skills, motivation and self-efficacy in nursing students (Roh & Kim, 2015). However, evidence is limited in student perception of confidence and understanding gained from simulation-based learning in the study of basic sciences. This study aims to assess student perception of clinical based skills training and simulations in students studying degrees in the field of Physiology.

This study was approved by the Science and Engineering Research Ethics Committee, Manchester Metropolitan University. Anonymous surveys were conducted with students enrolled on the unit “Cardiovascular Science”, a level 6, 15 credit undergraduate unit as part of the degree programmes BSc (Hons) Human Physiology or BSc (Hons) Sports Science and Human Physiology during 2020-21. Students were surveyed following clinical sessions, one after a skills session to practice Basic Life Support (BLS), another using high-fidelity patient simulators to diagnose signs of myocardial infarction (Sim). Surveys were conducted in Microsoft Forms. Each session survey assessed enjoyment, understanding/confidence, transferrable skills and problem solving, all assessed using a scale from 1 to 5 (1=negative, 5=positive). Students also surveyed on completion of all teaching (n=7), assessing perception of understanding, confidence, overall study experience and whether skills practiced add to students’ overall physiology skills, assessed on a scale of 1 to 3 (1=negative, 3=positive). Students were optionally asked for free text comment on all surveys. Free text was analysed qualitatively for themes. Quantitative data is presented as Mean±SD of responses.

Combined, (n=21 responses, BLS; n=12, Sim; n=9) both clinical skills sessions were enjoyed (Combined rating: 4.8/5±0.4) with students also reporting confidence and understanding in the theoretical basis of the skills practiced (Combined rating: 4.6/5±0.6). Students also felt these experiences provided transferable clinical skills relevant to employability (Combined rating: 4.7/5±0.6) and developed ability to solve problems (Combined rating: 4.4/5±0.7). Optional free text comments received were also universally positive and provide indication that students feel the sessions developed employability skills relevant to future career development (Table 1).

In the post unit survey (n=7, all self-reported attending at least one clinical skill session), all students surveyed reported that the addition of clinical skills sessions aided understanding of theory (3.0/3±0.0). Students reported confidence in applying theory to practical scenarios (2.7/3±0.5) and skills sessions positively added to overall study experience (2.9/3±0.4). Overall, students felt the skills practiced also positively added to overall physiological skills (2.7/3±0.5). Optional free text comments indicates students find the sessions useful as “...really informative

putting theory into practice”, “Practical application of theory”, “Interactive learning” and “Add more practical’s”.

These findings suggest students feel perceived confidence and improved understanding in the theoretical concepts when “traditional” lecture, lab and tutorial activities are supplemented by specific clinical skills not normally associated with the teaching and learning of theoretical concepts in physiological sciences.

Table 1: All free text comments received across both BLS and Sim sessions

Insightful
Really gave a fundamental understanding to CPR
Good for CV
Helpful, as this skill will probably be needed in the future
Really enjoyed session, skills useful for the unit as well as day to day life
Really useful, especially for those who want to pursue a career in clinical settings.
This clinical session was really interesting and I wish there was more clinical sessions like this
Great and useful life skills
Everything was understandable and thorough
Help me develop very important skills in first aid and showed understanding of carrying it out
Really enjoyed this session! Learnt a lot, would be really beneficial for future students
Would love to have this in other units
Excellent
Really help develop my skills and carrying practical work out helped explain it more

References: Roh YS & Kim SS. (2015). Integrating Problem-Based Learning and Simulation: Effects on Student Motivation and Life Skills. CIN: Computers, Informatics, Nursing 33, 278-284.
Steele KJ, VanRyn VS, Stanescu CI, Rogers J & Wehrwein EA. (2020). Start with the end in mind: using student career aspirations and employment data to inform curriculum design for physiology undergraduate degree programs. Advances in Physiology Education 44, 697-701.

PCB027

Challenges and opportunities in the phased introduction of R teaching for medical science students

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The growth of “big data” in medical sciences research in recent years has led to an increased demand for coding skills. This can clearly be observed in industry with employers actively seeking applicants with experience of open access languages such as R and Python. However, teaching coding presents many challenges such as time constraints within existing degree programmes and lack of experience amongst teaching staff. Here we share our experiences of the phased introduction of R teaching to undergraduate medical science students.

Our first approach was to develop an optional online resource called “Introduction to R”. The format was short lecture recordings including slides covering theory and demos in RStudio. To allow students to code in their own time, we provided an R notebook which could be used as a self-directed tutorial. This contained the code used in the videos, the expected outputs and explanations of the syntax. While we targeted this resource at years 3 and 4, all medical science undergraduates had access through a shared VLE. In June 2022, we introduced a live introduction to R seminar to an existing summer research seminar series targeted at both undergraduate and postgraduate students. Survey feedback was very positive with >85% rating the session as interesting and 71% reporting they learnt something new. Free text comments indicated there was a demand for in person workshops.

In response to this, we have recently developed a 2 h face to face workshop. While intended as a follow on session to the R seminar, the workshop is targeted at beginners and assumes no prior knowledge. This covers basic syntax, installing R and RStudio, simple calculations, importing data and running a t-test. Feedback for this will be collected via anonymous surveys including both 10-point Likert scales and free text questions.

PCB028

Teaching and learning about physiology of death and dying

Laura Ginesi, Derek Scott

undefined

Death, loss and grief are fundamental and profound aspects of the human experience that have long been a long and contested construct (Blakemore and Jennett, 2001). Nevertheless, death tends to be a topic of conversation that is avoided – even feared - because most people have never been with a dying person or know what to expect (Mannix, 2018).

On inspection, we and our students have found that the taboo related to death and dying extends to most physiology textbooks - the basic reference texts for student healthcare professionals (Brown, 2022). Searches using key words like “death”, “dying” and “mortality” yield few or no results in their indexes, while “apoptosis” and “necrosis” yield detailed information about cellular death. Similarly, searches of curriculum documentation using the same terms yielded expectations of student knowledge and understanding of “end of life care”, “palliative care” and death certification. The data show clearly that there is a void in explanation of physiological mechanisms that underpin the changes that the human body normally goes through at the end of life.

We are our bodies. Just as conception, pregnancy and birth mark the beginning of a person’s life, dying is a physiological process with recognisable stages of progression leading to the final stage when vital activities of living cease. The trajectory of each unique death depends on whether death is brought about by old age, malnutrition, dehydration, major trauma or terminal injury like suicide or drowning. However, textbook explanations of clinical signs such as Cheyne-Stokes breathing, Cushing’s triad or “death rattle” do little to explain what a dying human body is actually experiencing.

We designed a workshop based around selected video clips from James Bond movies and core physiological concepts (Michael and McFarland, 2020) to enable students to learn about and discuss fundamental mechanisms that normally precede a person’s death. Trigger warnings were used in advance and at the start to help to prepare students ahead of the sessions, reduce anxiety and promote feelings of safety within the groups.

The workshop was structured around activities designed to help students to recognise the physiological changes that are characteristic of loss of essential characteristics of cellular homeostasis which in turn leads to failure of body organs as the person approaches the end of

life. Breakout discussions enabled students to share their ideas about death as an integral part of the human life cycle as well as some of the social aspects related to own culture.

Feedback from the pilot sessions has been remarkably positive.

Although there is little published literature addressing the effectiveness of learning about death and dying, some studies about psychological training have shown promising results (Silverdale and Katz, 2005; Weaver, Balkan and Decker, 2022). We share our thoughts about why sound understanding of core physiological concepts relevant for death and dying has the potential to reduce anxiety and help student healthcare professionals to be better equipped to communicate appropriately with dying and bereaved people.

Blakemore, C.; Jennett, S. Eds. (2001) *The Oxford Companion to the Body*. Oxford University Press. Brown, K (2022) We need to talk about death. *Physiology News* Issue 128 (Winter)

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PCB029

Encouraging students' development towards employability using a digital micro-activity

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Providing student employability support and guidance forms an integral part of education. Much academic and University Careers and Employability Services (CES) effort focusses on students' skills and competency development for future vocations. Numerous strategies are employed to promote interaction with centralised University services and 'career development learning' (see Bridgstock et al., 2019). Students on our Medical Physiology and Therapeutics degree programme mainly focussed on employability as they approached final year studies. We aimed to encourage CES engagement, careers awareness and preparation for employability from year one using a simple activity.

Technology has offered new opportunities to enhance student engagement (JISC, 2022). We introduced a rapid, user-friendly 'micro-engagement' digital monitoring system for the students to track their interactions with careers support and their personal development towards employment. Simply based on a Microsoft excel spreadsheet template, the activity generated a visual 'map'. Piloted from entry level and giving clear instructions, students were asked to self-rate their CES interaction and exploration of different careers around subject topics and areas that initially had piqued their interest. They were asked to gauge their engagement on 6 aspects using a scale of 0 to 6 that reflected minimal to maximal interaction.

Completion of the activity created a personalised visual map for each student with the shape of each map being dependent on the self-ratings. The 'signatures' reflect developmental needs and are anticipated to expand each year and provide a continuing, progressive record of active participation and CES proactivity in preparation for employment. The expectation is that exploration of career interests and gaining CES awareness in first year generates smaller area 'maps' compared to subsequent years which demands self- evaluation, preparation for career applications, and a more practice approach towards career applications.

The activity has been progressively introduced into the curriculum via three 'backbone' core skills modules that focus on study and academic skill development at each stage of our three-year Hons programme. Students are requested to include their maps in summative skills portfolios to incentivise completion, to provide a subtle reminder about the need for personal and professional development, and to also signpost the University CES support. Each year group has been asked to complete the activity using similar questions for familiarity, ease of use, and to enable simple comparison between their maps as they progress through the course.

To date, > 80% of year one students have completed the activity with informal positive commentary suggesting this is a useful way for them to reflect about their future employability, boost their awareness of professional services and consider their career development needs. Introducing this activity may also be a helpful aid for personal tutors to discuss and support their student's individual progress towards career aspirations. It also bolsters students' preferences for academic input regarding careers and employability support (AGCAS, 2022).

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PCB030

The Use of Digital Storytelling to Compensate for Flexible Physiology Learning

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This study aimed at using digital case scenarios created by students to improve

the process of learning Physiology. Additionally, it allowed students to visualize and understand clinical scenarios and the physiological reasons behind them while assessing how much they stand to gain from the experience. This study is a project

to implement FAIMER, ASU MENA-FRI Institute, Cairo, Egypt. In a foundation course for first-year medical students, the instructor utilized a variety of instructional methods including lecture, small group discussion, individual assignments, and reflection. The instructor had experience with prior use of a digital storytelling project, (Medical Workshop in IUPS, August 2017, Rio, Brazil). This study obtained

IRB approval from the Faculty of Medicine, Ain Shams Medical Ethics committee. The results reported by the students themselves revealed that the project helped them improve their skills in problem-solving, teamwork, active learning, communication,

planning, and time management. In addition, it also increased their confidence in their abilities to learn, face unexpected challenges, and achieve goals, while considering new life opportunities, those which became an option when the students searched by themselves and learned more about the different angles of medicine. This study concluded that compared to the traditional lecture format that focuses on memorizing definitions and theoretical structures, digital storytelling can be regarded as an innovative teaching tool and a unique medical education method that allowed students to participate more in the learning process. This article proposes an active learning method in undergraduate medical education.

Knowledge gained from group project

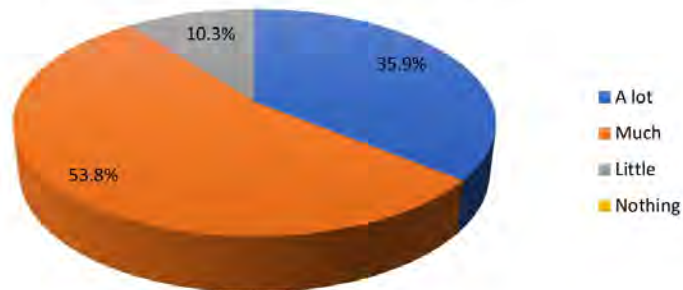


Figure 1.1: Students' responses analysis about knowledge gained from the project

Skills and knowledge gained from working in teams

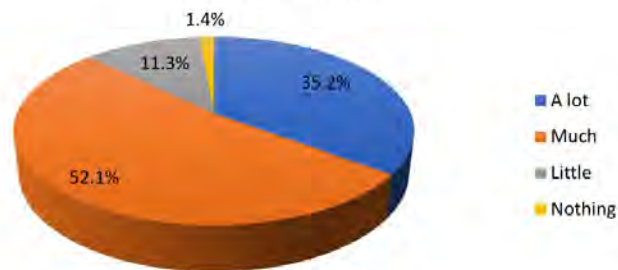


Figure 1.2: Students' responses analysis about knowledge and skills gained from the project

Group effect on achieving learning outcomes

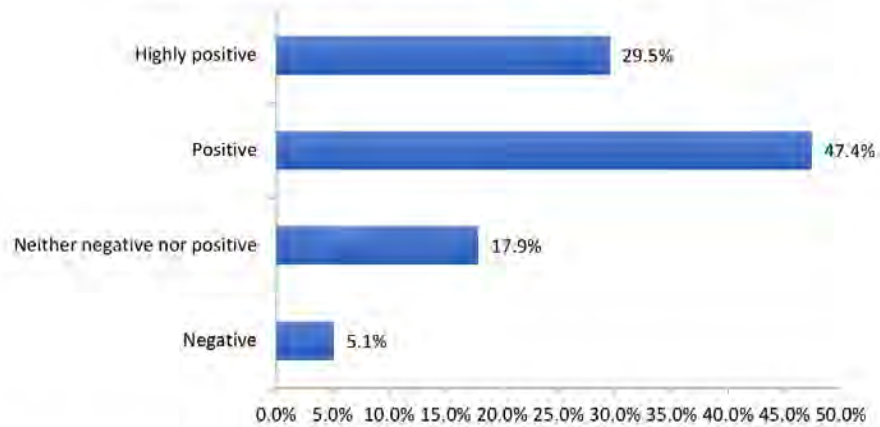


Figure 2: Analysis of students' responses about group effect on achieving learning outcomes

PCB031

ANTI-OXIDANT AND ANTI-INFLAMMATORY EFFECTS OF MELATONIN ON OBESE WISTAR RATS

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Obesity, a metabolic disorder is reaching an epidemic proportion and 20% increase in a normal body weight has been shown to be related to 20% increase in mortality rate. However, unsuccessful battle against this condition has led to the quest of finding a novel therapeutic agent (Timper and Brüning, 2017). Orlistat, a gastrointestinal lipase inhibitor has been recommended for the treatment of obesity. However, the gastrointestinal side effects have discouraged most patient from its continuous usage. Hence, there is a need for a more potent drug with less side effect (Jain et al., 2011). Melatonin, which are widely used alternative medicine have been reported to be effective in lowering the body weight with very little side effects. Therefore, the present study investigated the effects of melatonin in obesity model of male Wistar rats weighing between 110 – 130 g. It was hypothesized that melatonin is not an anti-obesitogenic therapy on obese rat model. Fifty (50) rats of ten (10) animals per group were divided into the following: control (untreated); high fat diet (HFD); high fat diet recovery (hfd); hfd + melatonin (4 mg/kg); and hfd + orlistat (30 mg/kg). Obesity was induced by exposing the rats to high fat diet for 16 weeks and confirmation was done using Lee index, which was determined by the formula: $4\sqrt{\text{body weight (g)} / \text{nose-anal length (cm)}}$. Rats with an index higher than 0.30 were considered obese and were used for the study (Adeyemi et al., 2020). Treatment started and lasted for 28 days after which the rats were anesthetized by intramuscular injection of 50 mg/kg of ketamine. Melatonin and orlistat were administered at 4 and 30 mg/kg b.w., p.o. respectively. Animals were cared for and used according to the University of Ilorin ethics guidelines. Diagnostic kits for the determination of the biomarkers were obtained from Abcam PLC, Cambridge, UK and the assays were performed according to the manufacturer's instruction. Data were analyzed using analysis of variance and LSD *post hoc* test at 0.05 level of significance. The results showed that the induced obesity was accompanied with significant increases in plasma glucose, but significant decreases in plasma insulin. Relative to the obese control, treatments with melatonin caused significant elevations in total antioxidant capacity (TAC), catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx), however, a significant decrease in interleukin 6 (IL-6) and tumor necrotic factor (TNF- α). Hence, it was concluded that melatonin could be beneficial in the management of obesity.

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PCB032

Effect of pulp extract of *Azanza garckeana* fruits on hematological and hormonal indices

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Introduction

Azanza garckeana is a tropical wild fruit plant that is found in Africa. It produces edible fruits that are used as food or herbal medicine.

Aim

In some African countries such as Nigeria, *Azanza garckeana* fruit products are used traditionally for the treatment of infertility, sexually transmitted diseases and for libido enhancement. The aim of this study was to investigate the effect of the extract of *Azanza garckeana* fruit pulp on haematological indices and reproductive hormones in male and female Wistar albino rats.

Method

This study was approved by the ethical committee of Faculty of Life Sciences, University of Benin, Nigeria. Forty-eight (48) Wistar albino rats comprising 24 males and 24 females were grouped into two control groups and six treatment groups. The treatment groups were administered 50, 300 and 2000 mg/kg body weight of aqueous-methanol pulp extract of *Azanza garckeana* fruits.

Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Lymphocytes (LYM) and Mean Corpuscular Volume (MCV) of these animals were assessed. Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin, Estradiol, Testosterone and Progesterone in the rats were determined.

Results

HGB & HCT were seen to significantly increase ($p < 0.05$) in 300 mg/kg fruit pulp treated female rats. Prolactin was seen to be significantly increased ($p < 0.05$) in 2000 mg/kg fruit pulp treated female, while progesterone was seen to increase ($p < 0.05$) in 300mg/kg and 2000 mg/kg fruit pulp treated male rats.

Conclusion

Results from this preclinical study, suggest that *Azanza garckeana* fruit extract may impact haematological and hormonal indices in Wistar rats, clinical studies would be done to confirm these trends in humans.

PCB033

Thyroid function is related to body mass index in post-menopausal women.

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Thyroid diseases increase with age, predominantly affecting women, thus they occur most often in post-menopausal and elderly women.¹ There is no consensus on universal screening for thyroid dysfunction in post-menopausal and elderly women worldwide, ² therefore this study was on thyroid function tests: serum TSH, thyroxine, (T₄), and tri-iodothyronine (T₃) in relation to body mass index (BMI) in post-menopausal women. It was a prospective cross-sectional study of post- menopausal women attending clinics at a Catholic hospital in Benin City, Nigeria. 40 pre-menopausal (control) and 40 post-menopausal healthy women (mean age 31.73±11.45 and 57.06±5.57 years respectively) were assessed. Informed consent was obtained, a pre- tested structured questionnaire containing their bio-data, gynaecology history and past medical history were obtained, and ethical approval was also obtained. At the end, blood samples were obtained following standard laboratory procedures.³ Results were expressed as mean±SD, analysed using SPSS version 20, and subjected to 't-test' and ANOVA. P< 0.05 was considered statistically significant. Post-menopausal women presented significantly higher (p<0.05) BMI than the control (27.60±4.32 kg/m² vs. 25.42±4.16 kg/m²). Serum TSH levels were significantly higher in post-menopausal than in pre-menopausal women (2.04±0.56 mIU/L vs 0.74±0.11 mIU/L.) Despite the higher TSH, T₃ and T₄ were significantly lower in post-menopausal women (1.02±0.16 ng/ml and 6.07ng/ml) than in pre-menopausal women (4.91±0.43 ng/ml and 10.13±0.25 ng/ml). These findings suggest that there might be reduced T₃ and T₄ receptors in post-menopausal women. The observed increase in BMI in post-menopausal women might be as a result of a fall in BMR, due to the decreased levels of T₃ and T₄.⁴

Table 1. Anthropometric profile of the sampled women

Groups	n	Age (years)	Height (m)	Weight (kg)	BMI (kg/m ²)
Pre-menopausal	40	31.73±11.45	1.51±0.37	60.49±17.17	25.42±4.16
Post-menopausal	40	57.06±5.57*	1.56±0.11	67.30±15.68*	27.60±4.32*

values are mean±standard deviation; * indicates significant difference at p<0.05 compared to the pre-menopausal women (control).

Table 2. Comparative evaluation of serum thyroid stimulating hormone (TSH), T₃ and T₄ levels between pre-menopausal and post-menopausal women

Groups	n	TSH (mIU/L)	T ₃ (ng/ml)	T ₄ (ng/ml)
Pre-menopausal	40	0.74±0.11	4.91±0.43	10.13±0.25
Post-menopausal	40	2.04±0.56*	1.02±0.16*	6.07±0.84*
% change		+175.68	-79.23	-40.08

values are mean±standard deviation; * indicates significant difference at p<0.05 compared to pre-menopausal women (control).

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PCB034

Hypoglycemic and antioxidant potential of *Phyllanthus amarus* leaf extract in Type 2 diabetic *Drosophila melanogaster* flies.

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INTRODUCTION. High sucrose diet has been reported to produce type 2 diabetic phenotypes in *Drosophila melanogaster*. Type 2 diabetes mellitus is a chronic metabolic disorder that is characterized by elevated blood glucose levels (hyperglycemia) caused by loss of insulin action (insulin resistance) (American Diabetes Association, 2014). Current management of this condition has been by the use of oral hypoglycemic drugs. However these drugs have their own side effects such as liver disorders, kidney toxicity, flatulence, abdominal pain, diarrhea (Lee et al. 2014) which has led to the search for natural compounds with hypoglycemic potential. This study was carried out to investigate the hypoglycemic and antioxidant potential of *phyllanthus amarus* leaf extract in high sucrose diet induced-type 2 diabetes in *Drosophila melanogaster*.

METHODS. Flies were divided into four (4) groups containing 50 flies per group. Group I served as control and they were reared on normal corn meal diet while group II were fed with 30% high sucrose via their diet. Group III flies were fed with 2.5mg of *phyllanthus amarus* (*P. amarus*) leaf extract. Group IV flies were co-treated with 30% high sucrose and 2.5mg of *P. amarus* via their diet for seven (7) days. Each experiment was carried out in five (5) replicates (n=5). At the end of the experimental period, the flies were homogenized and the supernatants were used to estimate glucose concentration and also assay for catalase (CAT), glutathione (GSH) and hydrogen peroxide (H₂O₂). A 15 days survival study was also carried out to investigate the effect of high sucrose diet on the survival rate of flies and the possible protective effect of *P. amarus*.

RESULTS. There was a significant increase in glucose concentration, hydrogen peroxide (H₂O₂) and a significant reduction in catalase, glutathione (GSH) and the survival rate of flies fed with high sucrose diet. However, in flies co-treated with *P. amarus* and high sucrose diet, there was a significant reduction in glucose concentration, hydrogen peroxide (H₂O₂) and a significant improvement in catalase, GSH and the survival rate of flies.

CONCLUSION. This study has shown that *P. amarus* possesses hypoglycemic and antioxidant potential and could be of therapeutic benefits in the management of type 2 diabetes mellitus.

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PCB035

Effect of ethyl acetate extract of *Amaranthus hybridus* on blood glucose, insulin, oxidative stress, and lipid profile in streptozotocin (STZ)-induced diabetic rats.

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Introduction

Diabetes Mellitus (DM) has become a global endemic with severe consequences such as cardiovascular diseases and end-organ damage. Considering the reported use of ethyl acetate extract of *Amaranthus hybridus* (EAH) as a treatment option in managing DM. It is therefore important to evaluate the outcomes of this plant on some consequences.

Aims /Objectives

This study is designed to evaluate the effect of ethyl acetate extract of *Amaranthus hybridus* (EAH) on blood glucose, insulin, lipid profile and oxidative stress in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Thirty male rats weighing 200g to 250g were randomly allotted into five groups (n=6). Group A (Control), B (DM), C; DM +300mg/kg body weight (BW) of Metformin (DMMET), D; DM+300mg/kgBW of EAH (DMEAH), E; 300 mg/kg body weight of EAH (EAH). Diabetes Mellitus was induced with 60mg/KgBW of STZ intraperitoneally. Dose of EAH and MET were administered daily via oral gavage for 14 days. Blood glucose was checked using a glucometer before and on the 7th and 14th day of treatment. On the 14th day, the rats were kept in diethylether fume chamber, blood samples were taken from the heart after cervical dislocation. Serum centrifuged from blood was used to estimate the superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), total cholesterol; low and high-density lipoprotein, triglyceride and insulin. Data were expressed as Mean $\pm$ SEM and compared using one-way ANOVA. Data was considered statistically significant when $P < 0.05$.

Results

Blood glucose level on day 7 was lower ($P < 0.05$) in Control (100.83 ± 3.66 mg/dl), DMMET (272.33 ± 45.70 mg/dl) and EAH (152.50 ± 48.27 mg/dl) when compared with DM (435.83 ± 34.46 mg/dl). On day 14, it was lower ($p < 0.05$) in Control (104.50 ± 3.63 mg/dl), DMMET (190.5 ± 43.3266 mg/dl), DMEAH (152.5 ± 48.66 mg/dl) and EAH (75.33 ± 2.3366 mg/dl) when compared with DM (460.67 ± 30.556 mg/dl). Insulin was higher ($p < 0.05$) in Control (5.1 ± 0.53 μ U/ml), DMEAH (1.55 ± 0.09 μ U/ml), and EAH (3.23 ± 0.26 μ U/ml) when compared with DM (0.81 ± 0.03 μ U/ml). SOD was higher ($p < 0.05$) in Control (2.94 ± 0.04 μ mol/ml), DMMET (2.67 ± 0.07 μ mol/ml), DMEAH (2.76 ± 0.06 μ mol/ml) and EAH (3.28 ± 0.07 μ mol/ml) when compared with DM (1.9 ± 0.04 μ mol/ml). CAT was also higher ($p < 0.05$) in Control (6.97 ± 0.13 μ mol/ml), DMMET (6.58 ± 0.08 μ mol/ml), DMEAT (6.77 ± 0.06 μ mol/ml) and EAH (7.25 ± 0.11 μ mol/ml) when compared with DM (5.65 ± 0.14 μ mol/ml). MDA was significantly higher in DM

(2.69 ± 0.04 $\mu\text{mol/ml}$) when compared with control and EAH (1.34 ± 0.27 ; 1.04 ± 0.30 $\mu\text{mol/ml}$). Total cholesterol was higher ($p < 0.05$) in DM ($2.08 \pm 0.16\text{mg/dl}$) than in control ($1.44 \pm 0.07\text{mg/dl}$) and EAH ($1.41 \pm 0.09\text{mg/dl}$). The level of LDL was significantly lower in control ($0.06 \pm 0.02\text{mg/dl}$), DMMET ($0.33 \pm 0.03\text{mg/dl}$), DMEAH ($0.33 \pm 0.02\text{mg/dl}$) and EAH ($0.09 \pm 0.02\text{mg/dl}$) when compared with DM ($0.52 \pm 0.05\text{mg/dl}$). Triglyceride was significantly lower in control ($0.72 \pm 0.11\text{mg/dl}$), DMMET ($1.1 \pm 0.05\text{mg/dl}$), DMEAH ($1.04 \pm 0.03\text{mg/dl}$) and EAH ($0.82 \pm 0.08\text{mg/dl}$) when compared with DM ($3.42 \pm 1.44\text{mg/dl}$). HDL was higher ($p < 0.05$) in groups control ($1.19 \pm 0.04\text{mg/dl}$), DMMET ($0.99 \pm 0.26\text{mg/dl}$), DMEAH ($0.76 \pm 0.01\text{mg/dl}$) and EAH ($1.13 \pm 0.04\text{mg/dl}$) when compared with group DM ($0.49 \pm 0.03\text{mg/dl}$).

Conclusion

The result demonstrated that ethyl acetate extract of *Amaranthus hybridus* improved blood glucose, Lipid profile and antioxidant enzymes in STZ-induced diabetic rats.

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PCB036

The role of electrogenic and electroneutral monocarboxylate transport in airway clearance.

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Introduction: The properties of airway surface liquid (ASL) are controlled by the coordinated activity of ion channels and transporters that mediate transepithelial absorption and secretion of ions. ASL homeostasis is dramatically altered in cystic fibrosis (CF) due to CFTR dysfunction that produces ASL dehydration and acidification, resulting in mucus-stasis, reduced airway clearance and favoring bacterial infections and inflammation. Monocarboxylates can be transported by an electrogenic Na⁺-coupled system that corresponds to SMCTs (SLC5 family) and an electroneutral H⁺-coupled system that correspond to MCTs, (SLC16 family). Previous work demonstrated the presence of MCT2 on the apical surface and MCT4 on the basolateral surface in human bronchial epithelium. But there is no evidence of SMCTs activity in this tissue. In here, we describe for first time that SMCT1/SCL5A8 is functionally expressed in mouse tracheal epithelium. We hypothesized that the activation of monocarboxylate transport might down regulate airway clearance as it is known that increased Na⁺ absorption reduce ASL, while increased H⁺ secretion acidify ASL. Then we tested if mucociliary clearance is affected by MCT and SMCT activity in mouse airways.

Methods: Short-circuit current in mouse tracheas was measured in Ussing chambers, expression of transporters by qRT-PCR, particle track speed (PTS) and mucus transport velocity (MTV) by videomicroscopy. Localization of MCT2 and MCT4 was evaluated by immunofluorescence. Animals (C57BL6/J) were housed at CECs-Animal facility under controlled temperature and humidity with free access to water and food. All protocols were approved by the IACUC (#CECs-2022-04), in accordance with relevant guidelines and regulations.

Results: 10mM apical L-lactate and D-lactate induced a negative current ($I_{sc} \sim -35 \mu A \cdot cm^{-2}$; n=4-5 for each group) that was dependent on apical Na⁺. qRT-PCR assay determined that SLC5A8/SMCT1 was highly expressed in airway epithelium compared to SLC5A12/SMCT2. In addition, L-lactate, which is transported by SMCT and MCT induced an increase in PTS (2.1 ± 0.1 control vs $3.28 \pm 0.4 \mu m \cdot s^{-1}$ n=3 each group; p<0.05 rank sum test) and inhibited by 1 μM of the MCT1/2 inhibitor AR-C155858 ($1.9 \pm 0.1 \mu m \cdot s^{-1}$). On the other hand, D-lactate that is exclusively transported by SMCT1 did not affected PTS ($2.03 \pm 0.1 \mu m \cdot s^{-1}$; p>0.05 compared to controls; n=3 each group), indicating that SMCT1-dependent Na⁺ absorption didn't modify airway clearance. Furthermore, analysis of MTV determined that L-lactate but not D-lactate increased the velocity of mucus (1.05 ± 0.1 control vs $1.29 \pm 0.1 \mu m \cdot s^{-1}$ for L-lactate and $1.07 \pm 0.02 \mu m \cdot s^{-1}$ for D-lactate; n=3 each group; p<0.05 rank sum test) and the increase of L-lactate was prevented by 1 μM of AR-C155858 ($0.98 \pm 0.1 \mu m \cdot s^{-1}$). Surprisingly, immunofluorescence determined that MCT2 and MCT4 are localized in basolateral membrane. Thus, our results indicate that the effect on PTS and MTV induced by L-lactate possibly is mediated by MCT1. Preliminary data indicated that in presence of MCT1 inhibitor (AZD3965) this effect on MTV was diminished.

Discussion: Monocarboxylate uptake by MCTs removes H⁺ from ASL, alkalizing the airway surface and improving airway clearance. The use of MCTs transportable substrates might help alleviate mucostasis in muco-obstructive diseases and will be tested in animal models of these diseases.

PCB037

Characterisation of the respiratory epithelium within Primary Ciliary Dyskinesia patients compared to healthy controls.

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Background: The human respiratory epithelium is lined with hundreds of millions of motile cilia that beat in a coordinated fashion to clear mucus, pathogens, and pollutants from the airways. Several diseases cause abnormal ciliary function, including severe asthma, COPD, cystic fibrosis and primary ciliary dyskinesia (PCD) (1). PCD is an autosomal recessive disorder that affects around 1:8000 new-born babies. Mutations in ciliary genes lead to protein defects within the cilia that cause them to beat in a dyskinetic fashion or become static. PCD patients demonstrate reduced mucociliary clearance, recurrent respiratory tract infections and progressive severe lung damage. However, our understanding of the epithelial biology of PCD patients remains incomplete (2).

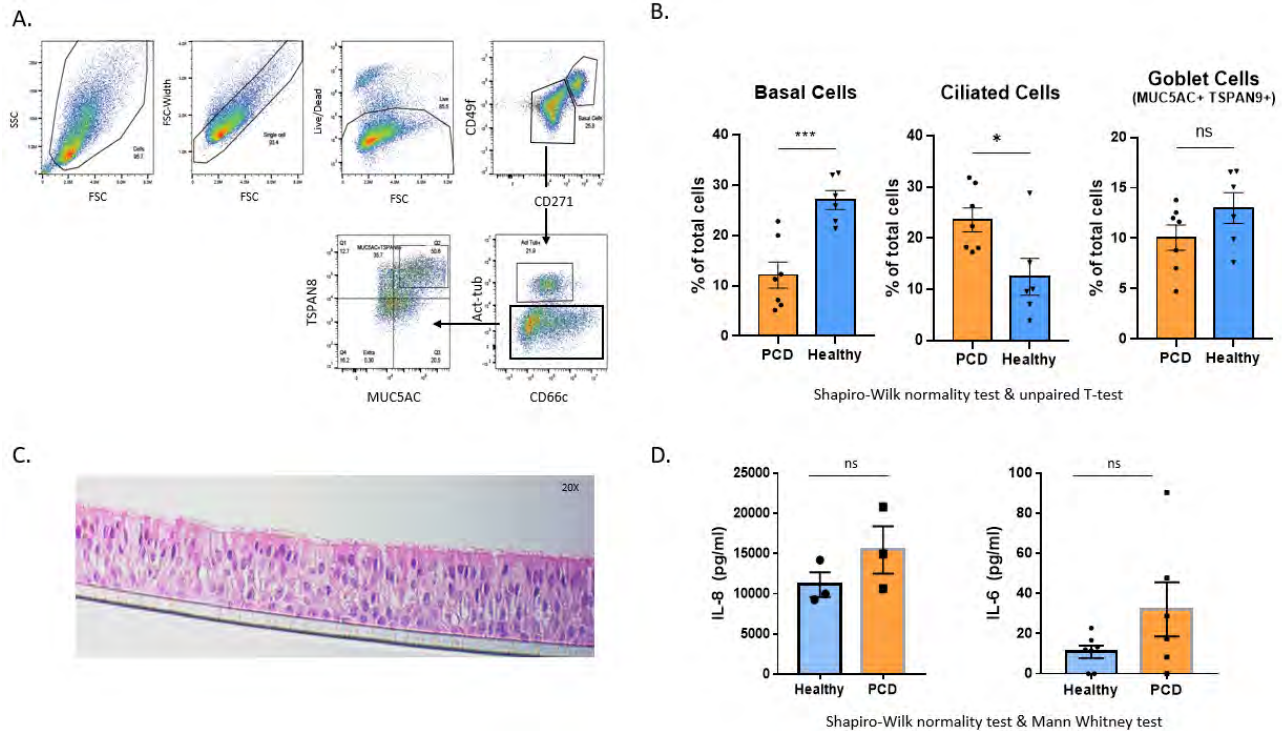
Aims:

- To determine if the cellular composition of ciliated epithelial cell cultures from PCD patients is similar to that of matched healthy controls.
- To determine if baseline cytokine and chemokine release from PCD and healthy control cultures differ.

Methods: Nasal brush biopsies from PCD patients with static cilia were cultured at air-liquid interface (ALI) to a ciliated phenotype (3). To characterise the cellular composition, we optimised a flow cytometry panel adapted from Bonser et.al, to quantify three major epithelial cell types: basal, ciliated and goblet cells (4). We used a histopathology approach, sectioning the ALI cultures to visualise epithelial populations using H&E (Figure 1). Finally, supernatants were analysed for cytokine changes using ELISA. Static PCD nasal brushings were age/sex matched with healthy controls. The mean and S.E.M was calculated, and statistical significance determined by firstly testing the normality of data using Shapiro Wilks test, followed by two-tailed T-test (flow cytometry) or non-parametric Mann Whitney test (ELISA).

Results: We confirmed the epithelial morphology of ALI is maintained during histology processing and H&E-staining (n=6 Healthy & n=PCD, 4 sections/donor). Flow cytometry showed PCD patients had fewer basal cells (n = 6 healthy, 7 PCD; p =0.0009) and an increased number of ciliated cells (p =0.0221) compared to age/sex match controls. Initial studies suggest a trend for increased IL-8 (n=3) and IL-6 (n=6) levels in PCD supernatants however increased donor number is required for statistical significance.

Summary: Flow cytometry showed a difference in the cellular composition of PCD ALI cultures, with increased numbers of ciliated cells and reduced numbers of basal cells. Preliminary ELISA results suggest static PCD may be associated with increased epithelial inflammation. Further characterisation is underway, increasing study numbers and processing cultured cells for RNAseq and mass spec to investigate apical secretions. Overall, this work will improve our basic understanding of PCD epithelial biology and identify potential PCD-specific therapeutic targets.



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PCB038

Insight into the mechanism of D-glucose accelerated exchange in GLUT1 from molecular dynamics simulations

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Atomistic molecular dynamics simulations demonstrate that when multiple β -D-glucose molecules are present within the GLUT1 transporter, simultaneous position exchanges frequently occur between adjacent ligands. These exchanges take place in the internal cavities and at both external and internal solution interfaces of the protein. They involve rotation of adjacent ligand positions along the central pore axis of the transporter with variable duration in the nanoscale (4 – 100 ns). Exchanges occurring at the extracellular protein interfaces involve fast displacements (2 – 10 ns) of D-glucose H-bonded to the protein interface by other D-glucose molecules present in solution. These examples of simultaneous D-glucose exchanges demonstrate that *accelerated exchange* is consistent with a multisite model for D-glucose transport within GLUTs where multiple D-glucose molecules move independently and stochastically within the transporter's tunnels, cavities, and the central pore.

Higher frequency of D-glucose exchange is observed in the membrane gel state, corresponding with D-glucose transport in human erythrocytes at low temperatures. The presence of multiple D-glucose molecules both within the transporter and in bathing solutions increases D-glucose penetration depths from the solutions into transporter intramembranous zones, particularly in the gel state.

That exchange frequency between adjacent ligands depends on the local D-glucose density within the transport pathway explains why accelerated exchange occurs more frequently in conditions where bottlenecks at the openings of the transport pathway are prolonged, at low temperatures (1), thereby augmenting ligand aggregation in the adjacent upstream regions.

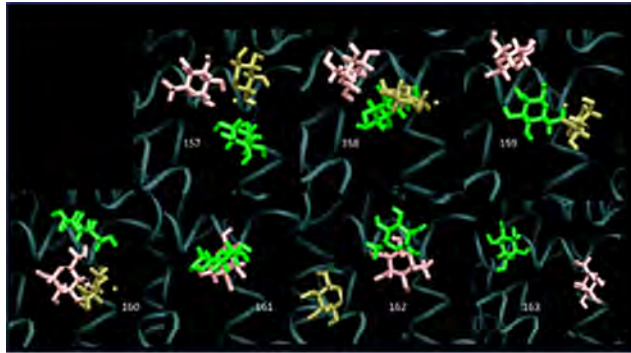


Figure 1A

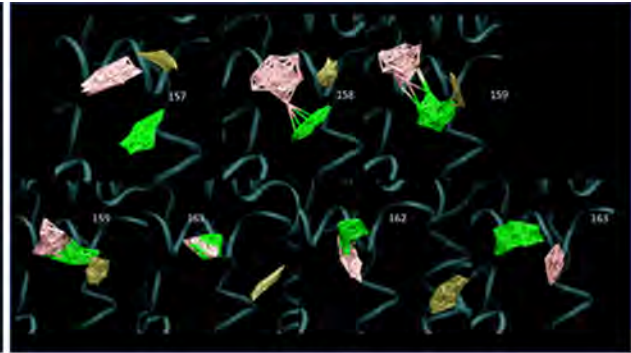


Figure 1B

The time stride is 500 ps between each successive frame number in the trajectory sequence frames 157 -163 shown in **Figure 1a** and **1b**. The pink glucose ligand, starts to the left of the green glucose ligand in frame 157 and finishes to the right of the green ligand in frame 163. The frame sequences show an example of D-glucose exchange with close contacts between the exchanging ligands. The ligand inversion occurs during a period of 3-4 ns.

Figure 1b Shows the same sequence as in **Figure 1a**, here the ligands are represented with dynamic bonds, where any bond $< 3.5\text{\AA}$ is shown as a shared bond. The shared dynamic bonds in frames from 158-162 indicate that the ligands are within $3.5\text{\AA}$ of each other at these times.

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PCB039

Developing a neutrophil trans-epithelial endothelial migration model of the human air-blood barrier to identify biomarkers for respiratory virus infection.

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BACKGROUND: Respiratory syncytial virus (RSV) is a major cause of bronchiolitis and pneumonia in young infants, with an estimated 33 million cases occurring globally each year (1). Despite the high prevalence of RSV, there are currently no licensed vaccines or effective antiviral treatments available and so supportive care remains the mainstay of therapy. A distinguishing feature of RSV bronchiolitis is the recruitment of an inflammatory infiltrate known as neutrophils into the airways of infants (2). Neutrophil-mediated factors such as neutrophil elastase (NE) and interleukin 8 (IL-8) in the blood and airways of infants hospitalised with RSV infection are thought to correlate with disease severity, although we currently do not know why (3,4). Clinical studies have also shown that neutrophils found in the systemic circulation of infants with RSV bronchiolitis contain RSV mRNA (5).

METHODS: Our lab has developed a novel trans-epithelial endothelial model of the human air-blood barrier to study neutrophil behaviour and function during RSV infection using animal-free components. To do this, primary paediatric airway epithelial cells (AECs) were grown at air-liquid interface (ALI) and co-cultured with human endothelial cells (ECs) before being infected with RSV expressing green fluorescent protein (GFP) (**Figure 1A**). After 24h, human neutrophils, obtained from a healthy donor, were added to the basolateral side. After 1h different sub-populations including basolateral, adherent and apical (migrated) neutrophils were recovered for subsequent analyses by flow cytometry.

RESULTS: Our data show that RSV infection led to a shift in the number of basolateral neutrophils to apical neutrophils, indicating movement across the EC/AEC barrier (**Figure 1B**). Exposure to RSV infected AECs led to increased expression of NE on basolateral neutrophils compared to the mock-infected control ($N=6$ neutrophil donors, Paired Two-Way ANOVA) (**Figure 1C**). This was accompanied by an increase in levels of pro-inflammatory chemokine and cytokines including interferon γ -induced protein 10 (IP-10), interleukin 6 (IL-6) and IL-8 in the apical supernatant of RSV-infected AECs compared to mock-infected control cells (**Figure 1D**) ($N=3$ neutrophil donors, Paired Two-Way ANOVA).

CONCLUSIONS: We have shown that neutrophils present on the basolateral (blood) side of RSV infected AECs increase expression of NE, which is similar to the finding of increased NE in the blood of infants hospitalised with RSV infection. These findings demonstrate that our *in vitro* model can replicate key human disease outcomes and can therefore be used to identify critical mechanisms that mediate epithelial cell damage and promote inflammation in children with severe RSV disease. Future work will investigate the mechanism behind this and compare the

phenotype of neutrophils isolated from our model to those from the blood of RSV-infected infants, thereby allowing us to characterise a neutrophil sub-population that can be used as a biomarker of severe infection.

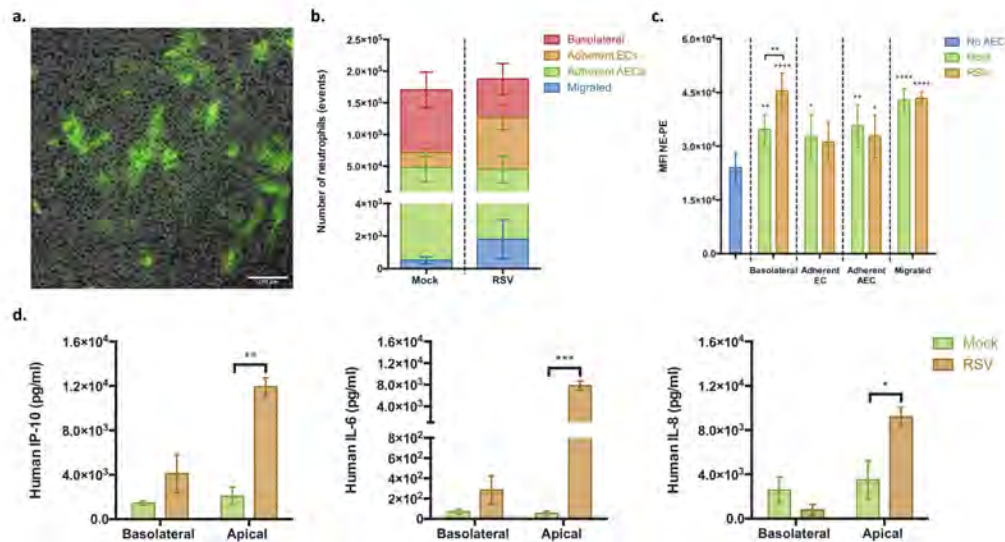


Figure 1. Neutrophils undergoing trans-epithelial endothelial migration increase their 'activation' status with enhanced expression of pro-inflammatory factors during RSV infection. **a.** RSV-infected AECs (green) at 24 hours post-infection (MOI 1). Scale bar = 100µm. **b.** Neutrophil recovery following migration for 1 hour from basolateral, adherent, and apical compartments. Absolute cell counts obtained by flow cytometry based on positive-gating for CD11b. **c.** Mean fluorescence intensity (MFI) of NE on neutrophils recovered after exposure to our model. Error bars represent mean ± SEM of N = 6 neutrophil donors. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ (Paired Two-Way ANOVA). **d.** Concentration of IP-10, IL-6 and IL-8 (pg/ml) in the apical supernatant of RSV-infected or mock-infected AECs. Error bars represent mean ± SEM of N = 3 neutrophil donors. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Paired Two-Way ANOVA).

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PCB040

Dietary regulation of ruminal UT-B2 urea transporters in adult male fallow deer bucks: effects of season and wildlife feeding activity.

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The urea nitrogen salvaging process (**UNS**) supports the symbiotic relationship between ruminants and their gastrointestinal microbiome by both supplying nitrogen and buffering bacterially-derived short chain fatty acids (**SCFAs**). Our previous studies have shown the importance of the UT-B2 urea transporters in the rumen of wild fallow deer living in Phoenix Park, Dublin. In this current pilot study, we investigated the effects on these transporters of seasonal changes and feeding behaviour in adult male deer bucks. Restricted, authorized culling of the deer population was performed by Irish Government bodies (Office of Public Works; National Parks & Wildlife Service) under strict national laws. Ruminal tissue samples were obtained from culled animals and ethical approval obtained for their use from UCD Animal Research Ethics Committee's (AR-EC-E-18-28). Initial investigation of the rumen papillae revealed that animals culled in January had significantly longer papillae (8.4 +/- 1.0 mm, N=5) than those culled just after the rutting season in November (5.3 +/- 0.7 mm, N=12) (p=0.0270, Unpaired T-test) [NOTE: All values are mean +/- S.E.]. In contrast, western blotting analysis showed that there was no significant difference in the abundance of UT-B2 transporters between these two groups (22 +/- 3, N=4, versus 34 +/- 9, N=4) (p=0.2416, Unpaired T-test). Adult males that had displayed consistent begging behaviour to obtain food from human visitors to the park had a higher papillae density (54 +/- 5 per cm², N=4) than non-begging adult males (40 +/- 2 per cm², N=7) (p=0.0128, Unpaired T-test). Furthermore, these animals had a significantly higher UT-B2 transporter to total protein abundance ratio (0.56 +/- 0.10, N=3, versus 0.13 +/- 0.04, N=3) (p=0.0183, Unpaired T-test). This increase was also shown, qualitatively by immunolocalization studies, to be predominantly in the stratum basale layer of the begging animals' rumen papillae (N=3). The findings of this novel study therefore improve our understanding of basic rumen physiological processes, but also add insight into the profound unseen effects that humans feeding wildlife may have.

PCB041

Regular exercise ameliorates anxiety and memory dysfunction due to combined oral contraceptive treatment in female rats: role of allopregnanolone and the vagus nerve

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The combined oral contraceptives (COCs) are a mixture of synthetic oestrogen and progestogen. In contrary to beneficial action of COCs in protecting uterine endometrium, a growing number of studies indicate an increased risk of cardiovascular, psychophysiological, and cognitive side effects through a reduction in vagal tone. These side effects are mediated by the catalysation of progesterone into allopregnanolone, which is known to attenuate vagal neurotransmission within brain stem (1). It is widely accepted that physical exercise stimulates the vagus nerve by a non-pharmacological intervention (2). Our objective in this study was to investigate the underlying mechanism in the therapeutic action of exercise as a vagal stimulator in COC-induced side effects that include anxiety, memory dysfunction, and cardiovascular damage. Adult female Sprague-Dawley rats were randomly divided into 4 groups as control, exercise, non-exercised COC and exercised COC groups (n=8 each). The treadmill exercise protocol required 25 m/min, 1 h/day, 3 days/week, for a total of 10 weeks, which corresponds to 60% of the maximum aerobic power (3). The groups received vehicle (distilled water, p.o.) or COC containing levonorgestrel and ethinylestradiol (1.0 µg/kg/day and 5.0 µg/kg/day, respectively) for 10 weeks. At the end of the 10th week, passive avoidance test (PAT) was performed to evaluate 72 h memory retention. Spatial memory was measured using the Y-maze. The hole-board test was performed to evaluate anxiety level. Vagal tone was assessed by heart rate variability (HRV) under an irreversible urethane anaesthesia (1.2 g/kg), after which the contraction / relaxation responses of the aortic rings were obtained with carbachol following precontraction with phenylephrine. Serum and brain tissues were obtained to measure allopregnanolone level by ELISA. The data were analysed using one-way ANOVA followed by post hoc Tukey test. The non-parametric Kruskal-Wallis test was applied when appropriate. $p < 0.05$ was considered statistically significant. Serum allopregnanolone level was increased in the exercised COC group ($p < 0.05$); however, brain tissue allopregnanolone levels were not different among experimental groups. The non-exercised COC group demonstrated a significant memory impairment in PAT as compared to the control group ($p < 0.05$), which was accompanied by memory deficits in the Y-maze score ($p < 0.05$). Despite that PAT score was increased in the exercised COC group, it was not statistically significant. Anxiety was increased in the COC group as compared to non-treated exercise group ($p < 0.01$). The lowest HRV scores were measured in the non-exercised-COC group, without reaching statistical significance. The aorta strips of all treatment groups showed decreased contractile responses to phenylephrine when compared to control aortae ($p < 0.001$), while relaxation responses were similar in all groups. Although COC treatment did not diminish cardiovascular functions, it increased anxiety and thereby inhibited exploratory behaviour. When added to COC treatment, exercise tended to increase allopregnanolone, which could have a role in increasing the vagal tone. Taken together, more research is needed to elucidate how exercise influences the metabolism of neurosteroids and brain stem autonomic circuits.

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PCB042

The reproducibility of dendritic cell and T cell counts to a 30-minute high-intensity cycling protocol as a tool to highlight overtraining

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Introduction: Intensified training and sufficient recovery is required to improve athletic performance. Heavy training has been reported as immunosuppressive in athletes, possibly due to reduced immunosurveillance with increased exercise stress. A maladaptation of the hypothalamic pituitary adrenal (HPA) axis has been shown after periods of intensified training; specifically, a blunted cortisol response to an exercise stress test has been reported. Activation of the HPA axis elevates dendritic cell (DCs) numbers. DCs are key antigen presenting cells that present antigen to T cells to initiate the required immune response. The link between training stress, the HPA axis and DCs has yet to be examined. Before investigating whether DCs become dysfunctional with intensified training, a reproducible response of these cells to an exercise stress test must be confirmed. Reproducible cortisol responses to alternating blocks of 1-minute cycling at 55% maximum power output ($W_{\max}$) and 4-minutes cycling at 80% $W_{\max}$ for 30-minutes (55/80) have been shown, and 20-minutes cycling at 80% $VO_{2\max}$ can elevate total DCs numbers. The 55/80 is based on percentage of work rate maximum, but large differences in homeostatic perturbations i.e. O_2 uptake kinetics and blood lactate responses are likely between participants in the 55/80. Therefore, submaximal anchors such as the ventilatory threshold (VT_1) should be used to prescribe intensity. **Aim:** Therefore, this study aims to assess the reproducibility of the DC and T cell responses to an adapted version of the 55/80 utilising VT_1 to prescribe intensity. **Methods:** 12 healthy males (age, 26.4 ± 5.8 years; $VO_{2\text{peak}}$, 48.58 ± 7.14 ml/kg/min) cycled for 1-minute at a work rate 20% below VT_1 and 4-minutes at 50% between VT_1 and $VO_{2\max}$ for 30-minutes (20/50 exercise test) with blood samples pre, post and 30-minutes post. This was repeated on two occasions, 2-7 days apart and at the same time of day. Using flow cytometry, total DCs were defined as Lineage- (CD3, CD19, CD20, CD14, CD56) HLA-DR+ and subsequently identified as plasmacytoid (CD11c- CD123+) (pDCs) or myeloid (CD11c+ CD123-) (mDCs). T-helper cells were identified as being CD3+CD4+ and T-cytotoxic cells were identified as being CD3+CD8+. Two-way repeated measures ANOVAs were used for all variables apart from pDCs which were analysed via a Wilcoxon signed-rank test for main effects of trial and post-hoc time effects, and a Friedmans test for main effect of time. **Results:** No significant effect of trial ($P > 0.05$) or interaction effects ($P > 0.05$) were found for any variable. A significant main effect of time for all variables were found with immune cell counts increasing from pre- to post-exercise and decreasing to baseline 30-minutes post-exercise ($P < 0.001$), apart from pDCs which remained elevated 30-minutes after exercise. Intraclass correlation coefficients showed excellent reliability for CD3+ T cells, CD8+ T cells and pDC responses between trials (ICC > 0.75) and good reliability for Total DCs, mDCs and CD4+ T cells (ICC 0.6-0.74). **Conclusions:** These results suggest that the 20/50 exercise test induces reproducible DC and T-cell count changes, which, implemented before and after a period of intensified training, may highlight the negative states of overtraining.

PCB043

The Role of Oestrogen in Female Skeletal Muscle Ageing: A Systematic Review

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Ageing is associated with a loss of skeletal muscle mass and function that negatively impacts the independence and quality of life of older individuals. Females demonstrate a distinct pattern of muscle ageing compared to males, potentially due to menopause where endogenous sex hormone production declines. This systematic review aims to investigate the current knowledge about the role of oestrogen in female skeletal muscle ageing. A systematic search of MEDLINE complete, Global Health, Embase, PubMed, SPORTDiscus, and CINAHL was completed from inception to 08/11/2022. The systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines and was registered in the international prospective register of systematic reviews (PROSPERO) (CRD42022374366). Studies were considered eligible if they compared a state of oestrogen deficiency (e.g. postmenopausal females) or supplementation (e.g. oestrogen replacement therapy) to normal oestrogen conditions (e.g. premenopausal females or no supplementation). Outcome variables of interest included measures of skeletal muscle mass, function, damage/repair, and energy metabolism. Quality assessment was completed with the relevant Joanna Briggs critical appraisal tool, and data were synthesised in a narrative manner. Thirty-two studies were included in the review. Nineteen studies (59%) had a low risk, 10 studies (31%) had a moderate risk, and three studies had a high risk (9%) of bias. Seventeen studies compared skeletal muscle outcomes in females across different menopausal stages. Overall, they showed that compared to premenopausal females, postmenopausal females display reduced muscle mass and strength, but the effect of menopause on markers of muscle damage and expression of the genes involved in metabolic signalling pathways remains unclear. Of 10 studies that investigated the effect of oestrogen supplementation, some suggest a beneficial effect of oestrogen replacement therapy on muscle size and strength, but evidence is largely conflicting and inconclusive, potentially due to large variations in the reporting and status of exposure and outcomes. The findings from this review points toward a potential negative effect of oestrogen deficiency in ageing skeletal muscle, but further mechanistic evidence is needed to clarify its role.

N/A

PCB044

Prevalence and translation of assessment and putative treatments for Long Covid-19 into clinical practice

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INTRODUCTION

Long COVID-19 (LC) is defined as the persistent symptoms ≥ 12 weeks following acute COVID-19 infection. The Office for National Statistics, ONS, estimated that there are 2M self-reported UK patients with (LC) ie 3% population (REF1).

METHODS & RESULTS

We currently have 400 adult patients in our teaching hospital NHS trust (which serves a population of 800 000) being managed with LC ie 0.0005% and another 120 on the waiting list. Applied nationwide, this suggests that only a small proportion of self-reported LC cases have the benefit of Hospital-based treatment. Raised BMI, associated type 2 diabetes, anxiety-depression and asthma were common co-morbidities as was seen in the national study.

A recent analysis by Subramaniam et al (2022) of national GP records of 486,149 non-hospitalized patients with some of 62 possible recorded LC symptoms during the first two surges of the pandemic produced three symptom cluster classes. The three classes were heterogenous symptoms, respiratory and anxiety-depression. Mean age was 43.8 years (s.d. 16.9), 55.3% of participants were female. 64.7% were white, 12.2% were Asian origin, 4.0% were Black Afro-Caribbean; 16.2% had missing ethnicity data. 53.8% were overweight or obese (BMI data missing for 13.0%), and 22.5% were current smokers (smoking data missing for 4.3%).

Separately, three leading (but not mutually exclusive) hypotheses and some putative treatments for each have been proposed (Couzin Frankel, 2022) :

1. Microvascular blood clots (identified by single photon emission computed tomography SPECT-CT or Hyperpolarised hyperpolarized xenon 129 MRI (XeMRI) to identify alveolar capillary diffusion limitation - DOAC anticoagulants
2. Persistent virus-antiviral therapy
3. An aberrant immune system - antihistamines

The results from the available clinical trials of treatment are awaited. Stimulate ICP. (Symptoms, Trajectory, Inequalities and Management: Understanding Long-COVID to Address and Transform Existing Integrated Care Pathways) tests the effectiveness of repurposed drugs (participants are allocated to (1) usual care, (2) famotidine/loratadine antihistamines, (3) Colchicine anti-inflammatory or (4) Rivaroxaban DOAC anticoagulant groups for 3 months). to treat long COVID. The effects of 3 months of treatment is measured on peoples' symptoms, mental health and other outcomes in patients attending 6-10 UK LC clinics. Cluster randomisation is at level of primary care networks so that integrated care pathway interventions are delivered as "standard of care" in that area. (Forshaw et al 2023)

CONCLUSIONS

Given the paucity of patients being referred to hospital, the majority of patients do not have ready access to advanced diagnostic techniques, associated tailored putative treatments or the above clinical trials. Underpowered clinical trials have in the past failed to provide reliable results applicable to the general population.

New approaches using analysis of real-world data of vastly larger patient numbers combined with machine learning may deliver reliable results faster in the future. Such initiatives are in line with UK government ambitions to provide more services out of hospitals, a larger primary care workforce and greater integration with social care, so that care is more joined up to meet people's physical health, mental health and social care needs.

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PCB045

Orai Ca²⁺ channels but not Ano1 or L-type Ca²⁺ channels contribute to adrenergic contractions of male mouse urethral smooth muscle

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Ca²⁺ dependent contractions of urethral smooth muscle (USM) prevent leakage of urine during bladder filling. In rabbit and pig, USM contraction relies on Ca²⁺ influx through L-type Ca²⁺ channels (Brading, 2006), and in rabbit this requires USM depolarization via Ca²⁺ activated-Cl⁻ channels (Ano1) (Fedigan *et al.*, 2017). Studies in mice demonstrated L-type channel inhibitors do not affect phenylephrine (PE) responses in male USM, but store-operated-Ca²⁺-entry (SOCE) via Orai channels was critical (Drumm *et al.*, 2018). However, this study only examined L-type channel inhibitors on responses to supramaximal PE doses (10 μ M). Thus, contributions of L-type or Ano1 channels by lower (more physiologically relevant) PE concentrations or nerve stimulation cannot be ruled out. We sought to examine potential roles for L-type and Ano1 channels in regulating male mouse USM across a range of PE concentrations and electrical field stimulation (EFS) frequencies. Intact USM rings from wildtype male C57 mice were mounted on an isometric force transducer inside a heated (37°C) organ bath, perfused with oxygenated Krebs solution. Contractions and relaxations of USM were monitored using LabScribe. Tissues were stretched to an initial 2 mN of tension and equilibrated for 1 hour before experimentation. EFS was delivered via two platinum electrodes either side of USM rings, at 1, 2, 5, 10, 20 Hz for 30 sec. USM rings contracted in response to PE (30nM – 30 μ M) in a concentration dependent manner, with an EC₅₀ of 1.3 μ M (n=60). EFS in the presence of L-NNA (nNOS synthase inhibitor), to prevent relaxations of USM due to nitric oxide release, evoked contractions whose amplitude was frequency dependent, with 20 Hz EFS evoking responses 80% larger than those at 2 Hz (n=113). The L-type channel activator FPL 64176 (300 nM) slightly (but significantly) increased the area under the curve (AUC) of PE-induced contractions (e.g. 1 μ M PE AUC increased 24.8% in FPL, P<0.05, n=6), without affecting EC₅₀. Similarly, EFS response amplitude was increased by FPL, e.g., contractions at 10 Hz increased 10% (n=6, P<0.05). FPL effects were reversed by the L-type channel inhibitor nifedipine (1 μ M, n=6, P<0.05), but nifedipine failed to significantly affect control PE or EFS responses (n=6, P<0.05) at any PE concentration or EFS frequency. In contrast, the Orai Ca²⁺ channel inhibitor GSK 7975A (10 μ M), reduced EFS-induced contraction amplitude by 50% at all frequencies (n=13, P<0.005). Upon subsequent addition of nifedipine in the continued presence of GSK 7975A, there was a further 10 - 20% reduction in residual EFS-induced contractions (n=13, p<0.05). The Ano1 channel activator Eact (10 μ M) and antagonist Ani9 (3 μ M) failed to affect PE dose-response curves or EFS responses at any frequency (n=6, P>0.05). Furthermore, nifedipine sensitive components of EFS-induced responses (unmasked when Orai channels were inhibited) were unaffected by Ani9 (n=6, P>0.05). In conclusion, in male USM, L-type channels can be activated by appropriate agonists (FPL) but inhibition of these channels does

not affect PE or EFS responses under normal conditions. Ca^{2+} influx via SOCE is the dominant Ca^{2+} influx pathway required for male USM contraction.

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PCB046

The impact of indoor carbon dioxide on human cognition and the physiological response

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Modern buildings often have automatized ventilation systems to reduce energy consumption, which leads to a higher air tightness of the building envelope and limit the control humans have over the ventilation. Therefore, there is an increasing research field on the impact of indoor air quality on human cognition and health. The indoor concentration of carbon dioxide is of particular interest because it can be used as a proxy for indoor air quality. However, past studies suggested that carbon dioxide itself can be a contributor to impaired cognition, but the findings are inconclusive due to several limitations [1]. Either a carbon dioxide exposure level higher than usually occurring indoors was used or the exposure time was short. Furthermore, studies differ in their applied cognition tests.

To cover these limitations, this interdisciplinary study used a cross-over design, in which 20 healthy office workers were exposed to two test days of eight hours to either 800 ppm carbon dioxide or 3,000 ppm carbon dioxide in a respiration chamber. Volatile Organic Compounds were filtered out from the air. Cognitive performance was measured using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Additionally, multiple price lists from economics literature were used to measure the risk and time preferences when faced with a financial decision-making problem [2]. A heuristics battery from psychology literature was used to measure sensitivity to bias behaviour in decision-making [3]. Lastly, subjects' satisfaction about the indoor air quality and their belief to which degree the air quality hinders their ability to answer the questionnaire was recorded on a 7-point Likert scale. Physiological parameters including oxygen consumption, heart rate, heart rate variability, respiration rate, blood carbon dioxide concentration, blood pressure, and skin temperature were measured continuously to investigate possible mechanisms. A linear model with group fixed effects on test subject and clustering of standard errors on the subject level was used. As robustness checks, the same linear model but with bootstrapped standard errors was estimated and a mixed linear model with a random intercept on the subject and a random slope on the carbon dioxide condition was applied. Multiple hypotheses testing has been applied to derive corrected p-values [4].

The statistical analysis indicated no significant effect of carbon dioxide on any test results of either the CANTAB test battery, the economic preferences, or the heuristics battery after applying multiple hypotheses testing. Furthermore, no significant effect on the physiological parameters could be found after correcting for multiple hypotheses testing. The same insignificance was derived using the other regression models in the robustness checks. Also, there was no significant difference in satisfaction levels with the air quality. However, subjects rated that in the high carbon dioxide condition the air quality hindered them less to answer the questionnaire ($p < 0.05$, after multiple hypotheses testing).

We could not replicate the negative effects of pure carbon dioxide exposure found in past studies. It seems that carbon dioxide is not causing the impaired cognition. The absence of a physiological response cannot confirm any adaptive behaviour which could explain why cognition is unaffected.

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PCB047

The effects of β -GPA on resistance training adaptations in rat skeletal muscle

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Resistance training induces not only muscle hypertrophy but also mitochondrial adaptation (Kitaoka et al. 2016). However, it is also known that resistance training-induced mitochondrial adaptation is limited and smaller than endurance training-induced mitochondrial adaptation (Wilkinson et al. 2008).

β -guanidinopropionic acid (β -GPA) inhibits creatine transport into the cell and decreases creatine in skeletal muscle. Previous studies have reported that β -GPA activates mitochondrial biogenesis and increases mitochondrial content markers in skeletal muscle (Williams et al. 2009).

However, the effects of β -GPA on resistance training are not clear. Therefore, the aim of this study was to investigate the effects of β -GPA on resistance training adaptation in rat skeletal muscle.

This experiment was approved by the Ethics Committee for Animal Experiments at Ritsumeikan University (BKC2022-009). 8-week-old male SD rats were randomized to (1) placebo group or (2) β -GPA group ($n = 5/\text{group}$). β -GPA (1000 mg/kg) was administered once daily in the β -GPA group by oral ingestion by a sonde. Resistance exercise was performed according to a previous study (Takegaki et al. 2019). Briefly, the ankle joint was positioned at 90 degrees, and a 3-second maximal isometric contraction was performed 10 times with a 7-second interval (1 set), for a total of 5 sets. Resistance exercise was performed only on the right gastrocnemius muscle and the left gastrocnemius muscle was treated as a resting sample. During exercise, rats were anesthetized with 2% concentration of isoflurane. Resistance exercise was performed for a total of 12 sessions, and 48 hours after the last exercise session, rats were anesthetized and gastrocnemius muscles were removed. Western blotting was used to evaluate the expression levels of proteins involved in mitochondrial biogenesis. Two-way ANOVA was used for statistical analysis, and multiple comparisons were made only when an interaction was observed. Unpaired t-test was only used for the analysis of the % change in muscle mass.

A significant interaction was found for protein expression levels of PGC-1 α , a key regulator of mitochondrial biogenesis ($p < 0.05$). Multiple comparisons showed that PGC-1 α protein expression was significantly higher in β -GPA + exercised leg (+82.5% vs placebo + rested leg) than in the placebo + exercised leg (+34.3% vs placebo + rested leg) ($p < 0.05$). A significant interaction was also observed in total OXPHOS (Complex I-V) protein expression, markers of mitochondrial content ($p < 0.05$). Multiple comparisons showed that total OXPHOS protein expression in β -GPA + exercised leg (+61.8% vs placebo + rested leg) was significantly higher than in exercised leg (+33.0% vs placebo + rested leg) ($p < 0.05$). Main effects of training and β -GPA were observed in muscle wet weight ($p < 0.05$). The % change in muscle mass with resistance training was significantly lower in β -GPA group (+3.92%) than in placebo group (+9.92%) ($p < 0.05$).

The study suggests that β -GPA, a creatine inhibitor, enhances resistance training-induced mitochondrial biogenesis. Furthermore, β -GPA can attenuate, but not completely abolish, resistance training-induced gains in muscle mass.

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PCB048

The effect of electrical pulse stimulation, an in vitro model of exercise, on metabolic characteristics of differentiated primary human muscle cells: comparison of two different protocols

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Introduction: Regular physical activity is capable of improving and maintaining human health. However, the molecular mechanisms of exercise-induced adaptations are still not fully understood. Electrical pulse stimulation (EPS) is used to induce visually detectable contractions of differentiated muscle cells, myotubes, to mimic exercise *in vitro*.

Aim: Our aim was to identify the EPS protocol that can induce significant physiological response in cultured human primary myotubes, by comparing the effectiveness of two EPS protocols, with 1. continuous, and 2. intermittent stimulation.

Materials and methods: Differentiated primary human skeletal muscle cells derived from healthy, lean men ($n=3$, 31 ± 2.45 yrs, $23,72\pm0.82$ kg/m²) were exposed to two EPS (Ionoptix, USA) protocols: (i) commonly used 24h continuous stimulation (frequency 1Hz, pulse duration 2ms), and (ii) 24h intermittent stimulation, where higher frequency stimulation (5Hz, pulse duration 2ms) is followed by subthreshold stimulation (0.2Hz, pulse duration 4 ms). This cycle was repeated 9 times in 24h period. Oxidation of radioactively labeled ¹⁴C-glucose and ¹⁴C-palmitate, incorporation of ¹⁴C-glucose into glycogen, changes in content of mitochondrial respiratory chain proteins (western blot) and in fiber-type specific gene markers (qPCR) were determined. Control cells were exposed to electrodes without EPS. Data are presented as average $\pm$ SEM and differences were analyzed with paired T-test (GraphPad Prism 9.4.1).

Results: Both types of EPS led to visually detectable contractions of myotubes and facilitated the incorporation of glucose into glycogen (continuous stimulation: 19.72 ± 5.09 pmol/3h/ μ g, $p=0.0063$; intermittent stimulation: 17.97 ± 4.88 pmol/3h/ μ g, $p=0.0051$ vs. control: 15.14 ± 4.79 pmol/3h/ μ g, $n=3$). However, oxidative glucose utilization tended to increase only after the intermittent stimulation (intermittent stimulation: 9.41 ± 1.76 pmol/3h/ μ g, vs. control: 7.58 ± 1.77 pmol/3h/ μ g, $p=0.0835$, $n=3$). Intermittent stimulation also led to significantly increased total glucose disposal (intermittent stimulation: 27.38 ± 6.49 pmol/3h/ μ g, vs. control: 22.72 ± 6.30 pmol/3h/ μ g, $p=0.0031$, $n=3$), while after continuous stimulation we observed only a trend (continuous stimulation: 26.48 ± 6.93 pmol/3h/ μ g, vs. control: 22.72 ± 6.30 pmol/3h/ μ g, $p=0.0744$, $n=3$). Importantly, we observed a 20% increase in total fatty acid oxidation in cells exposed to intermittent stimulation (intermittent stimulation: 983.5 ± 149.582 pmol/3h/mg, vs. control: 799.6 ± 98.8 pmol/3h/mg, $p=0.0669$, $n=4$), while continuous stimulation did not induce significant change due to high response variability (continuous stimulation: 1067.4 ± 249.0 pmol/3h/mg, vs. control: 799.6 ± 98.8 pmol/3h/mg, $p=0.33$, $n=4$). There were no changes in the protein content of respiratory chain complexes in response to two protocols ($n=4$, $p>0.1$). However, there was an increase in mRNA for MYH2 (marker of fast IIa fibers) specifically after continuous stimulation (continuous stimulation: 45.33 ± 5.16 AU, vs. control: 27.342 ± 4.34 AU, $p=0.0356$, $n=4$).

Conclusion: Electrical pulse stimulation is *in vitro* model used for studying exercise-related adaptative mechanisms in muscle cells. We identified intermittent EPS as more effective in inducing relevant physiological changes in metabolism, as exemplified by an improvement in glucose and palmitate oxidation.

Ethical approval: The study was approved by the ethics committee of the University Hospital Bratislava, Comenius University Bratislava and the Ethics Committee of the Bratislava Region Office and conforms to the ethical guidelines of the Declaration of Helsinki. All participants provided witnessed written informed consent prior to entering the study.

PCB049

Whole-body cardiopulmonary fitness and local skeletal muscle function in people living with Long COVID compared with healthy controls

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Introduction

The mechanisms underlying symptoms of persistent fatigue and severe exercise intolerance which can follow SARS-CoV-2 infection, termed 'Long COVID' (LC), are not fully understood. The objective of this study was to compare whole-body cardiopulmonary fitness ($\dot{V}O_2$) and local skeletal muscle measures of oxygen consumption ($\text{mus}\dot{V}O_2$), oxidative capacity (τ) and microvascular reactivity (post-occlusive reactive hyperaemia (PORH)) between individuals with LC and healthy controls (HC).

Methods

Participants with LC were recruited from the University College London Hospital (UCLH) Long COVID Clinic and HC from students and staff at University College London. Cardiopulmonary fitness was measured using analysis of expired gases during a sub-maximal (85% of predicted maximum heart rate) ramp cardiopulmonary exercise test (CPET) performed on a semi-recumbent cycle ergometer. Near Infrared Spectroscopy (NIRS) was applied to the gastrocnemius in combination with arterial occlusions and a short bout of muscle contractions for assessment of local skeletal muscle oxygen consumption ($\text{mus}\dot{V}O_2$), oxidative capacity (recovery of $\text{mus}\dot{V}O_2$, τ) and microvascular time to 95% PORH. Descriptive statistics are presented as n(%) and mean $\pm$ standard deviation. Outcome measures were compared between LC and HC using potential outcome means (POMs) calculated by an augmented inverse probability weighted estimator with a linear outcome model and logit treatment model. Estimates were adjusted for potential confounders (age, sex and ethnicity) and are summarised as POM(95% confidence intervals). The level of significance was set at $p < 0.05$.

Results

Analysis includes 32 adults (10(31%) men, 44 $\pm$ 12 years old) with LC and 19 HC (6(32%) men, 40 $\pm$ 13 years old). In patients with LC, cardiopulmonary fitness was lower ($\dot{V}O_2$ at Anaerobic Threshold (AT): 12.8(11.7,13.9) versus 16.3(15.0,17.5) ml/Kg/min, $p < 0.001$; AT: 47.7(44.1,51.2) versus 56.6(53.1,60.0) % of predicted $\dot{V}O_{2\text{max}}$, $p < 0.001$; $\dot{V}O_2$ Work Rate: 8.5(8.1,9.0) versus 9.4(9.0,9.8) ml/min/W, $p = 0.008$), oxidative capacity was poorer (τ : 38.7(31.9,45.6) versus 24.6(19.1,30.1) seconds, $p = 0.001$) and resting $\text{mus}\dot{V}O_2$ was lower (0.11(0.08,0.15) versus 0.15(0.12,0.18) $\mu\text{M/s}$, $p = 0.09$) compared to healthy controls. There were no observed

differences for time to 95% PORH between the groups (28.0(23.7,32.3) versus 27.3(22.3,32.4) seconds, $p=0.86$).

Conclusion

Results from this study suggest that, compared to healthy controls, individuals living with Long COVID have lower whole-body cardiopulmonary fitness and lower local skeletal muscle measures of oxygen utilisation and oxidative capacity but similar skeletal muscle microvascular function.

PCB050

Sex differences in the effect of physical activity throughout adolescence on VO₂max in early adulthood

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Motivation

Low cardiopulmonary fitness (CPF) predicts future morbidity in adults. Participation in physical activity (PA), particularly moderate to vigorous physical activity (MVPA), can improve CPF but the impact of MVPA throughout adolescence on CPF in early adult life remains unclear and potential differences in this association between men and women have not previously been quantified. We investigate sex-differences in the impact of MVPA throughout adolescence on early adult CRF.

Methods

Participants enrolled in a UK birth cohort (*The Avon Longitudinal Study of Parents and Children*, ALSPAC) undertook measures of physical activity at 11, 13, 15 and 24 years (y) old using a hip-worn accelerometer. Time in MVPA (average minutes/day) was derived from accelerometer data at each time. Cumulative MVPA participation was calculated as a life-time average across all measurement time points. CPF (VO₂max) was estimated from a Tecumseh step test at age 24. Ethical clearance for all procedures was granted by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee and all participants provided written and informed consent.

Structural equation modelling was used to compare associations between MVPA measured at each time and VO₂max at age 24 within the same model and cumulative MVPA participation and VO₂max. Direct and indirect effects of MVPA at each time point on VO₂max are presented. The full information maximum likelihood method was used to account for missingness under the assumption of missing at random. Maternal socioeconomic group was included in models as a predictor of missing observations. Skewed data were log transformed. Analysis was sex-stratified.

Results

Participants who undertook the step test at 24 and had undertaken at least two PA measurements during adolescence were included in this analysis (n=1347 (476 men)). Cumulative MVPA was positively associated with VO₂max in both men and women, but the association was slightly stronger for men (Table 1). In men, after adjustment for MVPA at each measurement time (direct effects), only MVPA at 24 remained strongly associated with VO₂max at 24 (Table 1). In women, we observed strong direct effects of MVPA at age 13 and age 24 on VO₂max at 24 (Table 1).

Conclusion

Cumulative MVPA participation throughout adolescence is an important determinant of CRF in early adulthood. These data also suggest that MVPA in early adolescence is an important determinant of early adult CPF in women independent from MVPA participation in early adulthood.

Total <i>n</i> (M/F <i>n</i>) 1347 (476/871)	B-coefficients(95%CI) & p-value of log daily mins of MVPA at each age on est.VO ₂ max at age 24			
Measurement age (years)	Men		Women	
	<i>Direct effect</i>	<i>Indirect effect</i>	<i>Direct effect</i>	<i>Indirect effect</i>
11	0.14(-0.55,0.83) 0.690	0.43(0.11,0.75) 0.008	0.12(-0.45,0.68) 0.688	0.26(0.08,0.45) 0.005
13	0.71(-0.06,1.48) 0.069	0.27(-0.08,0.62) 0.128	0.61(0.11,1.12) 0.018	0.05(-0.22,0.32) 0.720
15	0.62(-0.13,1.36) 0.105	-0.04(-0.26,0.18) 0.727	-0.12(-0.69,0.46) 0.694	0.22(0.04,0.39) 0.017
24	1.34(0.22,2.47) 0.019	-	1.24(0.38,2.10) 0.005	-
Cumulative (total effect)	1.58(0.76,2.40) <0.001	-	1.12(0.54,1.70) <0.001	-

Table 1. Direct and indirect effects (B-coefficients(95%CI), p-value) are presented for associations between time in moderate-vigorous physical activity (MVPA) at each age and estimated VO₂max (est.VO2max) at age 24.

PCB051

Evaluation of the Vapor Phase Toxicity of the Thyme Essential Oil and Thymol in Fibroblasts and Lung Tumor Cells.

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Essential oils (EO) are source of compounds with several pharmacological activities, which due to their volatile components can be directly delivered to the lung tissue by inhalation, an interesting characteristic for the lung cancer treatment. The species *Thymus vulgaris* L. (thyme) has been used for a long time in traditional medicine due to its therapeutic potential. In addition, the antitumor activity of thyme EO has already been demonstrated *in vitro* and *in vivo* assays. The main component of thyme EO, thymol and p-cymene, also present the cytotoxic activity against cancer tumor cell lines, but the effects of the vapor phase remains unknown. The aim of this study was to evaluate the cytotoxicity of the vapor phase of the *Thymus vulgaris* L. (thyme) EO and thymol, on the lung cancer cells A549 and H292, as well on the MRC-5 line, a model of lung fibroblast non-tumor. To perform the treatments with the vapor phase, was used the methodological strategy described by Seal *et al.* (2012) with some modifications. Cells were seeded (1×10^4 cells/well) in the 8 central wells of 24-well plates. In the 16 wells free from cells, was added 1 mL of solubilized thyme EO (62.5-1000 $\mu\text{g/mL}$) or thymol (31.25-500 $\mu\text{g/mL}$) diluted in different concentrations. The cells were treated 48 h. The cytotoxicity was assessed by MTT reduction and Sulforhodamine B staining assays and expressed as concentration capable of generating vapor to generate 50% reduction of cell viability (ICV_{50}). The chemical analysis of thyme EO by CG/MS confirmed thymol (41.84%) as the major compound. Both OE and thymol were cytotoxic to the three cell lines in a dose dependent manner, however thymol showed greater toxicity. The A549 cells were more responsive than H292. The ICV_{50} calculated to thyme OE for A549 was 305 $\mu\text{g/mL}$ and to thymol was 150 $\mu\text{g/mL}$, for the H292 it was 648 μg and 138 $\mu\text{g/mL}$, respectively. The vapor phase of the thymol was less toxic for the non-tumor cells (MRC-5), with the selectivity index (SI) equal to or greater than 2. To evaluate the effect of thymol in association with radiation, A549 cells were treated for 6 hours with thymol (125 $\mu\text{g/mL}$) before exposure to 9 Gray (Gy) of radiation. After 48 hours, cell viability assays were performed. The vapor phase of thymol reduced the viability of tumor cells in synergy with the radiation. The AO/EB staining (fluorescent microscopy) revealed that the association of treatments (thymol + IR) resulted in cell death by necrosis. In the group control (cells irradiated) was observed also necrotic cells, however a high number of apoptotic cells were found. Corroborating with these results, the caspase assay (CellEvent™ Caspase-3/7 Green Detection Reagent) showed a high activation in only control group. The results indicate that the cytotoxicity of the vapor phase of the thyme OE is due mainly to its major compound, thymol, which was more cytotoxic and more selective than the thyme OE, and it could be a great adjunct when used in association with radiation.

Seal, S *et al.* (2012). PLoS ONE, 7(10), 1–11.

PCB052

The impact of a mixed exercise countermeasure on muscle proteomic dynamics and muscle atrophy in active healthy older adults undergoing a 14-day head-down tilt bedrest

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Introduction Prolonged muscle disuse, as is often observed in long-term hospitalization, can have deleterious effects on muscle health. The aging population, which is more prone to lengthy hospital stays, has shown an increased risk of suffering from the impact of bedrest and presents greater rate of muscle atrophy and muscle weakness. An exercise countermeasure therefore presents itself as a prime solution to mitigate the effect of bedrest. Yet, little to no studies have investigated the impact of an exercise countermeasure on muscle atrophy, strength loss and muscle proteome dynamics in an aging population subjected to prolonged muscle disuse.

Methods We therefore sought to verify how a mixed exercise program affects muscle health and performances in active older adults aged 55 to 65 (n=20, 9 men and 11 women) subjected to 14 days of continuous head-down tilt bedrest. Half of the participants engaged in a daily mixed exercise program for an hour in addition to receiving passive mobilizations, while the other half received only passive mobilizations. Total muscle mass was estimated with DXA, and muscle volume and fat infiltration were measured with MRI. Knee extensor strength was measured isometrically with Biodex system at a 90° angle. To better understand the mechanisms at play, muscle biopsies were taken on the outer part of the quadriceps muscle on day 1, 3, 8 and 14 of bedrest and muscle proteomics analyses were done by tandem mass spectrometry. Finally, inflammatory markers (TNF- α , IL-6, IL-8), myostatin, and heat-shock protein 27 and 72 were measured in plasma before and after bedrest. Follow-up measurements were repeated after 4 weeks and 4 months. The effect of time and group, and their interaction was verified with mixed-effect models.

Preliminary results 14 days of continuous head-down bedrest induced changes in knee extensor strength in both group ($p < 0.001$) without a group-by-time interaction ($p = 0.37$). Total lean mass estimated with DXA decreased after bedrest ($p = 0.004$) and returned to normal after 4 weeks without a group effect ($p = 0.93$). MRI however showed a group-by-time interaction for changes in quadriceps muscle volume (Change from baseline to end of bedrest: Control: -6.2% CI [-8.3, -4.0%]; Exercise: -0.8% CI [-2.5, 0.9%], $p < 0.01$), with exercise mitigating the impact of bedrest. So far, the changes in protein abundance between day 1 and 14 of bedrest are related to biological processes of innate immunity and inflammation. These changes in protein abundance were however not reflected in systemic inflammation as there was no change from baseline in IL-6, IL-8 or TNF- α throughout the duration of the study ($p \geq 0.2$ for all).

Conclusion Altogether, our preliminary analyses show that engaging in an hour of mixed exercise everyday mitigated the effect of 14 days of head-down bedrest on quadriceps muscle volume. It did not, however, counter the loss of strength. Additional analyses will be done in

muscle biopsies and in plasma to better understand the mechanisms at play and full data will be presented at the Physiological Society Conference.

Ethical statement Participants gave their informed and written consent before participating in this study.

PCB053

Influence of anxiety, breathing vigilance and respiratory muscle strength on respiratory load perception

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Introduction: Respiratory effort perception is a complex phenomenon, essential to maintenance of homeostasis, and is influenced by a number of factors. Anxiety is associated with greater breathlessness in clinical populations. Anxiety and vigilance towards respiratory sensations have previously been shown to be inter-related but have not yet been explored in a controlled laboratory setting. Perceived breathing effort is often viewed in the context of load-capacity balance, but the influence of the absolute magnitude of pressure generation is under-explored.

Methods: Maximum inspiratory pressure (P_Imax) was measured with a differential pressure transducer during a sustained maximal inspiratory effort against an occlusion and calculated as the greatest one-second mean pressure. Breathing vigilance and anxiety were assessed prior to loading using the Breathing Vigilance Questionnaire (BVQ) and State-Trait Anxiety Inventory (STAI).

Inspiratory threshold loading was applied at 30%, 50% and 80% of each participant's P_Imax using an inspiratory muscle training device (POWERbreathe Plus IMT). A "sham" load at 4cmH₂O was delivered using an inverted Philips Threshold PEP device.

Each load was applied for twelve breaths, with five minutes of tidal breathing between loads. Order was randomised. Participants rated breathing difficulty using a 100mm visual analogue scale (VAS-D) immediately after each load. Friedman's ANOVA with Dunn's post hoc test using Bonferroni correction for multiple comparisons was used to examine differences in VAS-D at each load. Linear mixed effects modelling (LMM) was used to quantify the relationship between load and VAS-D, and the influence of BVQ score, STAI score and P_Imax.

Results: Thirty healthy adults (eighteen female) were studied (median (IQR) age 32.0 (24.3 – 44.5) years, mean (SD) P_Imax 119 (48)cmH₂O. Mean (SD) BVQ score was 10 (4), STAI-state was 24 (7), STAI-trait was 32 (6). Only three and seven participants respectively scored above the accepted threshold of 37 for "no or low anxiety".

Median (IQR) VAS-D varied with IMT dose (Baseline: 4 (0 – 10)mm, sham 11 (3 – 18)mm, 30% 29 (12 – 54)mm, 50% 41 (28 – 66)mm, 80% 73 (47 – 94)mm), $p < 0.001$). Individual values are shown in Table 1. On post hoc testing, all VAS-D values differed significantly from one another (p values 0.048 to < 0.001), with the exception of baseline *versus* sham and 30% *versus* 50% ($p = 1.00$ and $p = 0.604$ respectively).

LMM showed a significant ($p < 0.001$) relationship between VAS-D and load: slope (95% confidence interval) 0.72 (0.61 – 0.83)mm/%P_Imax. Neither BVQ nor STAI score influenced this relationship significantly. P_Imax significantly influenced the load-perception relationship: slope

(95% CI) of load *versus* VAS-D in the combined model 0.37 (0.09 – 0.65)mm/%P_Imax, p=0.01), additional influence of baseline P_Imax 0.003 (0.001 – 0.005)mm/%P_Imax/cmH₂O, p=0.009.

Conclusions: Perceived difficulty of breathing increases with applied threshold load. In a population with low state and trait anxiety and low levels of breathing vigilance, this relationship is not modulated by anxiety or breathing vigilance scores. Underlying respiratory muscle strength does however exert a significant relationship on load perception, suggesting that absolute magnitude of imposed load in addition to the fraction of the individual's capacity determines response to sensory feedback from the respiratory system.

PCB054

Lower Limbs Motor Index (LLIMI-DXA): a standardisation parameter of muscle mass in professional football players

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Introduction

Currently, the somatotype of elite football players has become towards a greater mesomorphic and ectomorphic component¹. In the UEFA Expert Group Statement² research, the range of fat mass has been established by Dual Photon X-ray Absorptiometry (DXA) between 8 and 13%, but no reference values for lean mass have been calculated. At present, there is a lack of useful reference parameters for setting lean mass targets in lower limbs of professional football players^{3,4}.

For this reason, the aim is to obtain lower limb lean mass reference ranges for this elite population.

Methods

The total sample consisted of 31 elite football players (Table 1), from the first team of Udinese Calcio (Italy).

Table 1. Characteristics of the players evaluated. n=31.

Variable	Mean ± SD
Age (years)	26,11 ± 4,73
Weight (kg)	82,31 ± 8,39
Height (cm)	184,76 ± 7,65

Full body DXA analysis was performed with a GE Healthcare Lunar iDXA device during the competitive stage of the 21/22 and 22/23 seasons, gathering a total of 298 measurements. Injured players were excluded from the total sample.

Lower limb lean mass was assessed using the index: lower limb lean mass/height² which we have named LLIMI-DXA.

This work was approved by the Ethics Committee of the Pablo de Olavide University.

Results

In our group we obtained mean reference values of the LLIMI-DXA index of 8.13 ± 0.86 kg/m². Approximately 70% of the players are in the range ($x \pm SD$) of 7.27-8.99 kg/m². Approximately 96% of our players are in the range ($x \pm 2SD$) of 6.41-9.85 kg/m². The evolution of this index during the 21/22 and 22/23 seasons is shown in table 2.

Table 2. DXA measurements season 21/22 and 22/23. n=31.

	LLIMI-DXA Evolution			
Period	July-August	September to December	January - February	March to May
Number of measures	62	128	91	17
LLIMI-DXA				
Mean $\pm$ SD	$8,07 \pm 0,87$	$8,12 \pm 0,84$	$8,15 \pm 0,89$	$8,41 \pm 0,80$

LLIMI-DXA: Lean mass/stature index² by dual photon X-ray absorptiometry on lower limbs. Total number of DXA measurements: 298

Conclusions

The LLIMI-DXA index has proved very useful in our elite football players for monitoring the evolution of the lean mass of the lower limbs during the season, so we consider this index to be very useful for analysis by the technical and health staff of a professional football club. Given that this is a novel index not evaluated in other teams, our data from the interval in which 70% of the players are found can serve as a reference for adequate values of this index, while the second interval can serve as a reference for a more exhaustive monitoring of muscle mass.

However, it would be advisable for each club to obtain the average values of the LLIMI-DXA index in order to be able to compare and analyse their athletes.

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PCB056

The protective mechanisms of Osteoprotegerin on C2C12 myogenic differentiation.

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Skeletal muscle mass progressively decreases with advancing age, increasing the risk of developing Sarcopenia, characterised by low appendicular muscle mass (Dufresne., 2016; Ageing and health., 2022). This currently poses a significant cost to global healthcare systems. Low skeletal muscle mass is more likely to cause a loss of independence and, ultimately, a reduced longevity. Osteoprotegerin (OPG), a decoy receptor for RANK/RANKL is known to prevent bone resorption within the bone remodelling cycle, yet it has been suggested to exhibit protective effects on dystrophic skeletal muscle in animal models (Dufresne et al., 2015).

To further understand the potential of OPG as a therapeutic target to combat age related muscle loss, a better mechanistic insight is required. Therefore, we aimed to identify if OPG exhibits protective effects in C2C12 myoblasts in the presence of TNF α , used to represent the inflammatory response associated with ageing, having previously shown deleterious properties in C2C12 cells. To achieve this aim we had 3 objectives 1; Ascertain concentrations of both TNF α and OPG that will exhibit observable effects on C2C12 myogenic differentiation, 2; develop a protocol to grow and treat differentiating C2C12 myoblasts with OPG and TNF α and, 3; treat C2C12 myotubes with TNF α and OPG, simultaneously, in order to examine any effect OPG may exhibit on differentiation parameters.

C2C12 myoblasts were seeded at a density of 5000 cells/cm² in growth media (GM) and grown until confluent. A titration of OPG and TNF α was conducted, concluding with 30ng/ml⁻¹ TNF α and 20ng/ml⁻¹ OPG as the most effective treatment doses for differentiation. Cells were then seeded in 24 well plates and grown to confluence in GM (n=4) after which GM was replaced with Differentiation Media (DM). Experimental treatments were then added at 0 and 24 hours of incubation to observe OPGs effects on the formation of myotubes with TNF α present. Mean myotube diameter and mean number of myonuclei per myotube were measured as differentiation measurement parameters. Mixed effects analysis of variance and One-way ANOVA tests were used and significance accepted at p<0.05.

Between controls (DM) and groups treated with TNF α and OPG simultaneously, significant differences in mean myotube diameter and mean number of myonuclei/myotube were observed (p<0.05). In all conditions containing both TNF α and OPG, mean myotube diameter was non-significantly increased when compared to conditions containing only OPG or TNF α (p>0.05). Significant increases in mean number of myonuclei per myotube between groups treated with TNF α or OPG at 0h, and groups treated with TNF α or OPG at 0h followed by OPG or TNF α at 24h (p<0.05) were also observed (Figure 1). The data herein provides a sound, viable method for investigating the effects TNF α and OPG exhibit on differentiating C2C12 myotubes. In addition, OPG demonstrates protective effects on C2C12 differentiation in a model replicative of the ageing skeletal muscle. It is clear OPG has the potential to interact with bone and muscle, however, further insight is required to understand its mechanistic actions to confirm OPG as a therapeutic target to help overcome the musculoskeletal declines throughout the ageing process.

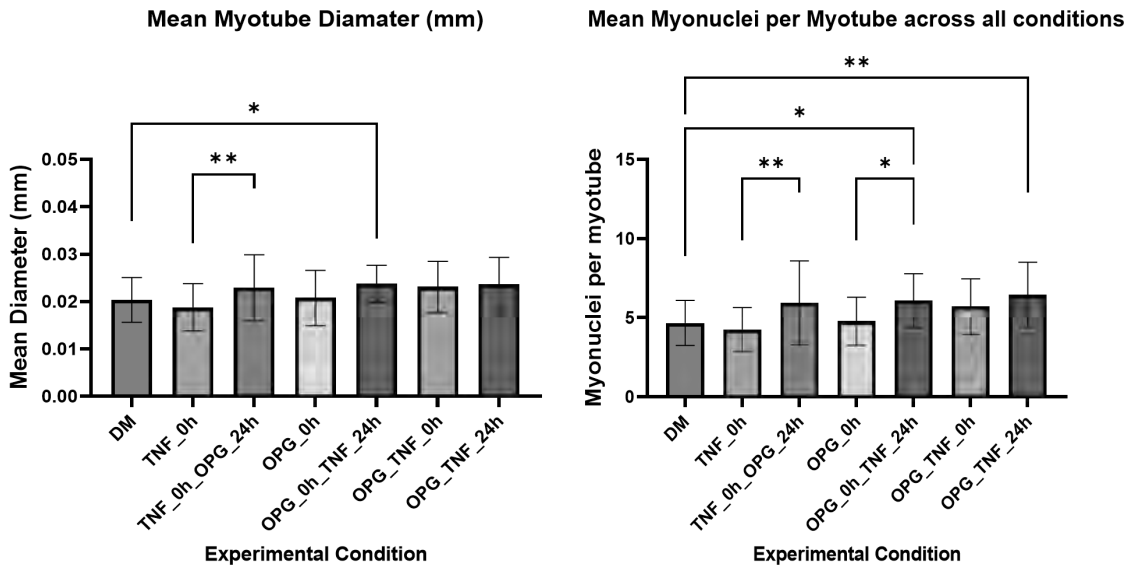


Figure 1: Mean myotube diameter and mean myonuclei per myotube data for each experimental condition plotted against untreated (DM) control. * and ** indicate the level of significance displayed by the data.

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PCB057

End Stage Liver Disease is Associated with Increased Quadriceps Intermuscular Adipose Tissue Compared to Age and Sex Matched Controls

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Background

The investigation of muscle quality is important to understand underlying muscle health and pathology in disease states, such as End Stage Liver Disease (ESLD). Myosteatorosis, i.e., fat infiltration into skeletal muscle, is a key indicator of muscle quality and may negatively affect muscle function. Previous studies have shown that myosteatorosis occurs in patients with non-alcoholic fatty liver disease¹. However, most studies involving patients with ESLD have typically assessed myosteatorosis in L3/L4 muscle groups^{2,3}. Thus, an in-depth investigation of myosteatorosis in the lower limbs of patients with ESLD, and in particular the quadriceps, remains to be completed.

Aims and objectives

The primary aim was to investigate whether quadriceps intermuscular adipose tissue (IMAT) differs between patients with ESLD and healthy age/sex-matched controls (HC), and whether IMAT differs based on anatomical location and/or quadriceps muscle head. A secondary aim was to explore the impact of IMAT on muscle function.

Methods

33 patients with ESLD (55.0±10.5 years) and 17 HC (49.6±15.4 years) participated in this observational study. Quadriceps IMAT was estimated at 20,40,50,60 and 80% of muscle length (distal = 0%) via Magnetic Resonance Imaging (MRI) Dixon technique. Vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris IMAT was also calculated at 50% of muscle length. Bioelectrical impedance analysis and maximal knee extensor isokinetic assessments were also completed to assess body composition and muscle strength respectively. Finally, wrist worn accelerometers were worn for up to 14 days to assess habitual physical activity. The study was approved by the Health Research Authority - West Midlands Solihull (REC reference: 18/WM/0167) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. ClinicalTrials.gov identifier: NCT04734496.

Results

Two-way ANOVA showed a main effect of anatomical location ($P < 0.0001$) and condition ($p < 0.0001$), in addition to a significant interaction (i.e., location x condition) ($P < 0.01$) for quadriceps IMAT. Šidák's post hoc comparison showed quadriceps IMAT was greater in ESLD at all anatomical locations compared to HC. Similarly, when comparing individual quadricep muscles, two-way ANOVA showed a main effect for muscle ($P < 0.0001$) and condition ($P < 0.0001$) with a significant interaction (i.e., muscle x condition) ($P < 0.01$). Šidák's multiple comparisons revealed significant differences between quadriceps muscles in ESLD but not HC. Pearson r correlation showed significant positive correlations between quadriceps IMAT (at 50% muscle length) and BMI ($r = 0.62$, $P < 0.0001$, $n = 50$), body fat percentage ($r = 0.65$, $P < 0.0001$, $n = 50$) and age ($r = 0.36$, $P < 0.01$, $n = 50$). In addition, negative correlations existed between quadriceps IMAT and both maximal knee extensor strength ($r = -0.50$, $P < 0.001$, $n = 50$) and habitual physical activity ($r = -0.51$, $P < 0.001$, $n = 42$).

Conclusions

Quadriceps IMAT is greater in patients with ESLD compared to HC, irrespective of the anatomical location or muscle analysed. Correlations suggest that quadriceps IMAT is positively associated with overall body fat percentage and BMI, and negatively associated with physical activity. Importantly, quadriceps IMAT may negatively impact muscle function and strength.

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PCB058

Quercetin modulates renin-angiotensin-aldosterone axis under high altitude stress

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Introduction:

Exposure to hypobaric hypoxic environment such as high altitude regions induces a rise in blood pressure, which possess a direct connection with renin-angiotensin-aldosterone system (RAAS). RAAS is one of the major regulatory pathways involved in acclimatization and maintenance of blood pressure in sojourners ascending to mountains. Quercetin, a plant flavonoid is known to be a potent molecule for the amelioration of complications caused by hypoxia via curtailing oxidative stress and improving antioxidant system.

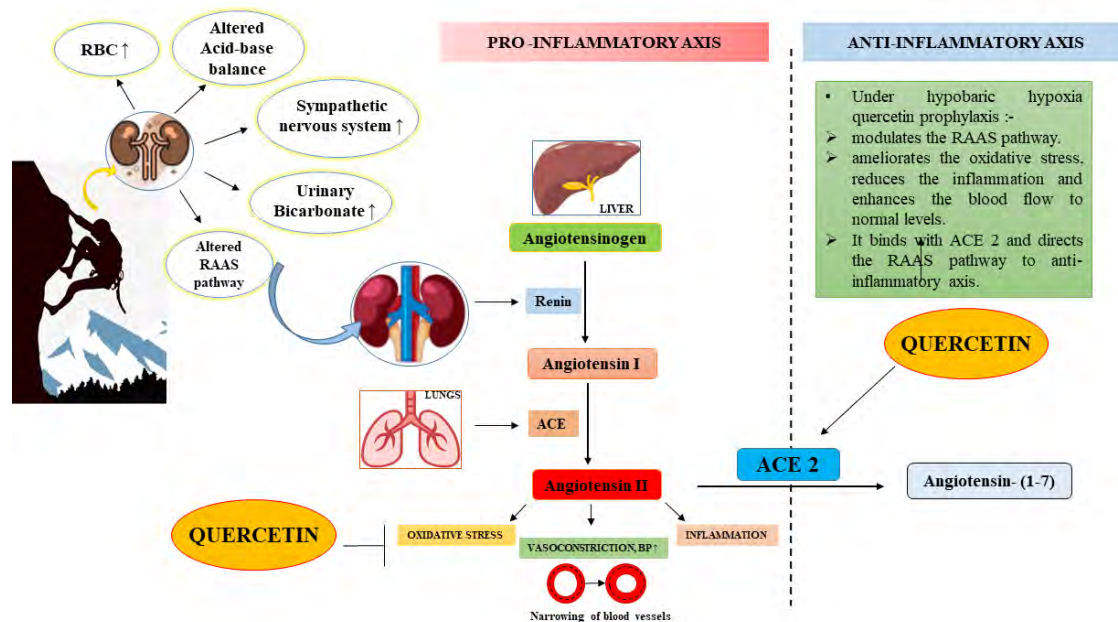
Aim: The study presented here aims at assessing the effects of hypobaric hypoxia on RAAS pathway and its components along with mitigation of anomalies with quercetin prophylaxis.

Methods: One hour prior to hypobaric hypoxia exposure, male SD rats ($n=6$) were orally supplemented with quercetin (50mg/kg BW) and acetazolamide (50mg/kg BW) and exposed them to 25,000 ft. in a simulated environmental chamber for 12 h at $25\pm 2^{\circ}\text{C}$. Different biochemical parameters like renin activity, aldosterone, angiotensin I, ACE 2 were determined in Plasma. Surface Plasmon Resonance Spectrometry was employed to analyze the binding efficiency of quercetin with ACE 2.

Results: As a conventional response to low oxygen conditions, oxidative stress parameters (ROS & MDA) were elevated along with suppressed antioxidant system (GPx & catalase) in plasma of rats. Quercetin prophylaxis significantly down regulated the hypoxia induced oxidative stress by reducing plasma ROS & MDA levels with efficient enhancement of antioxidants (GPx and Catalase). Further, hypoxia mediated regulation of renin and ACE 2 proves the outstanding efficacy of quercetin in repudiating altercations in RAAS cascade due to hypobaric hypoxia. Furthermore, differential protein expression of NFkB, IL-18 and endothelin-1 analyzed by western blotting approves the biochemical outcomes and showed that quercetin significantly aids in the reduction of inflammation under hypoxia. Studies conducted with Surface Plasmon Resonance demonstrated a binding among quercetin and ACE2 that indicates that this flavonoid might regulate RAAS pathway via ACE2.

Conclusion: The study promotes the prophylaxis of quercetin for the better adaptability under hypobaric hypoxic conditions via modulating the RAAS pathway.

Keywords: Renin; Angiotensin; Aldosterone; High altitude; Hypoxia; Oxidative stress



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PCB059

Modelling skeletal muscle ageing and repair in vitro

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AIM: One of the hallmarks of ageing muscle is the decreased ability to regenerate. This has been attributed to dysfunctional satellite cells that can result in reduced myogenic capacity. Although reduced myogenic capacity can impair muscle regeneration, other factors including restricted muscle repair programming are also at play (1). Muscle regeneration mirrors foetal development and thus, we hypothesise that the transcriptome (the full range of messenger RNA molecules expressed by an organism) involved in muscle development is affected by ageing, resulting in a diminished regeneration potential. The aim of this study was to develop a high-throughput *in vitro* model enabling cellular and molecular investigations of muscle regeneration across the life course.

Methods: Myotubes, differentiated from human myoblasts from an older donor (male, aged 68 years; from Promocell) and a younger donor (male, aged 20 years; from Lonza), were injured after exposure to 12% barium chloride. Myotube repair was assessed by morphological analysis of myotube fusion and width, cell cycle and the transcriptome. For morphological analysis, the myotubes were stained with phalloidin and DAPI (4',6-diamidino-2-phenylindole) to label the cytoskeleton and nuclei of muscle cells. This enabled us to estimate the fusion index and myotube width. The cell cycle was investigated using the EdU (5-ethynyl-2'-deoxyuridine) assay, which detects cells entering the S-phase (proliferative). In both morphological and proliferation assays, images were acquired with the Leica fluorescence microscope and analysed using ImageJ. Data were expressed as mean $\pm$ SEM. The transcriptome was assessed by RNA-seq. Timepoints for each analysis were pre-injury (control), post-injury, end of proliferation and end of differentiation (4 independent experiments). Statistical analyses of morphological and proliferation assays were performed using a two-sided unpaired t-test and RNA-seq using the DESeq2 R package (1.20.0).

Results: After repair, the fusion index ($p=0.04$) and myotube diameter ($p=0.0008$) were smaller in older myotubes compared to pre-injury (control). Younger myotubes exhibited a fusion index and width similar to their pre-injury state. With regards to the cell cycle, the number of EdU+ cells increased during the proliferation phase in myotubes derived from both young ($p=0.00003$) and aged ($p=0.0008$) myoblasts. Transcriptome analysis of older myotubes showed significant enrichment of the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, PI3K-Akt signalling, and of GO (Gene Ontology) biological processes associated with muscle development and the extracellular matrix. Many of the genes involved in muscle cell development were downregulated (Figure 1). In young myotubes, the most overrepresented KEGG pathways were cytokine receptor interaction and protein digestion and the most enriched GO biological process were extracellular matrix-related processes.

Conclusion: This model provides a high-throughput platform enabling cellular and molecular investigations of muscle regeneration across the life course. As expected, older myotubes showed impaired regeneration as evidenced by reduced myofusion index and width after repair. We postulate that this is due to the down-regulation of genes involved in muscle development and function.

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PCB060

Spectral decomposition of different pulse wave signals – a pilot study

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Pulse wave analysis (PWA) is commonly employed for the calculation of a multitude of physiological parameters related to cardiac pumping mechanics, arterial stiffness and peripheral vascular resistance. These parameters have been increasingly used in the last decades for the assessment of cardiovascular risk, which highlights their usefulness. In recent years new analytic strategies have focused on the spectral decomposition of pulse wave signals for the assessment of the dynamics of cardiac autonomic regulation, the best example being pulse rate variability (PRV) analysis. Spectral decomposition of pulse wave signals also provides insight into the mechanisms (central and local) regulating tissue perfusion through the assessment of the relative contribution of the different frequency intervals of the signals over time. Although there are striking differences between pulse wave signals from different anatomical regions, few studies have attempted to compare them on the basis of their frequency spectra. This study aimed to compare the frequency spectra of pulse wave signals obtained from the neck and fingertip regions and their respective underlying mechanisms. Ten young healthy subjects (23.4 ± 4.9 y.o.; 6 females, 4 males) participated in this study after giving informed consent. Pulse wave signals were obtained with photoplethysmography (PPG) sensors placed over a random common carotid artery and over the pulp of the second finger of the ipsilateral upper limb. PPG signals were recorded for 10 minutes while subjects were sitting upright and performing a simple postural modification – 5 min with both arms at heart level (phase I) and 5 min with one random arm placed 40 cm below heart level (phase II). The wavelet transform was used to decompose the raw PPG signals into their different frequency regions (high, low and very low frequency). The amplitude ratio of each frequency region was assessed over time and compared between phases of the protocol, as well as between signals. Nonparametric statistics were employed and a $p < 0.05$ was adopted. Significant differences in the amplitude ratio of the frequency intervals were identified between signals, highlighting their different physiological origin. Significant differences were also detected between the different phases, with the finger PPG signals showing more pronounced changes during the postural change when compared to the carotid signals. Although preliminary, our results show that the wavelet transform is a useful tool to provide a spectral decomposition of pulse wave signals from different anatomical regions. In addition, spectral analysis provides useful insights into the physiological origins of these signals.

PCB061

Suprasystolic limb occlusion and its impact on contralateral limb perfusion - an insight into flowmotion

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Suprasystolic limb occlusion (SLO) is a common challenge for assessing endothelial activity and cardiovascular risk. It is commonly carried out by inflating a pneumatic pressure cuff above systolic pressure for a certain period of time, during which perfusion in distal territories decreases significantly. Upon cuff release perfusion increases producing a well-known reactive hyperemia. Although it remains underexplored, the contralateral limb also responds to SLO. Previous results from our group suggest that this contralateral response is a sympathetically-driven perfusion decrease. This study aims to further expand the knowledge on the physiological response to SLO by exploring the physiological mechanisms underlying the contralateral vascular response. Ten healthy male subjects (mean 20.2 ± 2.3 y.o.) participated in this study after giving informed written consent. After acclimatization, subjects performed a standard SLO protocol on a random upper limb while sitting upright, as follows: 10 min resting with both arms at heart level (phase I), 5 min random arm occlusion (200 mmHg, phase II) and 10 min recovery in the initial position (phase III). Photoplethysmography (PPG) signals were acquired from the second finger of the occluded (test) and non-occluded (control) arms and then decomposed into their main frequency components (cardiac, respiratory, myogenic, sympathetic, endothelial) with the wavelet transform (WT). The electrodermal activity (EDA) was also acquired from the third and fourth fingers of both hands. Nonparametric statistics were used for comparing the activity of each frequency between phases and arms ($p < 0.05$). As previously reported, occlusion caused a significant decrease in cardiac, respiratory, myogenic and sympathetic activities together with a significant increase in NO-dependent and NO-independent endothelial activities in the test arm. The contralateral arm responded to occlusion with a significant decrease in perfusion, however no significant changes in the signal components. Nevertheless, the cardiac activity decreased, whereas the myogenic, sympathetic and endothelial NO-dependent increased. In contrast, the respiratory and endothelial NO-independent activities remained unchanged. Therefore, the perfusion decrease of the contralateral limb should be explained by the decrease of the cardiac and by the increase of the sympathetic activities. EDA increased significantly in both limbs during occlusion. These results show an overall agreement between EDA and the PPG sympathetic activity, reinforcing the usefulness of WT for assessing the mechanisms underlying perfusion regulation. They also highlight that only the non-occluded arm can be used for measuring the sympathetic nervous activity during SLO with decomposed PPG signals.

PCB062

Exploring the fractal behaviour of photoplethysmography perfusion signals

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The regulation of blood flow to any organ results from a complex regulation from several mechanisms, both central and local. Several mathematical tools have contributed to the better understanding the interaction between these mechanisms by exploring more detailed features of perfusion signals. Fractality is one of such features and can be defined as the self-similarity of a signal at different scales. Photoplethysmography (PPG) is a non-invasive and low-cost technology that allows the recording of skin/muscle perfusion over time. Previous publications have shown that PPG signals result from the contribution of the different physiological activities that affect perfusion (cardiac, respiration, myogenic, sympathetic, endothelial), each with its specific frequency interval. This study aimed at characterizing the fractal behaviour of PPG signals obtained during a suprasystolic limb occlusion (SLO) protocol. Ten healthy male subjects (mean 20.2 ± 2.3 y.o.) participated in this study after giving informed written consent. After acclimatization, subjects performed a standard SLO protocol on a random upper limb while sitting upright, as follows: 10 min resting with both arms at heart level (phase I), 5 min random arm occlusion (200 mmHg, phase II) and 10 min recovery in the initial position (phase III). PPG signals recorded from the index finger of both occluded and non-occluded limbs. These signals were first decomposed into their respective frequencies with the wavelet transform. Then, both the raw signal and the components were processed with a detrended fluctuation analysis (DFA) algorithm and the alpha exponent was calculated for each phase. The Wilcoxon signed rank test was used for comparing the alpha exponents between phases and the Mann-Whitney test for independent samples for limb comparisons ($p < 0.05$). The magnitude of alpha exponents increased with decreasing frequency of the PPG components. Furthermore, occlusion significantly changed the alpha exponents of the PPG signal and several of its components from the occluded limb. These results show that PPG perfusion signals exhibit fractal behaviour and that the DFA-derived alpha exponent could serve as new descriptor of perfusion regulation phenomena.

PCB063

Lifelong exposure to high-altitude hypoxia in humans is associated with improved redox homeostasis and structural-functional adaptations of the neurovascular unit

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Background: The neurovascular unit (NVU) is a functionally integrated cellular network responsible for maintaining structural integrity of the blood-brain barrier (BBB) and regulation of cerebral blood flow (CBF) via neurovascular coupling (NVC). Upon exposure to high-altitude (HA) in lowlanders born and bred at sea-level (SL), BBB integrity may become compromised due to autoregulatory breakthrough subsequent to local elevations in oxidative-nitrosative stress (OXNOS) reflected by a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability (Bailey *et al.*, 2009a; 2009b). Furthermore, MRI evidence of extracellular (vasogenic) edematous brain swelling (Kallenberg *et al.*, 2007) combined with hemosiderin deposits (Kallenberg *et al.*, 2008) implying erythrocyte extravasation, have been collectively interpreted to reflect BBB disruption (Bailey *et al.*, 2009a), predisposing to impaired cerebral bioenergetic function and cognitive decline (Bailey *et al.*, 2019). The aim of the present study was to determine how the hypoxia of HA across the temporal continuum of chronic through to lifelong exposure impacts the NVU phenotype and to what extent this is subject to altered redox homeostasis.

Methods: Nine male lowlanders were examined at SL (~344m) and after 14-days acclimatisation to 4,300 m (chronic HA) in Cerro de Pasco (CdP), Péru, alongside nine sex, age and body mass index-matched healthy highlanders native to CdP (lifelong-HA). Venous blood was assayed for serum proteins (S100B, neuron specific enolase [NSE], glial fibrillary acidic protein [GFAP], neurofilament light-chain [NFL], ubiquitin carboxy-terminal hydrolase-L1 [UCHL-1] and Total-tau [T-Tau]), reflecting NVU integrity via automated high-sensitivity clinical grade ELISA and single molecule array (Simoa) technology. Free radicals and NO were determined

using electron paramagnetic resonance spectroscopy and ozone-based chemiluminescence, respectively. Regional cerebral blood flow (CBF) was examined in conjunction with cerebral substrate delivery, dynamic cerebral autoregulation (dCA, transfer function analysis of spontaneous oscillations of middle/posterior cerebral artery blood velocity [MCA_v/PCA_v] and mean arterial blood pressure [MAP]), cerebrovascular reactivity to carbon dioxide (CVR_{CO2}, +9 mmHg end-tidal partial pressure of carbon dioxide) and NVC (PCA_v responses to visual stimulation) using Transcranial doppler (MCA_v/PCA_v) and Duplex ultrasound (internal carotid/vertebral artery blood flow: ICA_Q/VA_Q). Global cerebral blood flow (gCBF) was calculated as (ICA_Q + VA_Q) × 2, and substrate (oxygen/glucose) delivery as: gCBF × arterial oxygen content/glucose. Psychomotor tests and the Montreal Cognitive Assessment (MoCA) were employed to examine cognitive function.

Results: Compared to lowlanders at SL, highlanders exhibited elevated basal plasma and red blood cell NO bioavailability ($P = 0.003$ and $P = 0.026$, respectively), improved anterior and posterior dCA (↓MCA and PCA LF Gain, $P = 0.029$ and $P = 0.017$, respectively), elevated anterior CVR_{CO2} (↑MCA and ICA CVR_{CO2}, $P = 0.036$ and $P = 0.042$, respectively), preserved cerebral substrate delivery and NVC (all $P = >0.050$). In highlanders, S100B, NFL and T-tau were consistently lower ($P = 0.018$, $P = 0.037$ and $P = <0.001$, respectively) and cognition comparable all ($P = >0.050$) to lowlanders following chronic-HA.

Conclusions: These findings highlight novel integrated adaptations towards regulation of the NVU in highlanders that may represent a neuroprotective phenotype underpinning successful adaptation to the lifelong stress of HA hypoxia.

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PCB064

The effect of hypoxia on physiological and behavioural outcomes during simulated driving in healthy subjects

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Hypoxia is a common condition mainly caused by neurological, cardiac and respiratory disease. Hypoxia is known to affect cognition and driving is dependent on satisfactory cognitive ability. Currently those who are prescribed supplemental oxygen have not been given any guidance from regulatory bodies on whether to use supplemental oxygen while driving. There is very limited research on the possible effects that hypoxia may have on persons whilst driving. This study investigates whether hypoxia influences driving behaviours in healthy subjects using simulated driving. Breathing frequency, oxygen saturation (SpO₂), heart rate variability (HRV) and subjective comments were also collated.

52 healthy subjects participated in this study with written informed consent. The study was approved by the Science & Engineering Ethical Committee, University of Plymouth and procedures were in accordance with the Declaration of Helsinki. Inclusion criteria were ≥18 years old and no history of cardiorespiratory or chronic disease. All subjects had their anthropometric and resting blood pressure, heart rate and SpO₂ data collected. Then they were attached to ECG electrodes, a chest plethysmograph, and an oximeter before they started the simulated driving on an Xbox 360 game console using Forza Horizon 4 software. Each subject had four driving sessions; a 10-minute practice and three randomised interventions: 20-minute normoxic room air (FIO₂ 21%), 20-minute normoxic medical air (FIO₂ 21%) and 20-minute hypoxic air (equal to 15% FIO₂). Driving behaviours (DB) were assessed by the sum of positive and negative scores for each session. HRV and breathing frequency were collected by using LabChart software. Short term HRV was assessed using time domain (heart rate - HR, standard deviation of the RR interval - SDRR), frequency domain (low and high frequency - LF and HF) and Poincaré analysis (SD1 and SD2). The results were statistically analysed in SPSS by repeated measures ANOVA. $p < 0.05$ was considered as significant.

HR ($p < 0.0001$), SDRR ($p = 0.03$), SD1 ($p < 0.0001$), breathing rate ($p = 0.01$) and SpO₂ ($p < 0.0001$) were all significantly different over the three gas interventions ($n = 52$). LF, HF, DB all showed no significant difference. Pairwise comparisons showed that during hypoxia HR increased, while SDRR, SD1, breathing rate and SpO₂ were lower, when compared to both normoxic interventions.

The main finding of our study was that hypoxia did not significantly affect simulated driving behaviours in our subjects. Therefore, we believe that the level of hypoxia (FiO₂ 15%) used in

the present study, may not have a great impact during driving. These findings add important significance for legislators and policy makers when making decisions with regard to the road safety of hypoxic patients who drive. Interestingly, HRV was negatively affected by hypoxia whilst driving and provides a starting point for conducting further research on the impact hypoxia may have on driving performance for patients with cardiovascular disease.

PCB065

Moving calculations into clinic - application of a flow volume loop analysis model in respiratory diagnostics

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Introduction

Numerical spirometry measurements have been used to define abnormality for some time in clinical respiratory physiology. Analysis of the flow volume loop allows unique patterns to be identified but is less established outside of respiratory medicine. The purpose of this pilot study was to assess the performance of a graphical data model in a real world scenario.

Method

A Flow volume Analysis Model (FAM) was constructed including a 100 point model of analysis, area under the flow volume curve and pattern of flow decline after peak flow. The FAM was run on 50 examples. Cases compatible with various abnormalities (detailed in Table 1) were also scored by a Human Panel (HP). The HP was made of various roles including consultant physicians, registrars, junior doctors, physiologists, physiotherapists, and physician associates (n=22, x experience: 8 years, range: 2-31 years). For the purposes of creating a predicted curve, normal limits were taken from GLI dataset for Volume (FVC) and from ECSC for maximal flows. Ease of scoring and perception of automated spirometry analysis was assessed with a questionnaire using a 0-10 score.

Results

The most prevalent mistake in the HP cases involved classification of mild and moderate obstruction. The most common error in the FAM was scoring UAO as severe obstruction. Perceived ease of scoring task was inversely related to experience with a wide range. Support of automatic FVL analysis implementation in primary care and secondary care was neutral. The use of automated analysis to assist human interpretation was positively supported.

Conclusion

Both FAM and HP were relatively successful at identifying examples of normal shaped FVL. The FAM outscored the HP in most cases. More modelling data is required to improve automatic scoring of UAO and severe obstruction.

Table 1. Percentage of cases correctly classified by human and model flow volume loop scoring

	Abnormality evident on Flow Volume Loop (FVL)									
	'Normal'			'Restriction'			'Obstruction'			
	A	B	C	D	E	F	G	H	I	J
Human:	96	96	82	77	77	96	68	82	68	91
Model:	100	100	100	100	80	100	100	80	80	60

Key: **A:** Preserved FVL with normal FVC. **B:** Preserved FVL with supra-normal FVC. **C:** Preserved FVL with reduced FVC. **D:** Mild obstruction on FVL with reduced FVC. **E:** Severe obstruction on FVL with reduced FVC. **F:** Mild obstruction on FVL with normal FVC. **G:** Moderate obstruction on FVL with normal FVC. **H:** Severe obstruction on FVL with normal FVC. **I:** Moderate obstruction on FVL with supra-normal FVC. **J:** Upper Airway Obstruction (UAO).

PCB066

Identification of a miRNA Signature as a Diagnostic and Prognostic Marker in Clear Cell Renal Cell Carcinoma

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Clear cell renal cell carcinoma (ccRCC) is the most common type of renal cell carcinoma and is associated with high morbidity and poor prognosis. Micro-RNAs (miRNAs) have emerged as promising biomarkers for cancer diagnosis and prognosis due to their involvement in cancer progression and development (Ghafouri-Fard et al., 2020). The integration of big omics data from GEO and TCGA, along with data mining and machine learning, has revolutionized the identification of reliable diagnostic and prognostic signatures for various types of cancer. The present study aims to identify a diagnostic and prognostic signature for ccRCC using miRNA data from microarray and NGS experiments in GEO and TCGA.

Differentially expressed miRNAs (DEmiRs) in ccRCC samples compared to normal renal tissue were identified using GEO2R packages in R, with adjusted $P < 0.05$ and $\log_2FC > 1.5$ as cutoff criteria. The overlapping DEmiRs were identified, and the target genes of these DEmiRs with strong experimental validation were obtained using miRTargetLink 2.0 (Kern et al., 2021), and the pathway enrichment analysis was performed using ClusterProfiler package in R with KEGG annotation database (Wu et al., 2021). Kaplan-Maier (KM) survival analysis was performed to correlate the survival of patients with higher or lower expression of the identified miRNAs (Lánczky & Györfy, 2021). A support vector machine model was trained and cross-validated to classify tumor samples from matched solid normal tissue samples.

Six datasets, namely GSE11016, GSE12105, GSE47582, GSE73342, GSE151423, and a dataset from TCGA were chosen for the analysis. Results revealed that 14 DEmiRs were consistently differentially expressed in RCC tissues in the microarray datasets, and 26 DEmiRs in the NGS datasets. We identified 9 mRNAs that exhibited a consistent expression trend across all datasets included in the study. We identified 637 genes as targets of the miRNAs under investigation. Pathway enrichment analysis demonstrated that these target genes were significantly enriched in several crucial pathways, including but not limited to AGE-RAGE signaling, MAPK signaling, cellular senescence, toll-like receptor signaling, TNF signaling, PD-L1 expression, and PD-1 checkpoint pathways. Survival analysis revealed that among the 9 signature miRNAs, higher expression of 4 and lower expression of 5 miRNAs were significantly associated with poor survival. Based on these findings, we hypothesize that these identified key miRNAs have the potential to serve as prognostic biomarkers for patients with ccRCC. Using the nine miRNAs identified earlier as features, we trained a support vector machine model on the TCGA dataset. The results of the 10-fold cross-validation demonstrated a high accuracy of $99.23 \pm 0.89\%$ and an AUC of 0.99 ± 0.007 . These findings suggest that the model can accurately and reliably classify tumor samples from normal solid tissue samples.

In summary, this study has identified a nine-miRNA signature that is associated with poor survival outcomes in patients with ccRCC. Moreover, our machine learning model, based on this signature, is capable of distinguishing between tumors and normal tissue samples. Further

validation of this model in a clinical cohort would aid in translating our findings into clinical practice, potentially leading to earlier detection and improved follow-up care for ccRCC patients.

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PCB067

Effect of obesity on cognitive functions in school children

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Background: The prevalence of overweight and obesity in school children between 10-18 years old in Khartoum state in Sudan in 2010, was 10.8% and 9.7% respectively [1]. Obesity is a major risk factor of many health problems. Recently, researchers suggested that increased adiposity is associated with poor cognitive performance, independently of associated medical conditions [2]. This study aims to investigate the effect of obesity on cognitive function in primary school children.

Methods: This is a descriptive, cross sectional study that included 290 primary school children (150 boys and 140 girls) between 9 and 14 years old. Participants were chosen for the study using stratified multistage random sample technique from two of the biggest primary public schools in Omdurman city. Brief medical history was taken and general examination was done to exclude any abnormalities. Blood pressure (BP), weight and height were measured and body mass index (BMI) was calculated. Students were classified according to WHO BMI chart percentiles 2007 into: underweight (< 3rd), normal weight ($\geq 3^{rd}$ -< 85th), over weight ($\geq 85^{th}$ - < 97th), and obese ($\geq 97^{th}$). Cognitive function was assessed using Mini-Mental State Examination Test (MMSE) [3].

Results: Results of the study showed that 15.51% (n= 45) of students were obese and 17.93% (n= 52) were overweight. The mean BMI was 18.95 ± 4.68 kg/m². BMI showed insignificant difference between the three socioeconomic status (P=0.538). Mild cognitive impairment was detected in 9.3% (n=27) of students, 0.7% (n=2) has moderate cognitive impairment. Only 2 out of the 45 (4.4%) obese students had mild cognitive impairment, and one (2.2%) had moderate cognitive impairment. The association between cognitive impairment and BMI was insignificant (P=0.098). Mother's education showed a significant positive association with language and praxis (P=0.027). Whereas father's educational level had correlated positively with orientation (P =0.001) and with MMSE test results (P =0.037).

Conclusion: Obesity seems to have insignificant effect on cognitive function in obese school children. Parent's educational background has a major effect on their children's cognition.

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PCB068

Plasmodium berghei infection associated with adverse birth outcomes in pregnant Swiss albino mice

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Introduction: Malaria in pregnancy has been seen to cause poor pregnancy and foetal outcomes.

Aims/Objective: In this study, mice infected with *Plasmodium berghei* (*P. berghei*) during the second and third stages of pregnancy were examined for their pregnancy's outcome and changes in their blood's biochemical composition after delivery. Additionally, the physical and behavioural reactions of the mice's pups were also investigated.

Methods: Thirty pregnant female Swiss Albino mice were randomly divided into three groups; two received intraperitoneal injections of 10^6 *P. berghei*-infected red blood cells on gestational days (GD12 and 17), while the third group was left uninfected (control). The study was conducted with the approval of the department of pure and applied Zoology, Federal University of Agriculture Abeokuta, Nigeria. This study also followed the national institute of health guide for using and caring for laboratory research animals (NIH publication 8023, revised 1978). Data were reported as means and standard errors, and analysis of variance was used to determine a significant difference from the control group at $p < 0.05$.

Results: Pregnancy termination occurred in 20% of mice infected during GD12, whereas mortality before parturition occurred in 40% and 30% of mice infected during GD12 and GD17, respectively. Non-infected group's total protein and glucose concentrations were significantly higher ($p < 0.05$), while cholesterol and triglyceride concentrations were significantly lower ($p < 0.05$) when compared to the infected groups. The Mean birth weights (1.82 ± 0.37 g) of pups were higher ($p < 0.05$) in control mice compared to pups from infected groups. Offspring born to infected mothers exhibited poor physical and behavioural responses.

Conclusion: Mice infection by *P. berghei* during pregnancy resulted in adverse birth outcomes, altered measured biochemical parameters, poor physical and behavioural responses in their offspring and was more severe during the second stage of pregnancy.

PCB069

High-density lipoprotein protein composition differs between white Europeans and South Asians but is not related to its anti-inflammatory function

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Aims

Insulin resistance is known to alter high-density lipoprotein (HDL) composition and function (1). South Asians in the United Kingdom develop type 2 diabetes mellitus younger and at a leaner BMI than white Europeans(2). Our aim was to compare HDL composition and function with weight gain in young healthy white European (EU) and South Asian (SA) men.

Methods

White Europeans (n = 21) and South Asians (n = 14) were recruited with ethical approval from the University of Glasgow and NHS Greater Glasgow and Clyde according to the Declaration of Helsinki. These participants comprised the Glasgow Visceral & Ectopic Fat with Weight Gain in South Asians study (ClinicalTrials.gov Identifier: NCT02399423). Biochemical and anthropometric measures were taken at baseline and after ~7% weight gain over 6 weeks. Body fat distribution was measured by MRI. HDL was isolated from fasted plasma samples using sodium bromide sequential density ultracentrifugation. HDL apolipoprotein AI (ApoAI) was measured by ELISA. HDL cholesterol content was measured by commercial assay. HDL protein was measured by Bradford assay. HDL proteomics was performed by nano-liquid chromatography tandem mass spectrometry and protein levels expressed as a label free quantitation (LFQ) intensity. HDL anti-inflammatory function was assessed by measuring percentage inhibition of TNF α stimulated vascular cell adhesion molecule-1 expression in human microvascular endothelial cells. HDL was dosed onto cells at a concentration of 300 μ g/mL apolipoprotein AI. Data was analysed using mixed effects models followed by post-hoc Tukey test. Statistical significance was considered at $p < 0.05$ and for interaction effects $p < 0.15$.

Results

HDL ApoAI, cholesterol and protein content were unchanged by ethnicity or weight gain. Of 50 proteins identified on HDL, 12 were higher in South Asians irrespective of weight gain, including apolipoprotein A-IV (EU, LFQ 2.22×10^7 [1.49×10^7 , 3.53×10^7] SA, 3.63×10^7 , [2.60×10^7 , 4.94×10^7], $p = 0.011$, Median [Q1, Q3]). Five proteins had interaction effects where South Asians and white Europeans responded differently to weight gain, including apolipoprotein C-III, apolipoprotein D, apolipoprotein F and cholesteryl ester transfer protein (CETP) (Table 1). The change in apolipoprotein C-III LFQ intensity post weight gain negatively correlated with the change in liver fat fraction after weight gain (Spearman $r -0.42$, $p = 0.023$, Figure 1). HDL anti-

inflammatory function did not differ between white Europeans and South Asians or post weight gain.

Conclusion

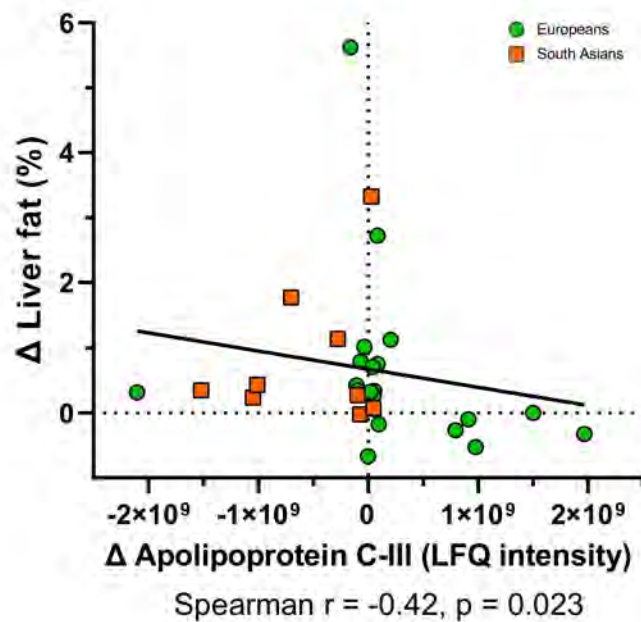
South Asians have a markedly different HDL protein composition to white Europeans which responds differently to weight gain. However, this did not affect HDL anti-inflammatory function. HDL protein composition may therefore act as a biomarker of systemic pathophysiology, in this case impaired lipid metabolism with weight gain in South Asians. In this light, these findings suggest CETP inhibition and reduced triglyceride transfer to HDL, and increased lipase activity in South Asians. This may favour triglyceride transfer down the lipolytic pathway and into adipose tissue. The association between the change in HDL apolipoprotein C-III and liver fat fraction after weight gain offers a potential mechanism of ectopic lipid overspill in South Asians (3). This may contribute to ethnic differences in type 2 diabetes mellitus aetiology.

	Baseline		Weight gain		p value		
	EU (n = 21)	SA (n = 11)	EU (n = 21)	SA (n = 13)	Ethnicity	Visit	Interaction
Alpha-1B-glycoprotein	8.16x10 ⁵ [0, 1.57x10 ⁶]	1.44x10 ⁶ [1.09x10 ⁶ , 2.91x10 ⁶]	6.47x10 ⁵ [0, 1.96x10 ⁶]	1.49x10 ⁶ [1.08x10 ⁶ , 2.38x10 ⁶]	0.027	0.42	0.63
Apolipoprotein A-IV	1.97x10 ⁷ [1.35x10 ⁷ , 3.20x10 ⁷]	3.41x10 ⁷ [2.41x10 ⁷ , 5.02x10 ⁷]	2.28x10 ⁷ [1.39x10 ⁷ , 4.06x10 ⁷]	3.85x10 ⁷ [2.63x10 ⁷ , 4.85x10 ⁷]	0.011	0.90	0.43
Apolipoprotein C-III	9.14x10 ⁷ [3.81x10 ⁷ , 1.96x10 ⁸]	2.92x10 ⁸ [7.89x10 ⁷ , 1.24x10 ⁹]	1.29x10 ⁸ [6.15x10 ⁷ , 7.82x10 ⁸]	8.15x10 ⁷ [2.59x10 ⁷ , 2.01x10 ⁸]	0.85	0.68	0.01
Apolipoprotein D	1.05x10 ⁸ [6.00x10 ⁷ , 2.54x10 ⁸]	1.14x10 ⁸ [9.03x10 ⁷ , 1.59x10 ⁸]	8.58x10 ⁷ [3.88x10 ⁷ , 2.17x10 ⁸]	1.87x10 ⁸ [1.46x10 ⁸ , 2.65x10 ⁸]	0.42	0.33	0.077
Apolipoprotein F	5.19x10 ⁶ [1.17x10 ⁶ , 1.22x10 ⁷]	0 [0, 5.73x10 ⁶]	3.31x10 ⁶ [0, 7.16x10 ⁶]	4.96x10 ⁶ [0, 6.80x10 ⁶]	0.16	0.071	0.033
Carbonic anhydrase	3.58x10 ⁵ [0, 6.76x10 ⁵]	3.09x10 ⁵ [0, 1.62x10 ⁶]	1.10x10 ⁵ [0, 5.17x10 ⁵]	6.68x10 ⁵ [1.54x10 ⁵ , 1.79x10 ⁶]	0.025	0.84	0.65
Cholesteryl ester transfer protein	0 [0, 6.75x10 ⁵]	2.93x10 ⁵ [0, 5.25x10 ⁵]	0 [0, 5.83x10 ⁵]	4.99x10 ⁵ [1.78x10 ⁵ , 5.96x10 ⁵]	0.45	0.68	0.12
Complement C3	9.46x10 ⁵ [0, 1.48x10 ⁶]	1.17x10 ⁶ [7.43x10 ⁵ , 1.57x10 ⁶]	1.18x10 ⁶ [0, 1.91x10 ⁶]	1.39x10 ⁶ [9.30x10 ⁵ , 2.34x10 ⁶]	0.038	0.29	0.44
Complement C4-B	1.25x10 ⁶ [5.44x10 ⁵ , 2.14x10 ⁶]	1.97x10 ⁶ [1.32x10 ⁶ , 2.61x10 ⁶]	8.58x10 ⁵ [0, 2.10x10 ⁶]	2.91x10 ⁶ [1.32x10 ⁶ , 3.64x10 ⁶]	0.040	0.23	0.048
Gelsolin	0 [0, 7.93x10 ⁵]	8.08x10 ⁵ [0, 1.66x10 ⁶]	4.95x10 ⁵ [0, 1.28x10 ⁶]	8.81x10 ⁵ [4.07x10 ⁵ , 1.29x10 ⁶]	0.043	0.46	0.43
Hemopexin	0 [0, 1.30x10 ⁶]	9.35x10 ⁵ [6.47x10 ⁵ , 2.97x10 ⁶]	0 [0, 2.10x10 ⁶]	2.50x10 ⁶ [1.63x10 ⁶ , 2.83x10 ⁶]	0.004	0.32	0.84
Ig-kappa chain C region	1.33x10 ⁶ [3.94x10 ⁵ , 2.78x10 ⁶]	1.88x10 ⁶ [1.11x10 ⁶ , 3.02x10 ⁶]	9.60x10 ⁵ [0, 2.23x10 ⁶]	2.27x10 ⁶ [1.41x10 ⁶ , 3.66x10 ⁶]	0.049	0.83	0.59
Pigment epithelium derived factor	3.16x10 ⁶ [1.99x10 ⁶ , 4.78x10 ⁶]	4.03x10 ⁶ [2.38x10 ⁶ , 6.32x10 ⁶]	3.90x10 ⁶ [1.76x10 ⁶ , 5.25x10 ⁶]	2.98x10 ⁶ [2.3x10 ⁶ , 5.21x10 ⁶]	0.20	0.52	0.076
Protein AMBP	1.58x10 ⁶ [8.05x10 ⁵ , 2.18x10 ⁶]	1.56x10 ⁶ [1.31x10 ⁶ , 3.68x10 ⁶]	1.67x10 ⁶ [5.99x10 ⁵ , 2.57x10 ⁶]	3.00x10 ⁶ [1.98x10 ⁶ , 3.5x10 ⁶]	0.023	0.32	0.64
Retinol-binding protein-4	1.10x10 ⁶ [0, 1.72x10 ⁶]	2.65x10 ⁶ [5.53x10 ⁵ , 5.93x10 ⁶]	1.08x10 ⁶ [0, 2.96x10 ⁶]	2.27x10 ⁶ [1.66x10 ⁶ , 3.77x10 ⁶]	0.048	0.67	0.73
Vitronectin	9.09x10 ⁵ [0, 1.26x10 ⁶]	1.35x10 ⁶ [8.60x10 ⁵ , 1.95x10 ⁶]	1.09x10 ⁶ [3.22x10 ⁵ , 1.34x10 ⁶]	1.36x10 ⁶ [8.80x10 ⁵ , 1.65x10 ⁶]	0.039	0.53	0.58
Zinc-alpha-2-glycoprotein	2.61x10 ⁶ [7.88x10 ⁵ , 3.30x10 ⁶]	3.84x10 ⁶ [2.45x10 ⁶ , 5.00x10 ⁶]	3.17x10 ⁶ [8.23x10 ⁵ , 4.17x10 ⁶]	3.41x10 ⁶ [2.76x10 ⁶ , 3.88x10 ⁶]	0.041	0.91	0.22

Table-1: Proteomic differences between white European and South Asian HDL at baseline and weight gain. Data expressed as median LFQ intensity [Q1, Q3]. Mixed effects models followed by post-hoc Tukey test. Statistical significance was considered at p < 0.05 and for interaction effect p < 0.15.

	Baseline		Weight gain		p value		
	EU (n = 21)	SA (n = 11)	EU (n = 21)	SA (n = 13)	Ethnicity	Visit	Interaction
Alpha-1B-glycoprotein	8.16x10 ⁵ [0, 1.57x10 ⁶]	1.44x10 ⁶ [1.09x10 ⁶ , 2.91x10 ⁶]	6.47x10 ⁵ [0, 1.96x10 ⁶]	1.49x10 ⁶ [1.08x10 ⁶ , 2.38x10 ⁶]	0.027	0.42	0.63
Apolipoprotein A-IV	1.97x10 ⁷ [1.35x10 ⁷ , 3.20x10 ⁷]	3.41x10 ⁷ [2.41x10 ⁷ , 5.02x10 ⁷]	2.28x10 ⁷ [1.39x10 ⁷ , 4.06x10 ⁷]	3.85x10 ⁷ [2.63x10 ⁷ , 4.85x10 ⁷]	0.011	0.90	0.43
Apolipoprotein C-III	9.14x10 ⁷ [3.81x10 ⁷ , 1.96x10 ⁸]	2.92x10 ⁸ [7.89x10 ⁷ , 1.24x10 ⁹]	1.29x10 ⁸ [6.15x10 ⁷ , 7.82x10 ⁸]	8.15x10 ⁷ [2.59x10 ⁷ , 2.01x10 ⁸]	0.85	0.68	0.01
Apolipoprotein D	1.05x10 ⁸ [6.00x10 ⁷ , 2.54x10 ⁸]	1.14x10 ⁸ [9.03x10 ⁷ , 1.59x10 ⁸]	8.58x10 ⁷ [3.88x10 ⁷ , 2.17x10 ⁸]	1.87x10 ⁸ [1.46x10 ⁸ , 2.65x10 ⁸]	0.42	0.33	0.077
Apolipoprotein F	5.19x10 ⁶ [1.17x10 ⁶ , 1.22x10 ⁷]	0 [0, 5.73x10 ⁶]	3.31x10 ⁶ [0, 7.16x10 ⁶]	4.96x10 ⁶ [0, 6.80x10 ⁶]	0.16	0.071	0.033
Carbonic anhydrase	3.58x10 ⁵ [0, 6.76x10 ⁵]	3.09x10 ⁵ [0, 1.62x10 ⁶]	1.10x10 ⁵ [0, 5.17x10 ⁵]	6.68x10 ⁵ [1.54x10 ⁵ , 1.79x10 ⁶]	0.025	0.84	0.65
Cholesteryl ester transfer protein	0 [0, 6.75x10 ⁵]	2.93x10 ⁵ [0, 5.25x10 ⁵]	0 [0, 5.83x10 ⁵]	4.99x10 ⁵ [1.78x10 ⁵ , 5.96x10 ⁵]	0.45	0.68	0.12
Complement C3	9.46x10 ⁵ [0, 1.48x10 ⁶]	1.17x10 ⁶ [7.43x10 ⁵ , 1.57x10 ⁶]	1.18x10 ⁶ [0, 1.91x10 ⁶]	1.39x10 ⁶ [9.30x10 ⁵ , 2.34x10 ⁶]	0.038	0.29	0.44
Complement C4-B	1.25x10 ⁶ [5.44x10 ⁵ , 2.14x10 ⁶]	1.97x10 ⁶ [1.32x10 ⁶ , 2.61x10 ⁶]	8.58x10 ⁵ [0, 2.10x10 ⁶]	2.91x10 ⁶ [1.32x10 ⁶ , 3.64x10 ⁶]	0.040	0.23	0.048
Gelsolin	0 [0, 7.93x10 ⁵]	8.08x10 ⁵ [0, 1.66x10 ⁶]	4.95x10 ⁵ [0, 1.28x10 ⁶]	8.81x10 ⁵ [4.07x10 ⁵ , 1.29x10 ⁶]	0.043	0.46	0.43
Hemopexin	0 [0, 1.30x10 ⁶]	9.35x10 ⁵ [6.47x10 ⁵ , 2.97x10 ⁶]	0 [0, 2.10x10 ⁶]	2.50x10 ⁶ [1.63x10 ⁶ , 2.83x10 ⁶]	0.004	0.32	0.84
Ig-kappa chain C region	1.33x10 ⁶ [3.94x10 ⁵ , 2.78x10 ⁶]	1.88x10 ⁶ [1.11x10 ⁶ , 3.02x10 ⁶]	9.60x10 ⁵ [0, 2.23x10 ⁶]	2.27x10 ⁶ [1.41x10 ⁶ , 3.66x10 ⁶]	0.049	0.83	0.59
Pigment epithelium derived factor	3.16x10 ⁶ [1.99x10 ⁶ , 4.78x10 ⁶]	4.03x10 ⁶ [2.38x10 ⁶ , 6.32x10 ⁶]	3.90x10 ⁶ [1.76x10 ⁶ , 5.25x10 ⁶]	2.98x10 ⁶ [2.3x10 ⁶ , 5.21x10 ⁶]	0.20	0.52	0.076
Protein AMBP	1.58x10 ⁶ [8.05x10 ⁵ , 2.18x10 ⁶]	1.56x10 ⁶ [1.31x10 ⁶ , 3.68x10 ⁶]	1.67x10 ⁶ [5.99x10 ⁵ , 2.57x10 ⁶]	3.00x10 ⁶ [1.98x10 ⁶ , 3.5x10 ⁶]	0.023	0.32	0.64
Retinol-binding protein-4	1.10x10 ⁶ [0, 1.72x10 ⁶]	2.65x10 ⁶ [5.53x10 ⁵ , 5.93x10 ⁶]	1.08x10 ⁶ [0, 2.96x10 ⁶]	2.27x10 ⁶ [1.66x10 ⁶ , 3.77x10 ⁶]	0.048	0.67	0.73
Vitronectin	9.09x10 ⁵ [0, 1.26x10 ⁶]	1.35x10 ⁶ [8.60x10 ⁵ , 1.95x10 ⁶]	1.09x10 ⁶ [3.22x10 ⁵ , 1.34x10 ⁶]	1.36x10 ⁶ [8.80x10 ⁵ , 1.65x10 ⁶]	0.039	0.53	0.58
Zinc-alpha-2-glycoprotein	2.61x10 ⁶ [7.88x10 ⁵ , 3.30x10 ⁶]	3.84x10 ⁶ [2.45x10 ⁶ , 5.00x10 ⁶]	3.17x10 ⁶ [8.23x10 ⁵ , 4.17x10 ⁶]	3.41x10 ⁶ [2.76x10 ⁶ , 3.88x10 ⁶]	0.041	0.91	0.22

Table-1: Proteomic differences between white European and South Asian HDL at baseline and weight gain. Data expressed as median LFQ intensity [Q1, Q3]. Mixed effects models followed by post-hoc Tukey test. Statistical significance was considered at p < 0.05 and for interaction effect p < 0.15.



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PCB070

Activation of pancreatic stellate cells evokes signalling and metabolic changes promoting cellular resilience

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Activated pancreatic stellate cells (PSCs) are involved in the excessive deposition of extracellular matrix (ECM) proteins resulting in the development of fibrosis in alcoholic pancreatitis or fibrotic stroma in pancreatic tumours [1]. In response to tissue injury, quiescent fibroblast-like PSCs undergo activation, that is assume a myofibroblast-like phenotype characterised by upregulation of α -SMA expression, increased contractile capacity and production of ECM components [2]. This phenotype transition significantly affects the physiology of PSCs, including changes in cell signalling and metabolism, which may have profound implications in diseases of the pancreas.

To investigate this, we used quiescent and TGF- β -activated (for 48 h or 7 days) human PSCs, in which we compared pathophysiological Ca^{2+} signals, mitochondrial potential and cell death in response to ethanol (EtOH) and palmitoleic acid (POA) – the major inducers of alcoholic pancreatitis. We also measured mitochondrial parameters using the Seahorse Cell Mito Stress Test and compared the expression of Ca^{2+} channels and pumps between the quiescent and activated phenotypes.

Our data show that, compared to quiescent PSCs, activated cells differ significantly, in terms of Ca^{2+} signalling and metabolic activity. Activated PSCs are much less prone to EtOH/POA-induced cytosolic Ca^{2+} overload and cell death, predominantly due to downregulation of the TRPA1 channel (a decrease to 17.4% and 23.2% in PSCs 48 h and 7 days post-activation, respectively) [3]. In quiescent PSCs, inhibition or silencing of TRPA1 expression reduces cytosolic Ca^{2+} responses to 50 mM EtOH / 50 μ M POA (from 4342.5 ± 486.9 a.u. to 1508.0 ± 205.4 a.u., $p=0.0051$) and protects these cells from cell death (a decrease of cell death from 70.5% to 19.8%, $p=0.0006$), mimicking the activated phenotype. In addition, activated PSCs had their basal respiration, ATP production and spare respiratory capacity increased (by approx. 1.6x, 1.4x and 12-16x respectively), compared to quiescent cells. EtOH/POA disrupted the mitochondrial potential in quiescent PSCs (an average decrease of 231.9 ± 15.8 a.u. below baseline levels), but this effect was inhibited in cells 7 days post-activation (30.1 ± 8.0 au, $p<0.0001$). Activated PSCs were also less sensitive to menadione-induced oxidative stress compared to quiescent cells.

Our results reveal significant changes in Ca^{2+} signalling machinery and the condition of mitochondria between quiescent and activated PSCs. These changes are directly responsible for the increased resilience of activated PSCs to noxious signals, which likely allows them to divide and deposit collagen and other components of the ECM even under harsh pathophysiological conditions such as ongoing inflammation. Better understanding of the

activation-induced alterations in the cellular physiology of PSCs provides new insights into the mechanisms of pancreatic disorders, particularly those associated with fibrosis.

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PCB071

Neurogenic activation of lipolysis in white adipose tissue ex vivo

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Motivation White adipose tissue (WAT) is a major energy store, endocrine organ and is critical to metabolic homeostasis. Adipocytes release stored energy through lipolysis; the hydrolysis of stored triglycerides to liberate free fatty acids and glycerol. WAT is known to be innervated by sympathetic and sensory nerves ^[1], though the direct contribution of nerves in controlling lipolysis is currently unclear. It is well established that lipolysis is stimulated by catecholamines through activation of β -adrenergic receptors expressed by adipocytes ^[2]. Sympathetic nerves innervating WAT are a potential source of norepinephrine, but evidence of their involvement in lipolytic control is limited. Here we have developed a model to study the effects of nerve stimulation on lipolysis in WAT *ex vivo*.

Methods Adult male C57BL/6J mice (8-10 weeks) were sacrificed by CO₂ asphyxiation. Inguinal fat pads were identified and *Immunocytochemistry*: whole-mount immunocytochemistry was performed on whole fat pads, following previously described methods ^[3] Paraformaldehyde-fixed tissue was imaged by confocal microscopy. Nerves were stained with a chicken polyclonal antibody against β 3-tubulin (1:500; Abcam), vasculature was stained with isolectin B4 (1:500; Invitrogen) and BODIPY used to visualise adipocytes. *Ex vivo lipolysis assay*: Sections (11 – 25mg) of inguinal fat pad were added to Dulbecco's Modified Eagle's medium containing 5.5 mM glucose and 2% (w/v) fatty acid-free bovine serum albumin. Tissue was challenged with pharmacological agents for 3 hours at 37°C, 5% CO₂ and 95% relative humidity. Media samples were removed to assay free glycerol as an indirect measurement of lipolysis. Glycerol was quantified by a colorimetric absorbance assay.

Results β 3-tubulin immunoreactivity revealed nervous innervation throughout the inguinal fat pad ($N=5$), with nerve bundles commonly observed tracking the length of the tissue. Finer innervation was mostly restricted to innervating large/medium-sized blood vessels. Parenchymal nervous innervation of white adipose was also observed ($N=3$). In lipolysis assays, it was observed that glycerol was constitutively released over 3 hours ($N=5$). Statistical analysis was conducted by ANOVA with post-hoc Tukey tests.

Norepinephrine (2 μ M) stimulated glycerol production above levels of constitutive glycerol production ($P<0.01$; $N=5$). We next employed veratridine to stimulate nerves, a natural product that inhibits voltage-gated Na⁺ channel inactivation, increasing nerve excitability. In these experiments, veratridine (100 μ M) stimulated glycerol production to the same level of norepinephrine ($P>0.05$; $N=5$). The effect of veratridine was abolished by tetrodotoxin (1 μ M; 30 mins preincubation) ($P<0.01$; $N=5$), with no significant difference observed from constitutive glycerol production ($P>0.05$; $N=5$).

Conclusions Our data reveal that white adipocytes and blood vessels of the mouse inguinal fat pad are innervated by nerves. Norepinephrine or pharmacologically increasing nervous excitability both stimulate lipolysis *ex vivo* in inguinal fat. Glycerol was also produced constitutively, suggesting basal lipolysis occurs in inguinal fat. Application of TTX was able to abolish veratridine-induced lipolysis to basal levels.

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PCB072

Age-related differences in skeletal muscle fibre-specific mTOR-mediated signalling proteins via immunofluorescent microscopy

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Background: Ageing results in a gradual loss of skeletal muscle mass and type II fibre atrophy, through dysregulated muscle protein synthesis (MPS). Traditional immunoblotting techniques have revealed dysregulated mTOR-mediated signalling as a mechanistic driver of impaired MPS in older adults. However, immunoblotting cannot determine fibre-type specific protein abundance, localisation, or translocation of these molecular regulators within cells. Immunofluorescence microscopy (IF) has the capacity to address the shortcomings associated with immunoblotting and uncover mechanisms of age-related MPS impairment and muscle decline. This study aimed to use IF to measure age-related and fibre-specific differences in the abundance and localisation of mTOR-mediated proteins.

Methods: Resting muscle biopsies from eight young males (YM; 24 ± 4 years, BMI; 24 ± 4 kg·m²) and seven older males (OM; 67 ± 5 years, BMI; 25 ± 2 kg·m²) were embedded in OCT compound, frozen in liquid nitrogen-cooled isopentane for IF subsequent analysis. Embedded muscle samples were cut using a microtome blade, and cryosections (7mm) were collected on room-temperature uncoated glass slides. Sections were fixed and incubated in MHC-I, Rheb, TSC2 or WGA, mTOR and Sestrin-2 primary antibodies and incubated in contrasting Alexa Fluor secondary antibodies. Images were captured at 10x for fibre type distribution and 20x for analysis of fibre-type immunofluorescent abundance. Full ethical approval was granted (18/EM/0004) and procedures were conducted in accordance with the Declaration of Helsinki.

Results: OM displayed a significantly ($P=0.0151$) lower proportion of type I fibres than YM (YM, $57.6 \pm 0.6\%$ vs OM, $47.6 \pm 2.8\%$). Mean CSA was not different between groups for type I (YM, 4745.8 ± 98.1 mm² vs OM, 6133.0 ± 408.6 mm²) or type II fibres (YM, 6556.7 ± 196.0 mm² vs OM, 5752.3 ± 246.8 mm²). Notably, a 2-fold higher abundance of TSC2 in OM compared with YM (YM, 12.71 ± 0.28 A.U. vs OM, 25.45 ± 0.64 A.U.; $P<0.001$) was observed. OM displayed a ~31% (YM, 68.86 ± 3.36 A.U. vs OM, 47.22 ± 2.77 A.U., $P=0.020$) and a ~63% (YM, 39.21 ± 2.15 A.U. vs OM, 14.49 ± 0.57 A.U., $P<0.001$) lower abundance in Sestrin-2 and mTOR, respectively, compared with YM. Rheb abundance was ~43% higher in OM compared to YM (YM, 18.66 ± 0.92 A.U. vs OM, 26.71 ± 0.58 A.U., $P<0.001$). There were no differences in protein target abundance between fibre types for either group. Notably, peripheral and membrane bound fluorescence was higher in OM.

Discussion: Using IF, it is possible to characterise fibre-specific molecular regulators of skeletal muscle. Whilst the selection of regulatory proteins we investigated were similarly abundant in type I and II fibres, differences in mean fluorescence relative to fibre area of mTOR, TSC2,

Sestrin-2 and Rheb may be implicated in age-related MPS impairment and muscle decline. IF could be used to identify dysregulated signalling events that underpin age-related anabolic resistance (e.g., impaired MPS response to amino acids and/or contraction).

Conclusion: IF characterisation of the fibre-type-specific abundance of key regulators of mTORC1 revealed age-related differences that may be linked to dysregulated proteostasis.

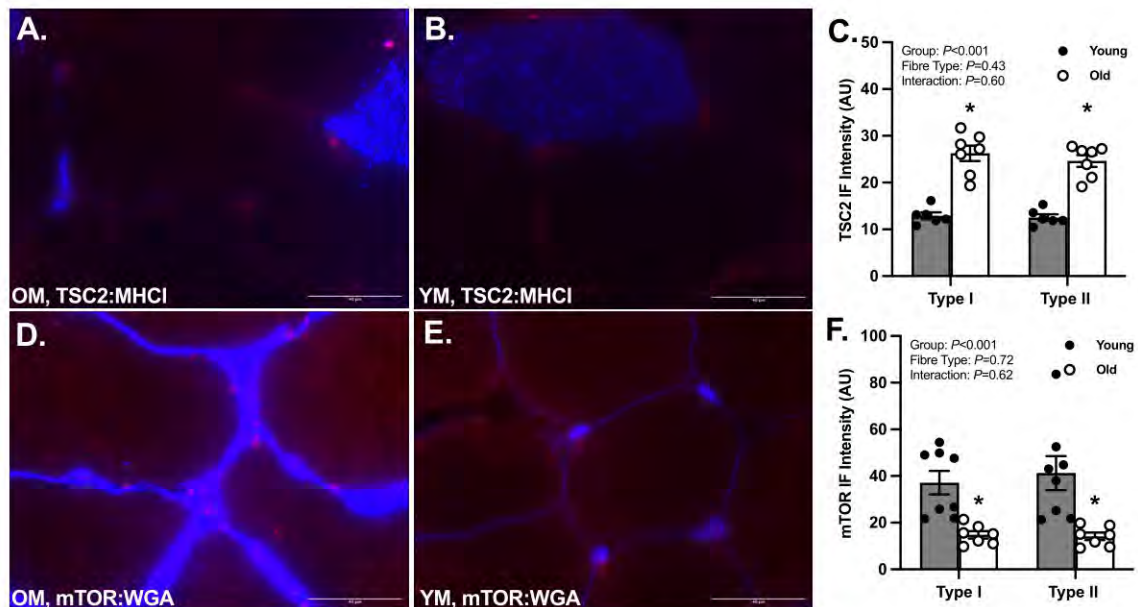


Figure 1. Immunofluorescent qualitative and quantitative comparison in different fibre types in young and old men.

TSC2 (red) MHC1 (blue) in muscle fibres in OM (A) and YM (B) with increased red fluorescence between fibres in old. 2-way ANOVA for between fibre comparisons in total TSC2 fluorescence for YM and OM (C). mTOR (red), WGA (blue) in OM (D) and YM (E) with red fluorescence most prevalent at the periphery in young and at the cell membrane in old. 2-way ANOVA outputs for between fibre comparison total mTOR fluorescence for YM and OM. Images captured 60x, scale bar = 40µm.

* indicates significantly different from YM.

N/A

PCB073

Pancreatic stellate cell activation elicits changes in mitochondrial fitness and dynamics that can be targeted in a mouse model of pancreatic cancer

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Pancreatitis and pancreatic cancer – severe diseases of the exocrine pancreas – are often associated with fibrosis, which can significantly impair organ functionality. This excessive collagen deposition is mainly driven by the activation of pancreatic stellate cells (PSCs) – a transition from a fibroblast- (quiescent) to myofibroblast-like (activated) phenotype (FMT). It has been established that FMT in PSCs induces substantial changes in gene expression and protein levels, particularly in extracellular matrix (ECM) proteins. Since the activated phenotype of PSCs is characterised by increased production and secretion of ECM proteins, we hypothesised that FMT likely affects mitochondrial functions and bioenergetics of these cells.

To test this, we have carried out real-time fluorescence imaging of mitochondrial dynamics and compared the characteristics of mitochondrial networks between quiescent and activated PSCs. Inhibition of mitochondrial fission was also tested as part of combination therapy with gemcitabine in an orthotopic mouse model of pancreatic cancer (Ethical Committee Approval no. 336/2022). Injection of the Panc02 pancreatic cancer cell line expressing luciferase allowed regular monitoring of tumour growth in vivo with a non-invasive bioluminescence imaging system (IVIS Lumina) during the experiment. Flow cytometry analysis was applied in order to assess the specific tumour composition after the treatment.

Our data indicate that the process of mitochondrial fission was potentiated upon cell activation, as shown by transmissive electron microscopy and fluorescence microscopy. Dynamin-related protein 1 (Drp1) required for mitochondrial division was upregulated upon activation of PSCs. Importantly, pharmacological inhibition of mitochondrial fission decreased the expression of PSC activation markers (eg. alpha-smooth muscle actin and collagen) as well as increased the incidence of cell necrosis in activated PSCs in vitro. Application of gemcitabine and mitochondrial fission inhibitor in vivo resulted in decreased tumour growth kinetics and tumour volume.

Our results reveal significant changes in the mitochondria between quiescent and activated PSCs, with mitochondrial fission playing a crucial role. This study suggests that the activation-induced alterations in PSC mitochondrial dynamics and metabolism could play an important role in pancreatic diseases, including pancreatic cancer.

PCB074

Higher stromal vascular fraction TGFB1 transcription and adipocyte overexpression of COL4A1 may explain reduced capacity for adipocyte expansion in pre-eclampsia

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Pre-eclampsia (PE) is a disorder of human pregnancy, which is defined as new onset hypertension with proteinuria. In recent years, a role for adipose tissue (AT) dysfunction has emerged in PE pathology. Women with PE display exaggerated insulin resistance in subcutaneous AT (SAT) at third trimester compared to normotensive (NT) controls, without evidence of hypertrophy¹. This may indicate a reduced capacity in PE to store gestationally-acquired fat within AT. We hypothesised that increased AT fibrosis in PE, defined as excessive accumulation of extracellular matrix components including collagens, could limit healthy AT expansion in PE.

Non-labouring pregnant women in the third trimester with PE, as defined by ISSHP guidelines, and age- and BMI-matched NT controls undergoing elective C-section at the Glasgow Royal Infirmary were consented for SAT and visceral AT (VAT) biopsy collections. Ethical approval was granted from the Glasgow Royal Infirmary Local Research Ethics Committee (06/S0704/14). Adipocyte diameter was assessed by manual microscopic sizing of a minimum of 100 adipocytes. Fibrosis was quantified in paraffin embedded AT sections using picrosirius red staining (PE n=11, NT n=6). Acquired images of stained whole tissue sections were converted to 8-bit images, background area subtracted and total red staining of collagen fibres (%) measured in ImageJ. Taqman qRT-PCR was used to measure mRNA expression relative to the endogenous control *PPIA* in whole AT (n=6 per group) and isolated adipocytes (n=9 per group). Data were analysed using repeated measures mixed-effects models of PE status (NT or PE) and AT depot (SAT or VAT) and their interaction; mean $\pm$ SD reported.

There was no difference in mean adipocyte diameter between PE (SAT: $113.6 \pm 9.6\mu\text{m}$, VAT: $90.6 \pm 10.6\mu\text{m}$) and NT (SAT: $111.7 \pm 7.9\mu\text{m}$, VAT: $88.2 \pm 8.3\mu\text{m}$) ($P_{\text{PE status}}=0.53$, $P_{\text{AT depot}}<0.001$, $P_{\text{interaction}}=0.90$). There was no difference in tissue collagen content between NT and PE ($P_{\text{PE status}}=0.56$, $P_{\text{AT depot}}=0.55$, $P_{\text{interaction}}=0.39$). Whole AT *COL6A3* ($P_{\text{PE status}}=0.41$, $P_{\text{AT depot}}=0.005$, $P_{\text{interaction}}=0.51$), *COL1A1* ($P_{\text{PE status}}=0.76$, $P_{\text{AT depot}}=0.16$, $P_{\text{interaction}}=0.21$) and *COL4A1* ($P_{\text{PE status}}=0.17$, $P_{\text{AT depot}}<0.001$, $P_{\text{interaction}}=0.20$) mRNA expression was not different between PE and NT. Interestingly, whole AT mRNA expression of *TGFB1* was higher in PE compared to NT ($P_{\text{PE status}}=0.012$, $P_{\text{AT depot}}=0.039$, $P_{\text{interaction}}=0.73$). Isolated adipocyte *COL4A1* mRNA expression was higher in PE compared to NT ($P_{\text{PE status}}=0.044$, $P_{\text{AT depot}}<0.001$, $P_{\text{interaction}}=0.64$). Adipocyte *TGFB1* expression did not differ between PE and NT ($P_{\text{PE status}}=0.91$, $P_{\text{AT depot}}=0.001$, $P_{\text{interaction}}=0.48$).

In conclusion, while there was no effect of PE on AT fibrosis, higher adipocyte expression of *COL4A1*, a key basement membrane component was observed. This has also been seen in adipocytes from obese individuals² suggesting similar localised restriction of adipocyte hypertrophy in PE which may contribute to impaired AT expansion. Higher whole AT, but not adipocyte, *TGFB1* expression (a regulator of *COL4A1* transcription) indicates that TGF β is

produced by stromal vascular cells. Understanding the relationship between TGF β and adipocyte collagen type IV may shed light on defective adipocyte differentiation in PE pregnancies.

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PCB075

The effect of ex vivo human serum on insulin signalling mechanisms in C2C12 skeletal muscle cells: a randomised parallel trial of diets differing in carbohydrate and fat content.

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Type 2 diabetes affects 462 million adults globally (1) and skeletal muscle has shown to be a key regulator of glucose homeostasis (2). Both high-carbohydrate (HC) and low-carbohydrate (LC) diets reduce markers of insulin resistance (3) however, the underlying mechanisms are yet to be elucidated. *In vitro* models provide insight to niche responses, however, in isolation, cannot elucidate the impact of the change in metabolic factors on insulin signalling. Conditioned serum, in combination with conventional cell culture methods, may allow for such cross talk to be investigated (4). Therefore, the purpose of this study was to investigate the effects of diet on insulin resistance markers in adults and how their serum impacts skeletal muscle insulin signalling. Procedures received ethical approval from LJMU Research Ethics Committee (16/ELS/029). Participants were randomly assigned to a HC (n=8, ≥50% of energy from carbohydrates) or LC (n=8, consume <50 g/day of carbohydrates) diet. At 0, 4 and 8 weeks, serum samples were analysed for insulin resistance markers (5). Skeletal muscle C2C12 cells (n=3) underwent fusion in differentiation medium (DM; 2% horse serum). Myotubes were next incubated with 2% pooled human serum from 0, 4 and 8 weeks of intervention. After 30 min, the impact of serum on cellular energy status was assessed by immunoblotting for p/t-AMPK^{thr172}. Following incubation of serum for 3 hours, cells were stimulated with 100nM of insulin for 20 min and immunoblotted for p/t-Akt^{ser473} while glucose uptake was assessed via measuring 2NBDG uptake. Data are presented as mean ± SEM and underwent a 3 x 3 mixed ANOVA. Both groups reduced ($P < 0.01$) markers of insulin resistance (5). Serum from LC and HC significantly ($P < 0.05$) increased p/t-AMPK^{thr172} from 0 to 30 mins. p/t-AMPK^{thr172} significantly ($P = 0.04$) decreased with serum from 0, (LC; 6.51 ± 2.16 , HC 4.54 ± 0.83), 4 (LC; 2.16 ± 0.43 , HC; 2.93 ± 0.64) to 8 (LC; 1.33 ± 0.2 , HC; 1.96 ± 0.44) weeks. Insulin stimulation significantly ($P < 0.001$) increased p/t-Akt^{ser473} with serum from 0 (LC; 3.25 ± 0.62 , HC; 3.33 ± 1.29), 4 (LC; 1.95 ± 0.23 , HC; 2.59 ± 0.42) and 8 (LC; 2.13 ± 0.31 , HC; 2.6 ± 0.60) weeks however, no change in glucose uptake was observed. The fold change of p/t-AMPK^{thr172} was positively associated ($r = 0.62$, $P < 0.01$) with insulin stimulated p/t-Akt^{ser473}. As AMPK can regulate insulin sensitivity, p/t-Akt^{ser473} at each week were relativised to their p/t-AMPK^{thr172}. The fold change in insulin-induced p/t-Akt^{ser473} now showed a tendency ($P = 0.067$) of increasing from 0 (LC; 0.5 ± 0.01 , HC; 0.73 ± 0.28) to 4 (LC; 0.9 ± 0.11 , HC; 0.89 ± 0.14) and 8 weeks (LC; 1.6 ± 0.24 , HC; 1.33 ± 0.3) with both diets. To conclude, human derived sera can impact the *in vitro* skeletal muscle response to insulin stimulation. Both a LC and HC diet can improve markers of insulin resistance and improve the cellular environment. Further research is required to determine the potential of ex vivo serum at elucidating cellular adaptations.

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PCB076

Adipose depot dependency of chloride channels expression in murine white fat adipocytes

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Plasma membrane ion channels are important for cell homeostasis with their combined properties often defining the function of that cell; however, for white fat adipocytes (WFA) their expression and associated roles remain unclear. Given the importance of chloride channels in WFA membrane potential (Pulbutr et al., 2007; Bentley et al., 2014), we aimed to identify these at the molecular level. We explored this issue in adipocytes isolated from different adipose depots of adult rats, mice and 3T3-L1 cells: a common adipocyte cell line model. RNAseq revealed the expression of various chloride channel isoforms in the epididymal WFA of adult male rats. Among these, putative plasma membrane chloride channels were the volume-regulated chloride channels *Lrrc8a/b/c/d* and *Ttyh2*, the calcium-activated chloride channels, *Ano1* and *Ttyh3* and the glutamate aspartate transporter, *Eaat1*. Relative expression was confirmed by RT-qPCR. Data are given as mean ratios (95% CI, number of determinations) relative to rSWFA. Statistical significance, with $p < 0.01$ to account for repeated measures, was determined by ANOVA with Dunnet's multiple comparison test relative to rSWFA. Protein expression was determined by western blot in undifferentiated and differentiated 3T3-L1 cells with statistical significance determined by unpaired T-test. Confocal immunofluorescence was used to identify the cellular location of chloride channels on WFA. To investigate the role of chloride channels in adipogenesis, the effect of their inhibitors on 3T3-L1 cells was determined by measuring Nile Red accumulation analysed using One-way ANOVA.

RT-qPCR showed no difference in depot expression for *Lrrc8a*, *Lrrc8c*, *Lrrc8d*, *Ttyh2* and *ANO1*, whereas, in rMWFA and rPWFA, *Lrrc8b* was expressed by 4.9 (2.5 to 13, $n=5$) and 3.3 (1.6 to 9, $n=5$) fold greater, respectively. Western blot demonstrated that *Lrrc8a* and *Ttyh3* were expressed in differentiated 3T3-L1 cells by 4.1 (3.0 to 5.1, $n=16$) and 5.4 (-6.4 to 17.2, $n=3$) compared to undifferentiated 3T3-L1 cells, respectively. Data also showed that *Eaat1* was exclusively expressed in differentiated 3T3-L1 cells while *Ano1* was exclusively expressed in undifferentiated 3T3-L1 cells. Immunofluorescence showed that *Eaat1*, *Ano1*, *Ttyh2* and *Ttyh3* are primarily located to the plasma membrane, while *Lrrc8a* was all around the cells. During 3T3-L1 differentiation, 25 μ M DCPIB, a selective blocker of the volume-regulated anion channels *Lrrc8a* and *Ttyh2*, and 5 μ M quercetin, which inhibits Ca^{2+} -activated Cl^- channels *Ttyh3* and *Ano1*, significantly reduced adipogenesis by 34% (16% to 53%, $n=5$) and 19% (6% to 31%, $n=5$) respectively. However, 50 μ M DIDS, which also inhibits *Ttyh3*, *Ano1*, and 10 μ M UCPH101, which inhibits *Eaat1*, were without effect.

This study provides evidence for the existence of chloride channels with various expression patterns among different WFA depots and in 3T3-L1 cells. *Lrrc8a*, *Ttyh2*, *Ttyh3* and *Eaat1* were all expressed in the plasma membrane of murine WFA, *Lrrc8a* was also found intracellularly. *Ano1* was expressed only in rat WFA. The observation that DCPIB and Quercetin significantly inhibited adipogenesis suggests that chloride channels play a role in adipocyte differentiation.

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PCB077

Effects of the administration of a combined oral contraceptive composed by ethinylestradiol and drospirenone on adiposity and liver histopathology in female mice in reproductive age

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Combined oral contraceptives (COC) is the type to birth control used for several women, but, little is known about its organic effects when it is combined with an obesogenic diet. Here, we aimed to investigate the effects of administration of a COC composed of 17 α -ethinylestradiol (EE2) and drospirenone (DRSP) upon obesity, glucose tolerance and non-alcoholic fat liver disease (NAFLD) in female mice of reproductive age. Eighty-days old Swiss female mice were fed on a standard diet (SD) or a high-fat diet (HFD) and daily received, via gavage, 0.2 mL of distilled water [CTL-SD (n=13) and CTL-HFD (n=16) groups, respectively], containing or not, 0.6 μ g EE2 plus 60 μ g DRSP [COC-SD (n=13) and COC-HFD (n=16) groups, respectively], for 65 days. At the end of the treatment, all female mice groups were submitted to an intraperitoneal (IP) glucose tolerance test (GTT) and two days after were weighted and euthanized by decapitation. Total blood was collected to measure plasma triglycerides. Total adipose tissue stores and the liver were excised for visceral adiposity evaluation and hepatic fat extraction and histology, respectively. Results were analyzed using two-way ANOVA followed by Bonferroni-Sidak's test ($p < 0.05$). Procedures performed in mice were in accordance with Brazilian's ethical standards and were approved by the animal use committee (certificate number: MAC039). Consumption of HFD increased total BW gain in CTL-HFD females (11.9 ± 1.2 g), when compared with CTL-SD (0.5 ± 1.0 g). In accordance, CTL-HFD exhibited increased final BW (49.1 ± 1.4 g) and total visceral adiposity (147.2 ± 9.6 mg/g BW) than those observed for CTL-SD (35.3 ± 1 g and 57.5 mg/g BW, respectively). COC administration attenuated obesity induced by HFD, since COC-HFD females displayed high visceral adiposity (88.1 ± 12.5 mg/g BW), but similar BW gain (4.4 ± 1.5 g) and final BW (36.4 ± 1.8 g) than COC-SD (47.0 ± 5.0 mg/g BW, 4.4 ± 1.5 and 35.8 ± 0.4 g, respectively). But all these parameters in COC-HFD were lower than those observed for CTL-HFD. Also, CTL-HFD displayed high total glycemia during the GTT (40031 ± 2763 mg/dL.min⁻¹) in comparison with CTL-SD (28577 ± 2000 mg/dL.min⁻¹). COC treatment prevented glucose intolerance induced by HFD, since COC-HFD females displayed lower total glycemia during this test (31113 ± 1439 mg/dL.min⁻¹) when compared to CTL-HFD. HFD augmented plasma (85.6 ± 5.7 mg/dL) and hepatic triglycerides levels (24.7 ± 2.1 μ g/mg), and increased NAFLD score (3.2 ± 0.1) in CTL-HFD liver, mainly in part due to increase the hepatic steatosis score (3.1 ± 0.1), but not, inflammation score (0.1 ± 0.1). COC-HFD females displayed lower hepatic TG content (16.2 ± 2.0 μ g/mg) of CTL-HFD, while their liver parenchyma displayed NAFLD associated features, such as hepatocytes with microvesicular steatosis (score = 1.6 ± 0.4), higher inflammatory foci (0.5 ± 0.2) and hyperemia. Therefore, COC administration to female mice of reproductive age, attenuated some HFD inducing obesity impairments, such as adiposity, glucose intolerance, but not prevented signs of liver damage that characterizes NAFLD development.

not applicable

PCB078

Effects of a resistance training programme in people living with HIV in Zimbabwe

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Purpose: Combination antiretroviral therapy (cART) increases life expectancy in people living with HIV (PLWH). However, receiving cART coupled with physical inactivity increases the risk of developing non-communicable diseases. Resistance training (RT) has also been found to increase muscle strength and mass, mitigate muscle wasting, improve psychological status, glucose tolerance, insulin sensitivity and lipid profile in PLWH. However, this has not been fully investigated in sub-Saharan Africa. Therefore, the purpose of this study was to investigate the effect of RT on body composition, laboratory analysis and strength values in PLWH receiving cART in Zimbabwe.

Methods: One-hundred and twenty-eight PLWH receiving cART, aged 18–45 years were purposely recruited to saturation. The study obtained ethical approvals from the University of Kwa-Zulu Natal's Biomedical Research Ethics Committee (BREC) BF293/15 and the Medical Research Council of Zimbabwe (MRCZ) MRCZ/B/948. All participants signed an informed consent following initial BL assessments. Two districts in Zimbabwe were used. Participants in Budiriro were randomly assigned for convenience to an experimental (EXP) group (n = 64) performing RT 3 days/week and participants in Mabvuku to a control (CON) group (n = 64) for 12 weeks of no exercise. Body mass index, waist-to-hip ratio, percentage body fat, lean body mass (body composition), laboratory analysis profiles and one-repetition maximum (1RM) strength were measured at baseline (BL) and after 12 weeks (W12) in both groups. Significance was set at $p < 0.05$.

Results: Lean body mass increased in the EXP group (n=64) from 52.42 ± 8.360 kg to 53.07 ± 8.225 kg (mean difference -0.65 kg), suggesting that the 12-week RE intervention programme significantly increased ($p < .001$) lean body mass in the EXP group. In the CON group, lean body mass reduced from 50.87 ± 6.340 kg to 47.43 ± 7.829 kg (mean difference 3.44 kg) during the same period. Fasting blood glucose decreased significantly ($p < .001$) in the EXP group from 4.440 ± 0.445 mmol/l to 4.240 ± 0.488 mmol/l (mean difference 0.2 mmol/l), compared to the CON group at BL 3.68 ± 0.711 mmol/l to W12 3.98 ± 0.818 mmol/l (mean difference -0.3 mmol/l). Fasting total blood cholesterol decreased significantly ($p < .0005$) in the EXP group from 4.440 ± 0.526 mmol/l to 4.240 ± 0.488 mmol/l (mean difference 0.2 mmol/l), compared to the CON group, which increased from 4.556 ± 0.445 mmol/l to 4.672 ± 0.497 mmol/l (mean difference -0.116 mmol/l). In the EXP group, 66% of participants improved resting blood pressure, a significant change from BL to W12 ($p < .0005$). In the EXP group, 1RM muscular strength increased significantly ($p < .001$) for bench press (mean difference -3.9 kg), squat (mean difference -26 kg), biceps curl (mean difference -7.84 kg) and leg curl (mean difference -11.44 kg) from BL to W12 compared to the CON group.

Conclusions: These findings highlight the benefits of RT for PLWH receiving cART. This demonstrates the need for additional public health initiatives involving RT in this population in sub-Saharan Africa.

Keywords HIV-infected · Resistance training · Body composition · Chronic disease · Strength

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PCB079

Altered cardiac mitochondrial respiratory complex activities in adult mice born to obese dams

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Introduction: Exposure to gestational obesity increases cardiometabolic disease risk in both human longitudinal cohorts and animal models. Male mice born to obese dams display cardiac hypertrophy and declining systolic and diastolic function. Despite alterations in cardiac mitochondrial respiratory function and ultrastructure in the offspring of obese dams, the role of mitochondrial metabolic changes in the programming of cardiac dysfunction by maternal obesity remains poorly defined.

Objective: To evaluate myocardial mitochondrial respiratory capacity in a well-established murine model of maternal obesity, we subjected banked cardiac tissue from 8wk-old offspring of both sexes to a protocol optimised for respirometry in frozen samples to profile electron transport chain (ETC) capacities.

Methods: Animal work was conducted in accordance with the UK Home Office Animal (Scientific Procedures) Act 1986 and following local ethical approval. Cardiac tissue was previously collected from 8wk-old C57BL/6J offspring mice of both sexes born to dams fed either a control (CC group) or obesogenic diet (OC group) for 10 weeks prior to mating and throughout gestation. Frozen cardiac tissue was homogenised in MiR06 respiration medium and transferred to Oxygraph-O2K chambers. A substrate inhibitor titration was initiated to determine uncoupled capacities of ETC complex I (CI; NADH), complexes I+II (maximal ETC capacity; NADH + succinate), complex II (CII; succinate + rotenone), and complex IV (CIV; TMPD + ascorbate). Respiratory oxygen fluxes (JO_2) were normalised to chamber homogenate dry weight and maximal ETC capacity, and analysed by two-way ANOVA for sex and maternal diet ($n = 7$).

Results: Uncoupled respiratory complex activities normalised to tissue weight did not significantly differ between the 2 offspring groups, suggesting no overt changes in mass-specific mitochondrial respiratory capacity, although the CI-linked JO_2 /maximal ETC capacity ratio was higher in the OC group (control: 0.37 vs. obese: 0.41, $P = 0.041$). Mass-specific CI-linked ETC activity (male: 171.97 pmol O_2 /[s×mg dw] vs. female: 117.29 pmol O_2 /[s×mg dw], $P = 0.077$) and CI/maximal ETC capacity ratio (male: 0.42 vs. female: 0.37, $P = 0.0065$) were higher in male offspring, with females exhibiting a trend towards lower CII activity normalised to maximal ETC (male: 0.69 vs. female: 0.73, $P = 0.054$).

Conclusion and further work: Despite no differences in mass-specific uncoupled respiratory complex activities, suggesting an overall preservation of cardiac mitochondrial respiratory capacity, CI activity as a proportion of maximal ETC capacity was increased in maternal obesity exposed offspring, potentially indicating an early remodelling of relative respiratory complex

activities. Further work is necessary to profile the stoichiometry of respiratory complex expression, and to define the wider mitochondrial metabolic phenotype in these samples.

PCB080

PUTATIVE GENETIC IMPACT ON DEPRESSION AND SUICIDAL IDEATION AMONGST A POPULATION OF YOUNG FEMALES WITH PREMENSTRUAL DYSPHORIC DISORDER

FREDDY AGOREYO, Ese Onuyoh-Adaitire, Blessing Agoreyo, B Onuyoh-Adaitire

undefined

Premenstrual Dysphoric Disorder (PMDD) is a pathological spectrum of emotional and somatic symptoms observed during the luteal phase of menstrual cycle interfering with the physical and social life of the individual. WHO in 2016 revealed that Nigeria had the highest suicidal number in Africa with over 17,000 lives lost to Suicide. The aim of this study was to evaluate the putative genetic impact on depression and suicidal ideation amongst a population of young females with premenstrual dysphoric disorder. The study was carried out across Benin metropolis. A total of 200 apparently healthy young female adults were recruited in this study with age range between 18 and 30 years. Subjects were grouped into 3 groups: Control subjects (without symptoms of PMS), subjects with symptoms of PMS only and those with PMS and Suicidal tendencies. To assess the subjects' subjective perception of health, each subject was asked to fill out the self-reporting luteal phase depression and distress measurements. Five (5.0) mls of whole blood was

collected and dispensed into 2.5ml DNA shield container. Analyses were carried out in the University of Benin, University of Benin Teaching Hospital (UBTH) and Federal University of Technology, Akure (FUTA), Nigeria. All data were presented as mean $\pm$ standard deviation.

Statistical analyses were done using graph pad prism 8.1. The data was evaluated using two-way

analysis of variance (ANOVA) utilizing the F test. Data was expressed as the mean value $\pm$ SD for the control and test groups. Differences within the groups were then assessed using least significant difference (LSD) and p-values less than 0.05 ($p < 0.05$) was considered statistically

significant. The Beck's Depression Inventory showed a significant rise in the test participants when compared with the control participants ($p < 0.05$). Cytochrome P450 -17 gene expression was significantly up regulated in the test participants compared to control participants during the luteal phase of the menstrual cycle ($p < 0.05$). The Extra sex comb/Enhancer of Zestes genes were significantly down regulated in the test participants when compared with the control participants ($p < 0.05$).

PCB081

Regulation of synaptic AMPA receptor function by TARP combinations

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AMPA receptors (AMPA) are responsible for fast excitatory synaptic signalling in the brain. A majority of AMPARs are heterotetramers composed of four pore-forming subunits (GluA1-4), co-assembled with transmembrane AMPAR regulatory proteins (TARPs). Six TARP family members have been described which differ in their effects on receptor trafficking and function (Jackson & Nicoll, 2011). While many central neurons contain more than one type of TARP, it is unknown whether different TARPs can co-associate within with the same receptor and further modify individual AMPAR function.

Structurally, TARPs possess four transmembrane domains and intracellular C- and N- termini. While the TARP's backbone mediates binding to AMPARs, its C-terminal tail (CT) binds to postsynaptic density protein (PSD-95) through a PDZ binding motif (Bats, 2007). Functionally, TARP family members fall into two categories, type I (gamma-2, -3, -4 and -8) and type II (gamma-5 and -7), according to their ability to rescue excitatory postsynaptic currents (EPSCs) in stargazer cerebellar granule cells (CGCs). Stargazer mice are a spontaneous mutant that lack gamma-2 (stargazin) expression in homozygous individuals. CGCs of these mice lack AMPAR-mediated synaptic currents, suggesting these neurons rely on gamma-2 for AMPAR surface expression and synaptic clustering. Transfecting any type I TARP in stargazer CGCs can compensate for loss of endogenous gamma-2. While only type I TARPs promote AMPARs surface delivery and synaptic expression, TARPs from both subtypes enhance AMPAR channel function. They do this by increasing the AMPAR deactivation- and desensitization time, increasing net cation influx. Of the TARPs, gamma-4 produces the slowest decaying AMPAR-mediated currents.

We made patch clamp recordings from CGCs cultured from postnatal stargazer mice. Transfection of a chimera containing the CT of gamma-2 fused to the backbone of gamma-4 gave rise to miniature EPSCs that decayed remarkably slowly when compared with wild type gamma-2 and gamma-4 (τ_w 22.7 ± 1.7 ms versus 3.2 ± 0.2 ms and 4.8 ± 0.6 ms; all $n = 5$; $p = 0.007$ and $p < 0.001$, two-sided permutation t-test, Ho, 2019). Such combined action of the gamma-4 backbone and gamma-2 CT within the chimera suggests that gamma-2 and gamma-4 may use different mechanisms to control AMPARs kinetics. We therefore asked whether gamma-2 and gamma-4 could co-associate within individual AMPARs to confer distinct kinetic properties. To address this, we expressed a chimera containing the CT of gamma-7 fused to the backbone of gamma-4 (gamma-4_7CT) in cultured CGCs. As this chimera lacks the ability to promote the trafficking of AMPARs to synapse, any increase in mEPSCs decay time is expected to reflect the synaptic insertion of receptors containing both gamma-2 and gamma-4_7CT. Indeed, mEPSCs in these neurons displayed significantly slower decay times than those seen in GFP-transfected controls (τ_w 4.2 ± 0.3 ms versus 2.2 ± 0.3 ms, $n = 5$ and 7 ; $p = 0.0026$). Together, these results suggest that, in CGCs that contain endogenous gamma-2 and transfected gamma-4_7CT, a population of synaptic AMPARs contained both TARPs, and that the receptors can show some features derived from each.

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PCB082

Cognition is selectively impaired in males with spinal pain: A retrospective analysis of data from the Longitudinal Study of Ageing Danish Twins

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Background: Cognitive decline and dementia represent a major health and social care challenge in the twenty-first century as a leading cause of morbidity and mortality affecting over 55 million people globally. Back pain (BP) and neck pain (NP), often referred to collectively as spinal pain, contribute to additional health challenges in the elderly and is acknowledged as the primary cause of years lived with disability often co-existing with cognitive decline and neurodegeneration. Despite their co-existence, our current understanding of the mechanisms that potentially link BP and NP to accelerated cognitive decline remains uncertain.

Hypothesis: We hypothesise that elderly adults reporting spinal pain would exhibit lower cognition scores, and that this would be more pronounced in females, given their established vulnerability to later-life neurodegeneration.

Methods: To investigate the potential relationships between BP/NP and cognitive decline, we conducted a retrospective cross-sectional analysis of the Longitudinal Study of Aging Danish Twins Database as part of the Danish Twins Registry adjusting for age, sex, educational and socioeconomic status. Ethical approval was granted by the Faculty of Life Sciences and Education Ethics Committee at the University of South Wales (#19DB0501LR).

Results: A total of 4,731 adults (2,788 females/1,943 males) aged 78±6 (SD) years were included in the analysis. We observed a one-month prevalence of 25% with BP, 21% with NP and 11% for combined BP/NP. While there were no differences in cognition scores for male and females reporting combined BP/NP, compared to those without combined BP/NP (34.38 points; 95% CI=31.88, 36.88 vs 35.72 points; 95% CI=35.19, 36.26; $P=0.180$; and 35.72 points; 95% CI=35.19, 36.26 vs 35.85 points; 95% CI=35.39, 36.31; $P=0.327$, for male and females respectively), an adjusted analysis revealed that males with combined BP/NP presented with lower cognitive scores compared to males without combined BP/ NP (79.48 points; 95% CI=70.31, 88.66; $P=0.043$ vs 81.26 points; 95% CI=73.80, 88.72, respectively).

Conclusions: In the current study, males reporting combined BP and NP exhibited lower composite cognitive scores compared to males without combined BP and NP when adjusting for age, sex, educational and socioeconomic status. The fact that male twins reporting both BP and NP presented with lower cognitive scores may be due to males carrying more cardio-cerebrovascular risk factors (i.e., greater vascular disease burden) than females. Accordingly, an elevation in cardiometabolic risk factors and the pattern of combined BP and NP symptomatology may have the potential to increase systemic oxidative-inflammatory-nitrosative stress (OXINOS). Given this was only observed in males, these findings suggest a 'sex-specific susceptibility' to cognitive decline and supports the notion that combined BP and NP may be considered as an additional cardio-cerebrovascular risk factor for later life neurodegeneration warranting further investigation.

PCB084

Use of SH-SY5Y cell line to develop an *in vitro* model to study autism spectrum disorders

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Valproic acid (VPA) is an environmental risk factor for Autism Spectrum Disorders (ASD), especially during the first trimester of pregnancy, that is widely used in experimental designs for its ability to model ASD both morphologically and behaviourally on animals but was not studied on cell lines. Cell lines provide cheaper, ethically less problematic and reusable models (Xicoy et al., 2017). The aim of this study was to use SH-SY5Y, a human neuroblastoma cell line, to develop an *in vitro* model for studying ASD and observing VPA's effects directly on cells during different stages of differentiation.

SH-SY5Y cells were seeded to a 96-well plate with 2500 cells per well. 24hrs after seeding, culture media was changed to differentiation media and this was accepted as the first day of differentiation. The differentiation protocol was 7 days in total (Kovalevich and Langford, 2013). Cells were treated with VPA for 24 hours on different days of differentiation and each treatment day had three different concentrations (1mM, 5mM and 10mM of VPA). Experimental groups were the following: Control group (vehicle); VPA on the first day of differentiation; VPA on the third day of differentiation; VPA on the fifth day of differentiation. The MTT assay was performed as triplicates to determine cell viability. Statistical analysis was performed with One Way ANOVA and on Sigma Plot.

MTT assay showed that VPA affects cell proliferation/viability when compared to the control group on the first day of differentiation for each dose group ($p<0,001$; $p<0,001$; $p<0,05$). Cells exposed to VPA at the third day of differentiation also had reduced cell viability when compared to control cells except for the lower concentration of VPA ($p<0,001$; $p<0,001$). VPA treatment on the fifth day of differentiation significantly affected cell viability, when treated with concentrations of 5mM and 10mM ($p<0,001$; $p<0,001$). However, the lowest concentration of VPA had the opposite effect, increasing cell counting when compared to control cells ($p<0,001$). Effect of VPA exposure on different days of differentiation was compared within the same dose groups. In 5mM and 10mM doses, VPA exposures on third day of differentiation resulted in significantly lower cell count compared to first and fifth day of differentiation ($p<0,05$; $p<0,05$).

Consistent with literature, our results showed that 1mM of VPA does not have negative effects on cell viability whereas 10mM showed detrimental effects (Jang et al., 2021). Our results also showed that third day of differentiation is the most vulnerable time for VPA exposure in terms of cell viability. We suggest that 5mM VPA should be used for further analysis for cellular and morphological parameters that are disrupted in *in vivo* models of ASD.

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PCB085

Characterising Lipopolysaccharide induced Tumour Necrosis Factor Alpha release by BV-2 cells in Normoxia and Hypoxia (1% O₂)

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Microglial cells are the key immunogenic cells in the CNS, and significantly contribute to overall brain function by participating in phagocytosis during development, homeostasis, and diseased states. It has been demonstrated that oxygen levels have significant impacts on microglial morphology and behaviour. Hypoxia is reported to either induce, or attenuate externally induced, inflammatory signalling. This study aimed to characterise the LPS induced inflammatory response, including its potential dose dependency, of BV-2 cells, and discover how this response is altered when stimulation occurs during hypoxia. BV-2 cells between passage 3-11 were exposed for 24h to LPS, at 1, 10, 100, 1000, or, 10,000 ng/mL, in normoxia (21% O₂), or hypoxia (1% O₂). Media samples were collected and assessed for inflammatory response, via TNF- α ELISA assay. Cell viability, via LDH assay, and metabolic activity, via MTT assay, were also determined. Lastly expression of inflammation and hypoxia associated genes was analysed via qPCR, using only 100ng/ml LPS as a dosage.

MTT assays demonstrated decreased metabolic activity in hypoxia at all LPS doses, with 10,000, 10 and 1ng/mL being significant at $p < .05$, $n=4$ (Figure 1). This decrease is likely the reduced oxygen limiting aerobic respiration, though cell death from hypoxia, suggested from our LDH results, may also be responsible. LDH concentration was significantly increased by hypoxia compared to normoxic cells when LPS was present, but not by hypoxia alone. All LPS doses 10ng/mL or higher showed significantly increased LDH concentration compared to hypoxic control (min sig .05), $n=4$ (Figure 2). Predictably, all normoxic LPS dosages, and all doses above 1ng/mL in hypoxia, showed significantly higher TNF- α than control, $n=4$ (Figure 3). Not all doses differed significantly, however increasing TNF- α release trended with increasing LPS concentration. The exception was normoxic 10,000ng/mL LPS where a biphasic high (1000-3000pg/mL,) or low response (<50pg/mL, excluded herein) appeared between technical replicates. We suspect this results from the cytotoxic effect of LPS at high dosages limiting TNF- α production. qPCR analysis showed LPS 100ng/mL induced increases in TNF- α (x41.58), GAPDH (x42.86), and PDK (x24.32) expression at 21% O₂, which were reduced when combined with Hypoxia (x5.62, x4.42, x1.30 respective) $n=4$ (Figure 4). TNF- α (x30.58) and IL-6 (x25.64) were also notably increased in LPS dosed hypoxia samples compared to hypoxic controls. This indicates that while LPS promotes a proinflammatory response, 24h of hypoxia suppressed this induction.

In conclusion, this study demonstrates LPS stimulates TNF- α gene expression in both normoxia and hypoxia and TNF- α release shows evidence of dose dependency, with a potency threshold as low as 1ng/mL regardless of hypoxic status. This work further shows the combination of LPS and Hypoxic stimulation may induce cell death where LPS stimulation alone does not. Lastly, we demonstrated that 24h hypoxia (1% O₂) reduced BV-2 cell metabolism, including reduced expression of metabolically associated genes and reducing the inflammatory response of BV-2

cells induced by LPS at some concentrations. With these results, this study furthers our understanding of neuroinflammation under hypoxic conditions.

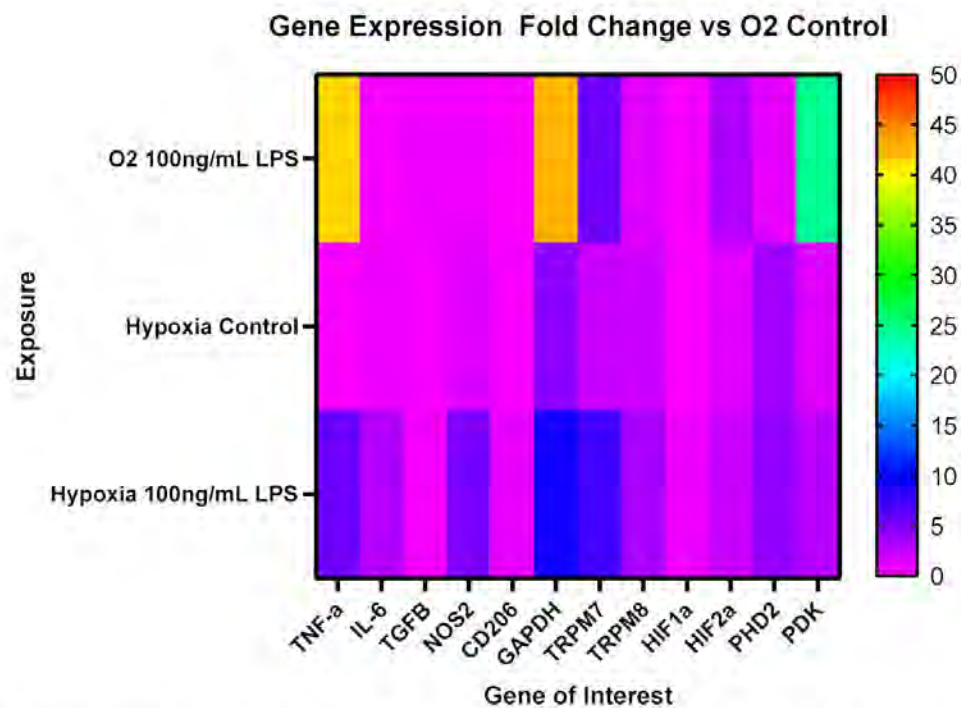


Figure4. Heat Map displaying Fold Change in expression of gene of interest compared to Normoxic (21% O₂) control. Results are given as average fold change for each exposure and gene of interest.

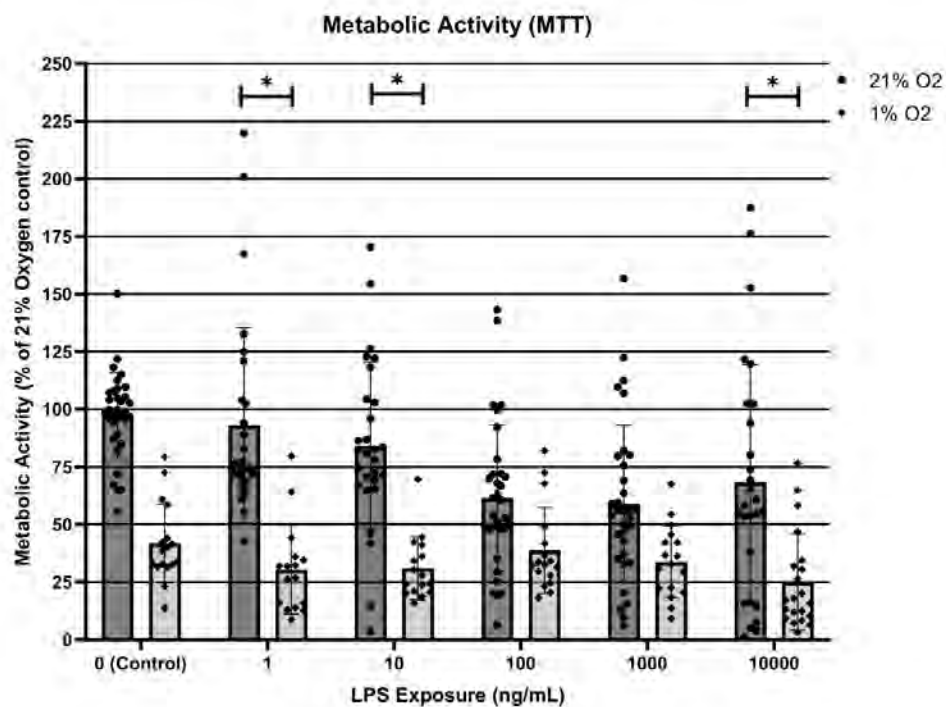


Figure1. Metabolic activity by exposure group. Graph displays average MTT result ($\pm$ SD) as a percentage of Normoxic (21% O₂) control. Significances are displayed above bars for corresponding LPS doses in Normoxia vs Hypoxia (*=P<.05.)

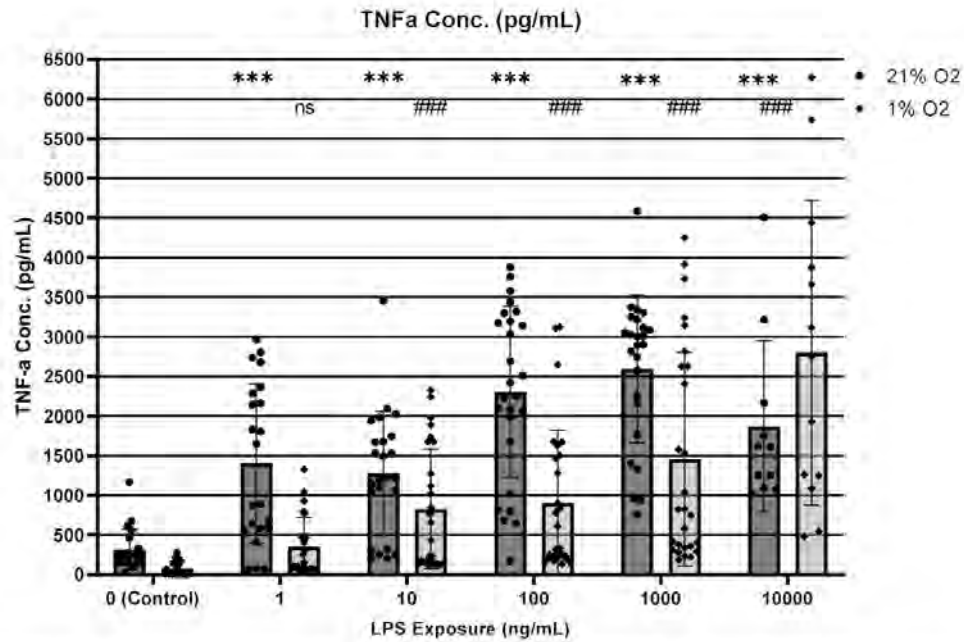


Figure 3. TNF-α concentration by exposure group. Bar displays average TNF-α Concentration ($\pm$ SD) in pg/mL with individual data points. Significances are displayed above bar compared to normoxic control (***) or Hypoxic Control (###), (ns= $P>.05$, ***/###= $P\leq.001$)

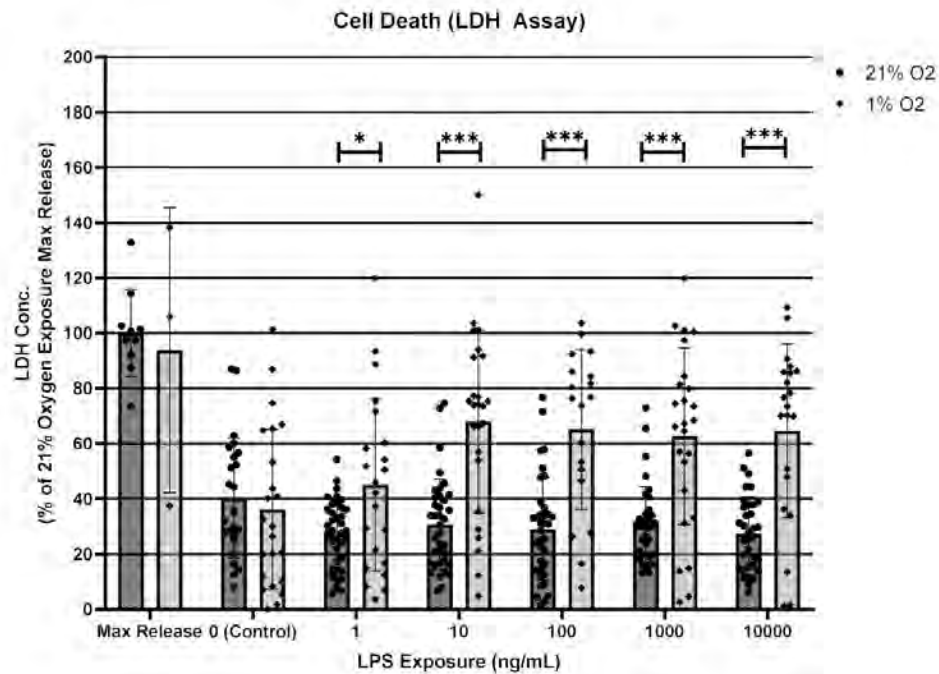


Figure2. LDH concentration by exposure group. Bars display average LDH concentration ($\pm$ SD) as a percentage of normoxic (21% O₂) control with individual data points. Significances are displayed above bars for corresponding LPS doses in Normoxia vs Hypoxia (*= $P < .05$, ***= $P \leq .001$)

PCB086

The efficacy of induced pluripotent stem cells in animal models of stroke: a systematic review

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Background: Stroke is a leading cause of death and disability which affects around 13 million people annually. Despite this, treatment options remain limited. The development of induced pluripotent stem cell (iPSC) technology by Yamanaka and colleagues offers a new opportunity for restoring function to stroke survivors. While iPSCs are showing potential in animal models, to the best of our knowledge no systematic review has yet been conducted.

Methods: We performed a keywords literature search in PubMed and Embase to identify relevant studies. Our inclusion criteria included controlled studies, any adult animal model of stroke and transplantation of iPSCs or iPSC-derived cells. Study quality and risk of bias was assessed using a 10-point CAMARADES checklist.

Results: Following screening, a total of 27 studies were included. The majority used human iPSCs (n=21) and induced ischaemic stroke using a middle cerebral artery occlusion (MCAO) model (n=19). The median score of the CAMARADES checklist was 5/10 (IQR: 4-7). While all studies were peer-reviewed and the vast majority complied with welfare regulations (88.9%), reporting of blinding to induction of stroke and assessment of outcome was low (44.4% and 48.2% respectively). A total of 21 studies reported that iPSCs lead to significant improvements in outcomes.

Conclusions: The results suggest that iPSCs show great potential for the treatment of stroke leading to improvements in neurological function and lesion volume. However, improvements in study design and reporting in future research are required.

PCB087

Blockade of hyperpolarization-activated channels with ivabradine attenuates mechanical, but not heat, hypersensitivity in two rat models of diabetic neuropathy

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Introduction: peripheral neuropathic pain (PNP), is associated with many types of injury/diseases, including diabetes mellitus (DM) that affects hundreds of millions of people worldwide. Hyperglycaemia in patients with longstanding diabetes can cause damage to peripheral nerves. This damage, known as diabetic neuropathy (DN), is the most common complication of both type 1 and type 2 diabetes mellitus, and is the most common cause of PNP. Indeed, up to 50% of people with diabetes have some degree of DN, and around 25% develop diabetic PNP (DPNP). DPNP usually experience a range of unpleasant symptoms including mechanical hypersensitivity. Despite its clinical importance, the pathophysiology of DPNP is still illusive. However, hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels, which have been implicated in the pathogenesis of other types of PNP are likely to be involved.

Aims of the study: To examine, in rat models of DPNP, whether blocking HCN channels with ivabradine, a peripherally restricted drug that is devoid of CNS side effects, and the only clinically available HCN channel blocker, would reverse/attenuate behavioural signs of DPNP.

Methods: Male Sprague Dawley rats (250-300 g, n=64) were used, and the experimental protocols were approved by University of Qatar Ethical review committee. Two models of DPNP were used: the streptozotocin (STZ) model of type 1 DM that involved a single injection of STZ (60 mg/kg, i.p.), and the high fat diet-fed, STZ (HFD/STZ) model of type 2 DM that involved a single injection of a low dose of STZ (35mg/kg, i.p. n=32 rats) after 2 weeks of feeding the rats on HFD (Skovso, 2014). Three groups of rats were used in each model: (1) vehicle (control) group (n=10); (2) Ivabradine group (10 mg/kg, i.p, n=12) and (3) Gabapentin (positive) group (n=10). Behavioural testing for mechanical and heat hypersensitivity was performed using a dynamic plantar aesthesiometer touch stimulator, and Hargreaves analgesiometer, respectively (Djouhri et al. 2019). Data were presented as the mean $\pm$ SEM, and One-way ANOVA with post hoc tests was used.

Results: Both STZ and HFD/STZ rats exhibited behavioural signs of mechanical and heat hypersensitivity as indicated by significant decreases ($P<0.001$) in the mean paw withdrawal threshold (PWT) and mean paw withdrawal latency (PWL) respectively at 35 days post treatment. A single injection of ivabradine caused a significant ($P<0.05$) increase in the mean PWT from 20.6 ± 2.6 g to 37.0 ± 2.1 g in STZ rats, and from 26.6 ± 2.9 g to 35.0 ± 2.2 g in HFD/STZ rats, at 2h, but not at 24h, post treatment. Ivabrdaine was as effective as the positive control gabapentin in attenuating mechanical hypersensitivity, but had not effect on heat hypersensitivity (no significant change in the mean PWL).

Conclusions: The findings suggest that HCN channels are involved in the mechanisms of mechanical, but not heat hypersensitivity associated with DPNP, and that their blockade with ivabradine may prove to be effective in treating DPNP in humans.

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PCB088

Minor effects of 12 week high-fat diet and/or prebiotic Xylo-oligosaccharides on cognition and brain metabolites in rats.

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Intro: Prevalence of obesity is expanding world-wide. Obesity is a severe risk factor for fatty liver and brain diseases, and problems in mental health. The role of gut microbiota (GM) in health maintenance is widely accepted; thus, the modification of GM owns huge potential for treating many diseases. *Feacaelibacterium prausnitzii* is one of the gut microbes associating with low hepatic fat (Munukka et al. 2014), and suggested to act as a possible psychobiont, a tool to modify and support mental health (Borkent et al. 2022). Because *F. prausnitzii* is extremely anaerobic, the gut level of it should be targeted via dietary means. Aims/objectives: We previously showed that a prebiotic XOS enhanced growth of *F. Prausnitzii* and ameliorated hepatic steatosis in rats (Lensu et al. 2020). Here we show the effects of high-fat diet and/or XOS supplementation on behavior and cognition, before and during the dietary intervention in adult male Wistar rats. Method: The ethical permission for the study was achieved from the National Animal Experiment Board of Southern Finland. To enhance the level of *F. Prausnitzii*, the diet of the rats (n=10 per group) was supplemented or not with prebiotic dose of XOS (0.12%, Shandong Longlive Biotechnology, CAS 87099-0), and the rats were having a simultaneous high-fat diet (60% energy from fat) to induce obesity or control diet (10% energy from fat, 'low-fat'), the details of the experiment can be found in Lensu et al. 2020. Behavior and cognition were studied with openfield, context-object recognition, and sucrose preference tasks. Untargeted metabolites from half of the brain tissue were measured by liquid chromatography – high resolution mass-spectrometry (LC-MS/MS) and curated using MS-DIAL and R. Data were tested with Generalized linear and mixed models (effect of high-fat diet and/or XOS, measurement time: pre-post) and between groups comparisons were done using non-parametric Kruskal-Wallis test. Results and conclusions: The activity of rats in the openfield-arena diminished during the 12-week intervention, independent of the group ($F [1,34] = 44.2$, $p < 0.001$), and low-fat diet enhanced anxiety-related behavior ($F [1,35.8] = 5.4$, $p < 0.05$). Low-fat diet attenuated the performance in the context-object recognition task in comparison to high-fat diet ($F [1,36] = 14.45$, $p < 0.001$), explained by increased preference of the familiar object in the low-fat group during the post-measurement. In the end of the intervention, rats having high-fat diet preferred sucrose more than those having low-fat diet ($F [1,36] = 6.59$, $p = 0.015$). For the brain metabolites, the high-fat diet group showed separation from the other groups in principal component analysis and t-stochastic neighbor embedding. Using the pathway analysis in Metaboanalyst, eight metabolic pathways were found to be significantly different ($q\text{-value} < 0.05$ and pathway impact > 0.1) between high- and low-fat groups, including purine, pyridimine, histidine and tryptophan metabolism. In conclusion, our 12-week intervention caused only minor effects on behavior and brain metabolites, and they were mainly affected by high-fat diet. Thus, our research suggests that XOS is not highly psychoactive prebiotic although it beneficially affects the GM and host's physiology.

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PCB089

Effect of GSG1L on the modulation of calcium-permeable AMPA receptor single-channel conductance by intracellular spermine

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Calcium-permeable AMPA-type glutamate receptors (CP-AMPA receptors) play essential roles in synaptic transmission and plasticity in the CNS. Type 1 transmembrane AMPAR regulatory proteins (TARPs) are CP-AMPA auxiliary subunits that slow gating, increase single-channel conductance and relieve channel block by intracellular polyamines (Soto et al., 2007). Conversely, the atypical auxiliary subunit GSG1L (germ cell-specific gene 1-like protein) (Shanks et al., 2012; Schwenk, et al 2012) decreases single-channel conductance and increases polyamine block, suppressing current flow and excitatory synaptic transmission (McGee et al., 2015; Gu et al., 2016). The aim of this study was to investigate the role of polyamines in the opposing effects of these different auxiliary subunits by examining the effect of spermine on CP-AMPA receptors expressed with and without GSG1L and the type 1 TARP $\gamma 2$. AMPARs were transiently transfected into Human Embryonic Kidney (HEK 293) cells and currents were recorded from outside-out patches in response to rapid application of glutamate (10 mM) achieved through piezoelectric translation of a theta-barrel application tool. Macroscopic current-voltage relationships were examined from -120 to $+100$ mV to characterise spermine-dependent rectification. To estimate the weighted mean single-channel conductance we used nonstationary fluctuation analysis (NSFA). We also analysed directly resolved channel openings in the tail of macroscopic currents at -120 mV. In the absence of auxiliary subunits, elevating intracellular spermine from $100 \mu\text{M}$ to 1 mM unexpectedly, decreased single-channel conductance of GluA2(Q), as measured with NSFA, by $\sim 50\%$ (from 20.6 ± 1.6 to 9.1 ± 2.2 pS; $p < 0.0001$; Welch t test; $n = 12$ and 5 , respectively). As TARP $\gamma 2$ greatly reduces the block of CP-AMPA receptors by intracellular spermine, we predicted that in its presence the polyamine-dependent reduction of GluA2(Q) conductance would also be reduced. This was indeed the case. With co-expression of $\gamma 2$ the effect spermine was eliminated (30.9 ± 2.3 and 27.4 ± 3.7 pS with $100 \mu\text{M}$ and 1 mM spermine, respectively; $p = 0.45$; unpaired Welch two-sample t test; $n = 10$ and 4). In contrast, GSG1L increased the effect of spermine on channel conductance. In the presence of just $100 \mu\text{M}$ intracellular spermine, the single-channel conductance was 12.7 ± 0.6 pS ($n = 18$; $p = 0.00031$ compared to GluA2(Q) alone and $p = 0.011$ compared to the spermine-free condition; $n = 14$). This reduction in NSFA-estimated single-channel conductance of GluA2(Q)/GSG1L by $100 \mu\text{M}$ spermine was mirrored by its effect on the mean amplitude of directly resolved single-channel events (reduced from 21.3 ± 1.4 to 15.7 ± 0.3 pS; $p = 0.016$; unpaired Welch two-sample t test; $n = 5$ and 5). Finally, we observed that GSG1L and spermine reduced channel conductance only when aspartate was present at the AMPARs Q/R +4 site in the channel's ion selectivity filter. Together, our results demonstrate that polyamines and GSG1L cooperate to attenuate CP-AMPA receptor conductance. Crucially, this unexpected property of intracellular polyamines is apparent at physiologically relevant negative membrane potentials.

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PCB090

The role of BK in glioblastoma multiforme membrane potential

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Introduction

Glioblastoma multiforme (GBM), is an aggressive brain tumour that accounts for nearly half of all glial brain tumours. Large conductance voltage and Ca^{2+} -activated potassium channels, BK, are overexpressed in GBM and are thought to play a role in their invasion and migration. Although these processes are modulated by changes in resting membrane potential, V_m , little is known about the origin of V_m and the role of BK. We have used cell-attached and whole-cell patch clamp in the glioblastoma cell line, SF188 to investigate the role of BK in GBM V_m .

Method

Single-channel BK currents were measured with cell-attached patch-clamp (CA). Pipettes contained 140 mM K^+ . Currents were measured with holding potentials from 0 mV to -90mV. V_m of intact cells were estimated from the reversal potential of CA single-channel BK current-voltage plots (i-V). V_m was then also measured with current clamp immediately after forming the whole-cell (WC) configuration. WC current-voltage-relationships (I-V) were characterised with voltage step protocol from a holding potential of -60mV. Data were normally distributed and are expressed as means $\pm$ S.D with n the number of cells. Statistical significance is defined as $p < 0.05$ with tests stated.

Results

At a pipette-potential of 0 mV, KB was spontaneously active in 23 out of 49 CA patches. CA I-V analyses indicated voltage-dependent activation of BK with a median slope-conductance of 202 pS. V_m estimated from the BK I-V reversal potential, -34.9 ± 10.9 mV ($n=21$) was similar ($p=0.9221$, Paired t-test) to that subsequently measured under WC current clamp: -29.7 ± 13 mV ($n=7$) ($[\text{Ca}^{2+}] = 45$ nM). With a high $[\text{Ca}^{2+}]$ pipette solution (1.5 mM) V_m became significantly hyperpolarized in WC current clamp (-46.4 ± 16.3 mV, $n=14$; $p=0.001$, Unpaired t-test). With the high pipette $[\text{Ca}^{2+}]$ the WC input resistance, R_m , was 221 ± 194 MW ($n = 8$; $p=0.0443$, Unpaired t-test); a value significantly smaller ($p=0.0443$, Unpaired t-test) to that measured with low pipette $[\text{Ca}^{2+}]$: 394 ± 194 MW ($n=8$). In 100% of CA patches, BK activity was abolished following perfusion of either 1 μM paxilline ($n=3$) or 200 μM quinine ($n=3$). With a low pipette $[\text{Ca}^{2+}]$, WC V_m was unaffected by 1 mM TEA ($n=15$) or 1 μM paxilline ($n=7$), but was significantly depolarised by 21 ± 3.3 mV with 200 μM quinine ($n=3$) relative to perfusion control ($n=4$; $p=0.0091$ ANOVA Dunnet's multiple comparison test).

Conclusion

GBM SF188 cells exhibit spontaneous K^+ channel activity in CA patches, with biophysical and pharmacological properties typical for BK. At low intracellular $[Ca^{2+}]_i$ BK does not appear to be responsible for the resting V_m , however the hyperpolarization of V_m associated with a decrease in R_m that is seen on elevation of $[Ca^{2+}]_i$ are indicative of BK activation. The reversal potential of BK in CA patches appears to be an accurate non-invasive measure of the resting membrane potential of SF188 cells. Further studies are required to determine what underlies the BK activation observed in CA patches on SF188 and to find under what physiological conditions does BK become activated to contribute to V_m in this cell line.

PCB091

Intra-Arterial Injection of Bradykinin In Femoral Artery Elicits Cardiorespiratory Reflexes Involving Perivascular Afferents In Rat Models

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Introduction: The physiology of baroreceptors and chemoreceptors present in the heart is well documented in regulation of cardiorespiratory functions. Since large blood vessels of the heart and peripheral blood vessels are of same origin, therefore, the involvement of the peripheral blood vessels in regulation of cardiorespiratory system can be anticipated. **Aims:** In this study, role of peripheral blood vessels in regulation of cardiorespiratory system was examined using bradykinin (BK) as a nociceptive tool. **Methods:** Ethical Approval was taken from the Institutional Ethical Committee, Banaras Hindu University, Varanasi, India prior to the beginning of the experiments. Role of perivascular sensory nerves in mediating cardiorespiratory responses produced after intra-arterial injection of BK (1 μ M, a pure nociceptive agent) was examined in urethane anesthetized male rats. Respiratory frequency, blood pressure, and heart rate were recorded for 30 min after the retrograde injection of BK/diclofenac/saline. Additionally, paw edema was estimated and water content was expressed as percentage of wet weight.

Results: The results are presented as mean $\pm$ SEM values. Injection of BK produced immediate tachypnoeic (86 ± 2.7 to 125 ± 5.6 per min), hypotensive (82 ± 4.1 to 49 ± 3.6 mm Hg) and bradycardiac (321 ± 4.2 to 266 ± 5.2 beats/min) responses of a shorter latency i.e. 5-8 s. Injection of equi-volume of saline did not produce any responses and served as time matched control. Paw edema was observed in the ipsilateral hind limb and contralateral hind limb as control. Ipsilateral femoral and sciatic nerve sectioning attenuated BK-induced tachypnoeic (87 ± 2.3 to 98 ± 3.2 per min), hypotensive (89 ± 3.5 to 75 ± 5.8 mm Hg) and bradycardiac (316 ± 6.2 to 303 ± 7.3 beats/min) responses significantly which indicate the origin of responses from the local vascular bed. Pretreatment with diclofenac sodium significantly attenuated the BK-induced tachypnoeic, (88 ± 3.1 to 94 ± 3.3 per min) hypotensive hypotensive (85 ± 1.8 to 78 ± 1.8 mm Hg) and bradycardiac (320 ± 5.1 to 308 ± 5.9 beats/min) responses and also blocked the paw edema. Post-Hoc correction using Dunnett's t-test (two sided) and Student's t-test for paired observations were used and a p value < 0.05 was considered as significant.

Conclusions: Administration of BK in the segment of an artery produced reflex cardiorespiratory changes by stimulating the nociceptors surrounding the blood vessels involving prostaglandins. This is a novel study exhibiting the role of peripheral blood vessels in regulation of cardiorespiratory system.

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PCB092

Testing the Albus model of cerebellar learning in human subjects

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The cerebellum is accepted to have a crucial role in classical conditioning. Following the classical work of Eccles and co-workers in elucidating its detailed neurophysiology (Eccles et al., 1967), several theorists developed computational models of cerebellar learning (Albus, 1971). In Albus' original conception, the so-called 'inactivation response' of a Purkinje cell (PC), a pause in PC spontaneous activity associated with a climbing-fibre response (CFR), could be interpreted as the internal neural representation of the overt unconditional response (UR). He further suggested that mossy/parallel fibre (MF/PF) activity produced by the conditional stimulus (CS) could be considered its internal neural representation. The effect of learning, by changing PF-PC synaptic weights with conjunctive CF/PF inputs, and the acquisition of a conditioned response would, he hypothesized, be accompanied by a conditioned pause of Purkinje neurones. Direct recordings from animal models have since provided evidence to support the Albus hypothesis. There is also strong evidence from the effects of lesions in humans that the cerebellum is required for the acquisition of classically conditioned eye blink responses. However, to date the Albus model has not been directly tested in intact human subjects.

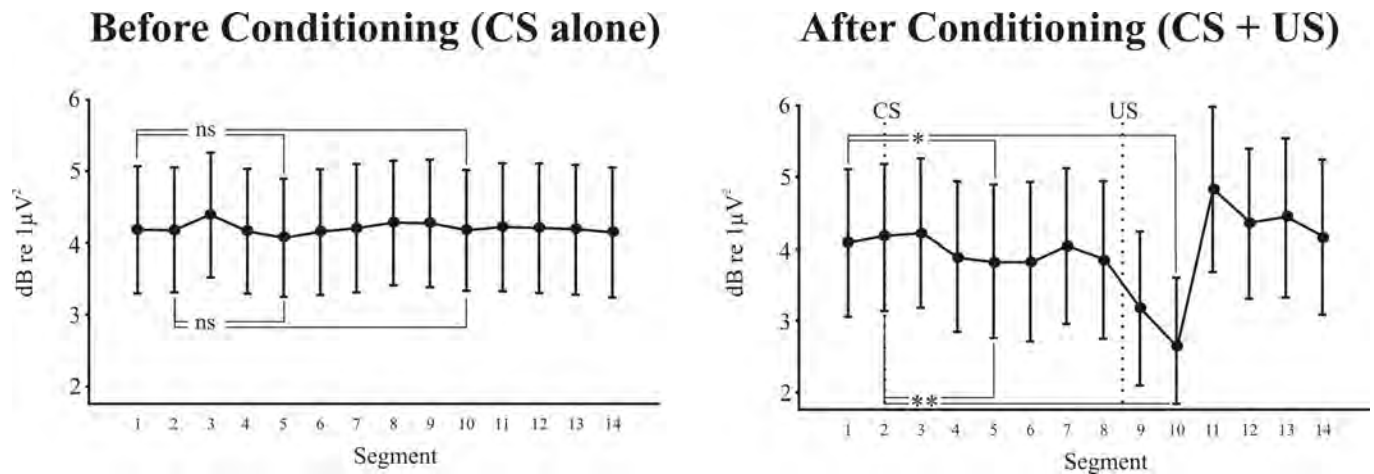
It had been widely thought that the cerebellum is particularly difficult to record from non-invasively. However, recent work using EEG/MEG techniques supports the view that non-invasive electrophysiology of the cerebellum is indeed viable. In our own work we have reported cerebellar evoked potentials produced by vestibular and axial stimuli from scalp electrodes from placed over the posterior fossa the properties of which are consistent with a CFR (Todd et al., 2017). In addition, we also observe an 'inactivation response' manifest as changes in the high frequency electrocerebellogram (ECeG: Todd et al, 2018) which has a higher frequency content than cerebral EEG. These observations suggest that it may be possible to directly test the Albus hypothesis in humans and this was the aim of the study reported here.

Electrophysiological activity was recorded in 14 healthy subjects (compliant with the Declaration of Helsinki) before, during and after a classical eye-blink conditioning procedure with a 500 ms auditory tone as CS and a maxillary nerve US (Todd et al 2023). Electrodes recorded EMG/EOG at peri-ocular sites, EEG over frontal eye-fields and the ECeG over the posterior fossa. ECeG high frequency power was computed using the continuous wavelet transform and each epoch segmented in time for statistical analysis before and after conditioning.

Of the 14 subjects half strongly conditioned while the other half were resistant. However, inhibition of cerebellar activity in the form of a significant reduction in high frequency ECeG power was observed in all subjects, prior to the CR, as shown in Figure 1. Pairwise comparisons of baseline, with conditioned and unconditioned pausing are given: *, $p < .05$, **, $< .01$, ***, $< .005$, ns = not significant.

We conclude that while conditioned cerebellar pausing may be necessary, it is not sufficient alone to produce overt behavioural conditioning, implying the existence of another central

mechanism. The outcomes of this experiment indicate the value of the non-invasive electrophysiology of the cerebellum.



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