

Physiology 2021

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Abstracts

Experiments on animals and animal tissues

It is a requirement of The Society that all vertebrates (and *Octopus vulgaris*) used in experiments are humanely treated and, where relevant, humanely killed.

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Abstracts of Communications or Demonstrations must state the type of animal used (common name or genus, including man). Where applicable, abstracts must specify the anaesthetics used, and their doses and route of administration, for all experimental procedures (including preparative surgery, e.g. ovariectomy, decerebration, etc.). For experiments involving neuromuscular blockade, the abstract must give the type and dose, plus the methods used to monitor the adequacy of anaesthesia during blockade (or refer to a paper with these details).

For the preparation of isolated tissues, including primary cultures and brain slices, the method of killing (e.g. terminal anaesthesia) is required only if scientifically relevant.

In experiments where genes are expressed in *Xenopus* oocytes, full details of the oocyte collection are not necessary.

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PL01

Teaching physiology: Past present and future

James Clark¹

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Educating our expanding undergraduate student cohorts in whole body physiology is complex, and to do this effectively can sometimes be a challenge. I was awarded the Otto Hutter Physiology Teaching Prize in 2019 (pre-COVID) and my oral presentation at Physiology 2020 was *going* to be looking at the role of online teaching in physiology education. However, the recent global COVID-19 Pandemic has hit physiology education hard with restrictions on face-to-face on-campus teaching and instructors have had to be more and more imaginative to delivery high quality online teaching. In this presentation, therefore we will explore the challenges of physiology education with a specific focus on practical skills and human physiology looking back at our traditional approach, how we responded to the COVID-Pandemic and the ways we might be educating our students in the socially distanced future within our Institutions.

PL02

Hidden clocks: circadian rhythms and the vulnerable fetus.

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Despite marking our age from the day of birth, our physiological journey starts 9 months before; assuming we went to full term gestation. During this time we build our existence upon the merging of two cells, growing an entire human being, the environment it lives in; the amniotic sac, and the organ of gas, nutrient and waste exchange; the placenta. An undeniably fantastic bioengineering feat, and one which provides the physiological foundation for our post-natal development to adulthood. However, our physiological journey from conception to birth can face adverse challenges, such as hypoxia and inflammation, leading to impaired development of organs and their functional control and injury, all of which has consequences for our post-natal physiology. Further, such adverse events may cause the fetus to be vulnerable to further challenges to its environment leading to death before birth, as is seen with stillbirth. To develop methods of detecting the at-risk-fetus to inform clinical decision making and targeting of perinatal treatments requires a greater understanding of the maturational changes in fetal physiology and pathophysiology. This includes the role of a key regulator of our own biology; circadian rhythms.

This talk will discuss how a preterm fetus can survive a significant hypoxic insult and continue to develop to full maturity, but with evolving brain injury. It will examine how injury and/or impaired brain development may make the fetus more vulnerable to further episodes of hypoxia. The talk will then explore how we can utilise information about the temporal changes in fetal physiology and behaviour after hypoxia to develop potential clinical biomarkers to detect an adverse event and determine phases of injury. This is vital for facilitating implementation of targeted therapies and inform clinical decision making. Here, time of day is an important factor as the fetus has robust circadian and ultradian rhythms, but their nature and functional role are poorly understood. The talk will present new data about fetal cardiovascular and neurophysiological circadian and ultradian rhythms. It will detail how they change with maturation and are altered by hypoxia and inflammation. The talk will highlight the importance of the time of day when utilising fetal diagnostic and prognostic biomarkers and the implications for the timing of routine clinical assessments. Finally, the talk will end with reflection upon the challenges we face to improve our understanding about the first 9 months of our physiological journey if we are to optimise our physiological development from birth to adulthood.

Acknowledgements :- The Fetal Physiology and Neuroscience Group, Department of Physiology, The University of Auckland

The Health Research Council of New Zealand

PL03

Regulation of blood flow at the capillary level in health and disease

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It is often assumed that local increases of tissue blood flow are mediated by relaxation of arteriolar smooth muscle but in many tissues, including the brain, heart, kidney and pancreas, capillary control of blood flow by contractile pericytes also occurs. In the brain, most of the adjustable resistance of the intra-cerebral vasculature is located in capillaries. I will demonstrate that neuronal activity mainly increases cerebral blood flow by dilating capillaries via pericytes, that this involves signalling via astrocytes, and that dilation of capillaries and of arterioles are mediated by different messengers. In the brain, heart and kidney, ischaemia leads to pericytes constricting, producing a long-lasting decrease of blood flow after ischaemia. Constriction of cerebral capillaries by pericytes also occurs at an early stage of Alzheimer's Disease, when it is expected to amplify the production of amyloid beta. The SARS-CoV-2 virus causing Covid-19 binds to ACE2 on cerebral pericytes and amplifies angiotensin II - evoked pericyte constriction by decreasing ACE2 function. Thus, awareness of the possibility of pericyte-mediated capillary constriction reveals new therapeutic targets to increase blood flow in numerous pathologies.

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PL04

The gut endocrine axis in the control of metabolism

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The gut endocrine system comprises a collection of enteroendocrine cells scattered throughout the intestinal epithelium, producing hormones that signal locally within the gut and distantly at tissues such as the brain and pancreas. In the field of diabetes and obesity, the best studied gut hormone is glucagon-like peptide-1 (GLP-1), which has been exploited therapeutically for the treatment of type 2 diabetes and obesity through the development of GLP-1 receptor agonists and DPP4 inhibitors.

Our research is focussed on gaining a molecular understanding the enteroendocrine system and its involvement in the control of metabolism and food intake. Technical advances now make it possible to apply live single cell recording and transcriptomic techniques to human enteroendocrine cells using intestinal organoid models engineered by CRISPR-Cas9 to express fluorescent sensors in specific cell types of interest. Mirroring previous findings from mouse models, we have shown that human GLP-1 secreting cells utilise a variety of signalling pathways for nutrient detection, including electrogenic glucose uptake and activation of specific G-protein coupled receptors by free fatty acids and amino acids.

Overall, we aim that our research will identify new drugs for type 2 diabetes and obesity that act by targeting gut endocrine cells, thus mimicking the gut endocrine consequences of bariatric surgery.

PL05

Local Iron Control- Mechanisms, Importance and Implications

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Our knowledge of iron homeostasis has grown enormously since the discovery of hepcidin at the turn of the century. Hepcidin was discovered as a liver-derived hormone that controls circulating iron levels by binding and degrading the iron exporter ferroportin in the gut and spleen, respective sites of iron absorption and recycling. Until recently, the consensus was that iron levels within tissues

were a function of circulating iron availability and controlled primarily at the point of uptake. Recent work from our laboratory has changed that consensus. We have discovered regulated iron export as an essential mechanism of local iron control in the heart, pulmonary vasculature, renal tubules and fetal liver¹⁻⁵. These tissues produce their own hepcidin for cell-autonomous regulation of ferroportin-mediated iron export. Disruption of local this iron control in a manner that increases or decreases iron levels in these tissues is sufficient to impair their physiological function, even against a background of normal circulating iron levels. Importantly, we found that the pathways mediating the pathophysiological effects of disrupted local iron control differ from tissue to tissue, revealing new, unexpected and diverse functions for local iron control in systems physiology. These discoveries change our understanding of iron disorders from the simplistic notions of “too little” or “too much” iron, to that of sophisticated tissue-level iron control. They also highlight local iron-regulated pathways as new therapeutic targets in certain pathophysiologies. Our aim now is to translate these discoveries into better clinical management of iron disorders, particularly iron deficiency of chronic disease.

Reference 1 :- **Lakhal-Littleton S** , Wolna M, Carr C, Miller J, Christian H, Ball V, Santos A, Diaz R, Biggs D, Stillion R, Holdship P, Larner F, Tyler D, Clarke K, Davies , Robbins P. 2015 Cardiac ferroportin regulates cellular iron homeostasis and is important for cardiac function. *Proc Natl Acad Sci USA* 10;112(10):3164-9

Reference 2 :- **Lakhal-Littleton S**, Wolna M, Christian H, Santos A, Diaz R, Biggs D, Davies B , Robbins P. 2016 An essential cell-autonomous role for hepcidin in cardiac iron homeostasis. *Elife*. Nov 29;5.

Reference 3 :- **Lakhal-Littleton S**, Crosby A, Frise M, Mohammad G, Carr C, Loick P, Robbins P. 2019 Intracellular iron deficiency in pulmonary arterial smooth muscle cells induces pulmonary arterial hypertension in mice. *Proc Natl Acad Sci USA*. 116 (26) 13122-13130.

Reference 4 :- Mohammad G, Matakidou A, Robbins P, **Lakhal-Littleton S**. 2021 The renal hepcidin/ferroportin axis controls iron reabsorption and determines renal and hepatic susceptibility to iron overload. *Kidney International* (in press)

Reference 5 :- Kammerer L, Mohammad G, Wolna M, Robbins P, Lakhal-Littleton S. 2020 Fetal liver hepcidin supports acquisition of iron stores in utero. *Blood* 136 (13): 1549–1557

Acknowledgements :- This work was supported by funding from the British Heart Foundation, the Medical Research Council, Kidney Research UK and Vifor Pharma.

PL06

Short Palate and Nasal Epithelial Clone 1 (SPLUNC1) is an Allosteric Regulator of Cation Channels

Robert Tarran¹

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Plasma membrane cation channels play critical roles in lung health: The epithelial Na⁺ channel (ENaC) is expressed in pulmonary epithelia where it regulates fluid homeostasis and mucus

clearance. The Orai1 Ca^{2+} channel is ubiquitously expressed and controls gene expression and inflammation, to list but two of its functions. SPLUNC1 (gene name BPIFA1) is a 256 amino acid long protein that is secreted from airway surface epithelia and glands into the lung lining fluid. We were initially interested in identifying secreted proteins that could regulate ENaC in airway epithelia. Using a proteomic screen, we identified SPLUNC1 as a protein that could interact with ENaC. Subsequent studies confirmed that SPLUNC1 bound to and internalized ENaC to reduce Na^+ and fluid absorption¹. Fluorescence resonance energy transfer (FRET) studies demonstrated that extracellular SPLUNC1 binding to ENaC caused intracellular changes in ENaC (i.e. allostery). We also found that SPLUNC1 binding caused the ubiquitin ligase NEDD4-2 to ubiquitinate ENaC and send it to lysosomes for degradation. Indeed, immobilized ENaC concatamers, or expression of a dominant negative form of NEDD4-2 were no longer internalized². We found that a region in human SPLUNC1's N-terminus, historically called the S18 region, was responsible for the ENaC-mediated inhibition, and peptides that corresponded to this region fully replicated this inhibition. Murine SPLUNC1 does not express the S18 region and cannot regulate ENaC. However, we noticed that SPLUNC1^{-/-} mice exhibited both spontaneous airway hyperreactivity and increased ex vivo airway smooth muscle contraction, which could be rescued with recombinant SPLUNC1³. Further study indicated that SPLUNC1 inhibited Orai1-dependent, store operated Ca^{2+} entry in both human and murine tissues. However, while ENaC inhibition was mediated by SPLUNC1's N-terminus, Orai1 inhibition was mediated by SPLUNC1's C-terminus. Surprisingly, SPLUNC1's C-terminus induced NEDD4-2-dependent ubiquitination, internalization and lysosomal degradation of Orai1, without affecting ENaC. Cystic fibrosis is an inherited disease where mutations in the CFTR anion channel lead to chronic airway infection and inflammation. We recently found that CF patients with reduced SPLUNC1 levels undergo more frequent acute exacerbations and have greater rates of hospitalization⁴. Similarly, reduced SPLUNC1 levels correlate with increased inflammation and reduced lung function in asthma patients⁵. These data suggest that restoring SPLUNC1 function would benefit both asthma and CF patients. We have developed proteolytically-stable peptides based on SPLUNC1's C-terminus, and found that they exhibit potent anti-inflammatory actions in asthma, acute infection and CF murine models, indicating that they are suitable for development as anti-inflammatory compounds. Work in this area is ongoing in the Tarran lab.

Funded by the NIH and the CF Foundation.

RT has equity in Eldec Pharmaceuticals.

1. Garcia-Caballero A et al. PNAS (2009).
 2. Kim CS et al. FASEB J. (2018).
 3. Wu T et al. Nat Commun. (2017).
 4. Khanal et al., ERJ, in press (2021).
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PL07

A Colourful Experience

Semir Zeki¹

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I will describe my discovery of the colour centre, area V4, in the primate brain, damage to which in the human brain leads to the syndrome of cerebral achromatopsia, or colourless vision. V4 was full of surprises, which challenged what we thought we knew about colour theory. As we explored it, it became increasingly difficult to deal with that discovery exclusively in terms of the classical colour theories of trichromacy and opponency, neither of which accounted adequately for the perceptual constancy of colour categories.

To explore V4 further we needed a new theoretical and methodological approach, using a more natural experimental paradigm than the traditional reduction screen, in which the colour of a 'point', or patch, isolated from its surrounds is manipulated by varying its constituent amounts of long, middle and short wave light. This was especially so since many V4 cells are selective for *hues* rather than to lights of specific wavelength. There thus appeared to be a major electrophysiological disconnect between the perceived colour and the stimulating wavelengths that was not evident when using old methods which relied on the colour of isolated 'points'. What was required was a clear electrophysiological demarcation, not made before, between colour and wavelength. The Land Colour Mondrian, a relatively simple abstract multi-coloured composition with no recognisable shapes, provided the methodological platform needed to explore this physiological distinction. The use of Land's paradigm allowed me to demonstrate that wavelength selective cells are not necessarily colour selective and that hue selective cells respond to a patch of their preferred hue regardless of the precise wavelength- energy composition of the light reflected from it alone, thus demonstrating a physiological, single-cell, counterpart to perceptual colour categorisation constancy. Moreover, I found that wavelength opponent cells can be made to give an "ON" or "OFF" response to a patch of any hue of the Mondrian display placed in their receptive fields, by simply adjusting the wavelength composition of the light reflected from the patch, without changing its colour category.

More recent use of the Colour Mondrian in perceptual experiments has shown that the colour of the after-image produced by viewing a patch in a Mondrian context is, like the colour of the patch itself, independent of the precise-wavelength energy composition of the light reflected from it (e.g. the after image of a green surface that is reflecting more red than green light is magenta, just like the after-image of a green surface that is reflecting more green than red light). The use of theoretical and experimental approaches that are better adapted to studies of cortical involvement in colour vision, has thus revealed new and unsuspected ways of how the brain constructs colours.

In short, one of the most venerable and foundational fields of natural philosophy and modern science remains a pioneering field, as open-ended and as important as before for our understanding of how we obtain knowledge of our world through colour.

SA01

Changes in cellular Ca^{2+} and Na^+ regulation during the progression towards heart failure

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This symposium talk aims to give an overview of the role played by the cardiac sodium channel in the decline in function of the heart as it begins to fail. The following summary will provide some background for the talk.

In adapting to disease and loss of tissue the heart shows great phenotypic plasticity that involves changes to its structure, composition and electrophysiology. In parallel, global cardiovascular adaptations occur involving stimulation of the sympathetic nervous system, release of natriuretic peptides and activation of the renin-angiotensin-aldosterone pathway. These adaptations provide compensation for the initial decline in cardiac function but, for reasons not understood, do not provide continued benefit in the longer term. The heart begins to fail in its task to produce sufficient cardiac output to meet the body's requirements for oxygen and nutrients. Heart failure is a chronic and progressive condition with very poor prognosis. Approximately 60% of people diagnosed with heart failure are dead within five years and, although exact percentages depend on the type of failure, up to half of those will die from disturbances of rhythm.

At the cellular level compensation responses involve a spectrum of changes to structure, electrophysiology and Ca^{2+} homeostasis. There is an increase in late Na^+ current and a decrease in Na^+/K^+ ATPase current. The cardiac myocytes gain Na^+ and this alters the balance of Ca^{2+} flux mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchange that limits early contractile impairment. Action potential duration prolongs as a result of the increase in late Na^+ current and changes in the expression and function of other ion channels and transporters, notably those carrying K^+ . Cytosolic Na^+ perturbations can disturb Ca^{2+} -dependent energy metabolism and reactive oxygen production in mitochondria reducing energy supply.

The normal spatial arrangements of Ca^{2+} -handling proteins are essential for efficient excitation–contraction (EC) coupling and the stability of this amplification mechanism. There is a reduction in T-tubule density in myocytes from failing hearts leading to an increased spatial separation of the junctional SR from the T-tubule membrane. These structural changes are thought to underlie temporal delays in EC coupling and an increase in spontaneous Ca^{2+} -release events (Ca^{2+} sparks).

It is likely that these structural and electrophysiological responses occur at the expense of (1) increasing the likelihood of arrhythmogenesis, (2) activating hypertrophic and apoptotic signalling

pathways and (3) decreasing the efficiency of EC coupling. The combination of action potential prolongation, altered Na^+ regulation, inconsistent Ca^{2+} release from SR and a less stable ventricular membrane potential provides a setting for triggered arrhythmias initiated either by early or delayed afterdepolarizations. The cardiac sodium channel plays an important role in the passage of the heart from function to dysfunction.

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SA02

Cardiac sodium channel (dys)function and arrhythmias: novel mechanisms and therapeutic targets

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The cardiac sodium channel Nav1.5, encoded by the *SCN5A* gene, is a key regulator of cardiac conduction, and sodium channel dysfunction may lead to cardiac arrhythmias and sudden cardiac death. Mutations in *SCN5A* are associated with a myriad of clinical syndromes, including Brugada syndrome, Long QT syndrome type 3, cardiac conduction disease, and overlap syndromes. The underlying mechanisms include a decrease in peak sodium current leading to conduction abnormalities and/or an increase in late sodium current causing repolarization disturbances. Yet, some Nav1.5-related disturbances, in particular structural abnormalities, may not be directly or solely explained on the basis of defective Nav1.5 expression or biophysics. An emerging concept that may explain the large disease spectrum associated with *SCN5A* mutations centres around the diversity, complexity and multifunctionality of the Nav1.5 complex. For instance, alterations in Nav1.5 may affect processes that are independent of its canonical ion-conducting role, including intracellular homeostasis. In addition, subcellular pools of Nav1.5-based sodium channels within the cardiomyocyte have been demonstrated, in particular at the intercalated disc and lateral membrane regions, where they associate with specific interacting proteins and display distinct functional properties. The trafficking pathways through which sodium channels reach their various subcellular destinations within the cardiomyocyte are now coming into focus. This lecture will address these recent insights and discuss the potential implications for the development of novel therapeutic strategies.

SA03

Structural and functional roles of the $\beta 3$ -subunit on the cardiac sodium channel: implications for arrhythmogenesis.

Samantha C Salvage¹, Christopher L-H Huang^{1, 2}, Antony P Jackson¹

The cardiac sodium channel, Nav1.5, drives the rising phase of the cardiac action potential. It comprises an ion-conducting α -subunit together with one or more regulatory β -subunits of which there are four main isoforms, β 1- β 4. The β 3-subunit is thought to influence Nav1.5 trafficking and gating; its mutations or knock-outs are associated with a variety of cardiac arrhythmic conditions including Brugada Syndrome (BrS; 1, 2). Available cryo-EM structures suggest multiple points of interaction in α - β subunit combinations. Previous evidence highlights a particularly important role for the extracellular immunoglobulin (Ig) domain of β 3 in functional modulation of Nav1.5 (3).

We have utilized a combination of electrophysiological, biochemical and cell-biological techniques to evaluate the functional behaviour of the Nav1.5- β 3-subunit complex *in vivo* (4, 5). We show that the β 3-subunit can form monomers, dimers and trimers within the plasma membrane independently of its binding of the Nav1.5 α -subunit. Using super-resolution stochastic optical reconstruction microscopy (STORM), we show that the Nav1.5 α -subunit assembles into localised, higher-order, two-dimensional clusters within the plasma membrane both in the presence and absence of the β 3-subunit. Although the β 3-subunit did not affect the average number of α -subunits within these clusters, it significantly increased the cluster radii (KS test, $P = 4 \times 10^{-9}$), suggesting that orientation and cluster packing of the Nav1.5 α -subunits is sensitive to the presence of the β 3-subunit. Whole-cell patch-clamp experiments identified a depolarising shift of steady-state inactivation in the presence of β 3 as well as an accelerated recovery from inactivation which would act to enhance channel availability. Following on from this we have used site-directed mutagenesis to investigate two unusual mutations in structurally distinct regions of the β 3-subunit. One mutation is a novel in-frame threonine deletion within the extracellular Ig domain recently identified in a BrS patient in the absence of any other causative mutations. Whole cell patch-clamp experiments highlighted a reduction in peak current compared to wild-type β 3 and a loss of the depolarising shift of steady-state inactivation. Yet this mutation did not abolish the Nav1.5- β 3 interaction. The second mutation is targeted at an unusually positioned and highly conserved glutamic acid residue within the transmembrane domain of the β 3-subunit. This mutation selectively abrogated the acceleration of recovery from inactivation that is normally exerted by wild-type β 3.

Our work highlights multiple structural and functional interactions between Nav1.5 and β 3-subunits in which the latter plays crucial roles, both in regulating normal Nav1.5 function and in the higher-order organisation of Nav1.5 within the plasma membrane. Future experiments will investigate the stoichiometry of these interactions and how mutations affect the oligomeric structures.

Reference 1 :- Hakim, P. *et al.* Scn3b knockout mice exhibit abnormal ventricular electrophysiological properties. *Prog. Biophys. Mol. Biol.* **98**, 251–266 (2008).

Reference 2 :- Hu, D. *et al.* A mutation in the beta 3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. *Circ. Cardiovasc. Genet.* **2**, 270–8 (2009).

Reference 3 :- Salvage, S. C., Huang, C. L.-H. & Jackson, A. P. Cell-adhesion properties of β -subunits in the regulation of cardiomyocyte sodium channels. *Biomolecules* **10**, 989 (2020).

Reference 4 :- Salvage, S. C. *et al.* Supramolecular clustering of the cardiac sodium channel Nav1.5 in HEK293F cells, with and without the auxiliary $\beta 3$ -subunit. *FASEB J.* **34**, 3537–3553 (2020).

Reference 5 :- Salvage, S. C. *et al.* Gating control of the cardiac sodium channel Nav1.5 by its $\beta 3$ -subunit involves distinct roles for a transmembrane glutamic acid and the extracellular domain. *J. Biol. Chem.* **294**, (2019).

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SA04

Structural insight to sodium channel function

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Voltage-gated sodium channels enable the translocation of sodium ions across cell membranes and play crucial roles in electrical signalling by initiating the action potential. In humans, sodium channel mutations give rise to a number of cardiovascular diseases, hence they are important targets for pharmaceutical drugs. The NavMs channel has been shown to be a good model for human channels, based on its high levels of sequence, structural and functional similarities to the human Nav1.5 isoform. Complexes of NavMs have been studied using crystallography, cryo-electron microscopy, and biophysical techniques including circular dichroism spectroscopy, bioinformatics, and molecular dynamics calculations, enabling identification of binding sites, molecular interactions, and the functional effects of both on-target and off-target drugs which bind to sodium channels. These studies can provide crucial information for the development of new pharmaceuticals and for the understanding of side-effects of currently-prescribed drugs.

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SA05

Overcoming amenorrhea in athletes with high training loads

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Menstrual function can be affected in female athletes with high training loads, ultimately resulting in functional hypothalamic amenorrhea. Can athletes overcome the effect of high training loads while maintaining a busy training and competitive schedule?

Since early reports of high prevalence of amenorrhea in athletes in the 1980s, decades of research support the idea that it is not training load *per se* that induces menstrual dysfunction. Instead, the aetiology of this condition may be the decreased energy available to sustain normal physiological function, what has also been referred to as 'low energy availability'.

To recover from the chronic effects of the energetic stress induced by low energy availability, it is theorised that athletes have to decrease training load, increase energy intake or both. However, due to the nature of competitive sports, elite athletes are often reluctant to decrease their training load so as to maintain a competitive advantage. Also, because access to elite athletes is very limited to researchers, there are no strictly controlled laboratory-based studies in elite population looking at recovery from low energy availability.

While decades of research have helped developing an understanding of the aetiology of menstrual disturbances in athletes, there is limited research on how to best recover from this condition and a definite answer to this question remains elusive. This invited talk will provide an overview of this topic and present evidence from a recent case study documenting recovery of menses of an amenorrheic elite road cyclist during a 5-year follow up after an episode of significant weight gain, while maintaining —and increasing— a high training load, physical capacity, and remaining competitive at an international level.

Reference 1 :- Loucks AB & Horvath SM (1985). Athletic amenorrhea: a review. *Med Sci Sports Exerc* **17**, 56–72.

Reference 2 :- Loucks AB (2020). Exercise Training in the Normal Female: Effects of Low Energy Availability on Reproductive Function. In *Endocrinology of Physical Activity and Sport*, ed. Hackney AC & Constantini NW, Contemporary Endocrinology, pp. 171–191. Springer International Publishing, Cham. Available at: http://link.springer.com/10.1007/978-3-030-33376-8_11 [Accessed March 31, 2020].

Reference 3 :- Areta JL, Taylor HL & Koehler K (2021). Low energy availability: history, definition and evidence of its endocrine, metabolic and physiological effects in prospective studies in females and males. *Eur J Appl Physiol* **121**, 1–21.

Reference 4 :- Areta JL (2020). Case Study: Resumption of Eumenorrhea in Parallel With High Training Load After 4 Years of Menstrual Dysfunction: A 5-Year Follow-Up of an Elite Female Cyclist. *International Journal of Sport Nutrition and Exercise Metabolism* **30**, 229–234.

SA06

The next step in female research; endocrine and methodological considerations for research in eumenorrheic females

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Data on eumenorrheic females is scarce and is often low to moderate quality, which is due to a number of reasons: (i) there is a shortage of specialist knowledge on female physiology, in particular endocrinology, in the sport and exercise science community; (ii) until recently, there has been a reluctance to include female participants in research studies, due to the numerous adaptations, to experimental design, needed to incorporate female specific considerations, such as the menstrual cycle; (iii) there is no consensus on the terminology and methodological approaches needed to produce high-quality studies. These issues have undoubtedly slowed the pursuit of knowledge in this field of research. The purpose of this talk is to detail the specific considerations needed when employing eumenorrheic women as participants in sport and exercise science-based research (Elliott-Sale et al., 2021). These considerations relate to participant selection criteria and adaptations for experimental design. This talk is intended to promote more high-quality research in eumenorrheic females.

Reference 1 :- Elliott-Sale KJ et al. (2021). Sports Med 51(5), 843-861

SA07

Oral contraceptive use and substrate metabolism during endurance exercise in women.

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While women's participation in leisure physical activities and competitive sports has progressively increased over the last century, many studies continue to exclude women, to overlook woman-specific features, or to extrapolate data obtained in men to women.

In recent decades, several research groups have stressed this weakness and called for a better consideration of women and of their hormonal specificities (Elliott-sale et al. 2021). Specifically, many adult women of childbearing age use hormonal agents that support menstrual regularity and provide birth control, with oral contraceptives (OC) being one of the most popular form. Due to their specific nature and concentrations, synthetic ovarian hormones contained in OC could influence physiological responses during exercise.

Substrate metabolism during endurance exercise is a key factor of health management but also of sport performance. Carbohydrates and fat are the two major fuels used by muscles for energy

production during endurance exercise, and it is acknowledged that substrate partitioning depends on several inter- and intra-individual factors, including exercise intensity and duration, body composition, training and nutritional status, physical activity level and sex. In addition, due to their specific characteristics, the exogenous hormones contained in OC are likely to influence substrate metabolism during exercise in women.

By analysing the available literature, we will try here to discuss the effect of OC use on substrate metabolism during endurance exercise.

Several studies conducted among women using different types of OC showed that substrate oxidation during moderate prolonged exercise was similar to what is observed in non-OC users (Bonen et al. 1991; Casazza et al. 2004; Isacco et al. 2012). Interestingly, some studies specifically reported increased lipolysis or decreased glucose flux without any change in substrate oxidation rates during endurance exercise in women using OC (Casazza et al. 2004; Isacco et al. 2012 and 2014). In addition, we previously reported that although OC use increases lipid mobilization, this effect was blunted by the lipolytic activity that occurs during endurance exercise (Isacco et al. 2014).

Results on the effect of synthetic ovarian hormones contained in OC on substrate metabolism during endurance exercise remain however unclear to date. While there are important health and performance implications regarding the use of OC among women, further studies are today needed to better understand this increased lipolytic activity without any substantial (or detectable) effect on substrate use during endurance exercise in OC users.

Reference 1 :- Bonen A et al. (1991). J Appl Physiol 70, 1917-1927.

Reference 2 :- Casazza GA et al. (2004). J Appl Physiol 97, 302-309.

Reference 3 :- Elliott-Sale KJ et al. (2021). Sports Med 51, 843-861.

Reference 4 :- Isacco L et al. (2012). Appl Physiol Nutr Metab 37, 646-656.

Reference 5 :- Isacco L (2014). Appl Physiol Nutr Metab 39, 1222-1229.

SA08

The influence of the menstrual cycle on substrate metabolism during exercise; applications for female athletes

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The fluctuating oestrogen and progesterone concentrations across the menstrual cycle in eumenorrhoeic women can alter substrate utilisation during exercise. Menstrual phase comparative studies (in the late 1990s to early 2000s) employed stable-isotopic tracers to study carbohydrate, fat and protein metabolism during exercise and provide the grounding for our current knowledge in the field. More recently, the complexed molecular signalling pathways of oestrogen and progesterone are being uncovered and lends clearer understanding to explain mechanisms and potential

magnitude of menstrual phase effect on energy metabolism during exercise. Multiple pathways of genomic and rapid non-genomic effects are described that vary by receptor isoform and tissue, thus explaining menstrual phase-specific differences in hepatic glucose production, whole body glucose uptake, tissue specific lipid storage and fat oxidation, and protein turnover during exercise (with added implications during fasted or carbohydrate-restricted training). For example, oestrogen suppresses, while progesterone either suppresses (in euglycaemic, insulin responsive state) or enhances (in glucose-deficient state) hepatic gluconeogenesis. This may drive the decrease in plasma glucose kinetics during exercise frequently reported in menstrual phases or conditions of elevated compared with suppressed ovarian hormones, despite oestrogen's capacity to increase exercise-stimulated glucose uptake and GLUT4 expression in an oestrogen receptor isoform-specific manner. Instead, oestrogen enhances long chain fatty acid uptake and oxidation in skeletal muscle and directs lipid availability away from adipose and toward skeletal muscle (while progesterone may partially antagonise some of these effects) explaining frequently reported increases in fat oxidation during exercise in late follicular or mid-luteal menstrual phases when oestrogen concentration is elevated. Oestrogen opposes, although progesterone's effect to promote protein catabolism appears to dominate during exercise in the luteal phase possibly aligning with the ovarian hormones' influences over gluconeogenesis and the need to provide gluconeogenic substrate during prolonged exercise. Furthermore, the chemical structure and properties of oestrogen affords cellular protection against the side-effects of metabolic flux by promoting plasma membrane stability, curbing free radical or lipid peroxidative damage and consequently modulating post-exercise inflammatory and exercise-induced damage responses or recovery time that is therefore menstrual phase specific. Thorough consideration of the current trends produces a sensible pattern of effect between menstrual phases that has practical application for future research design aiming to include eumenorrhoeic women and for athletes to optimise training or performance during racing.

SA09

Structural and functional characterization of the lipid scramblase TMEM16F

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The lipid scramblase TMEM16F catalyses the exposure of phosphatidylserine in platelets to initiate blood coagulation. TMEM16F is a member of the TMEM16 family comprising calcium-activated lipid scramblases and ion channels. We have combined cryo-EM with patch-clamp electrophysiology and liposomal assays to characterize the dual function of TMEM16F as a lipid scramblase and an ion channel. Cryo-EM analyses of the protein in the absence and presence of calcium reveal rearrangements induced by ligand binding. Functional characterization shows that both processes are activated by the same mechanism, but appear to be catalysed by distinct protein conformations.

Reference 1 :- Alvia C, Lim NK, Clerico Mosina V, Oostergetel GT, Dutzler R, Paulino C. Cryo-EM structures and functional characterization of the murine lipid scramblase TMEM16F. *Elife*. 2019;8.

Acknowledgements :-

SA10

The pros and cons of targeting TMEM16A in Cystic Fibrosis

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Recent work suggests activation of the Ca²⁺ activated chloride ion channel TMEM16A (anoctamin 1) as a genotype-independent (mutation-agnostic) treatment for cystic fibrosis, and also for other inflammatory airway diseases. This strategy is based on data obtained in studies performed almost exclusively *in vitro*, or under non-inflammatory conditions *in vivo*. It is assumed that activation of TMEM16A will induce airway electrolyte secretion, thereby compensating for the defect in CFTR-mediated Cl⁻ secretion. This should then lead to reduced airway mucus plugging and improved mucociliary clearance. However, in healthy lungs of human and mouse, TMEM16A is only weakly expressed, while in airways of patients with asthma and cystic fibrosis (CF), and in lungs of asthmatic mice, TMEM16A is upregulated particularly in glands and airway smooth muscle cells. Activation/potentialiation of TMEM16A in these cells may enhance mucus plugging and induce bronchoconstriction, which would favor a therapeutic use of inhibitors of TMEM16A, rather than activators. We analyzed expression of TMEM16A at different locations in human and mouse airways. We find upregulation of TMEM16A in lungs of people with CF or in asthmatic lungs, particularly in submucosal glands. However, in CF submucosal cells, stimulation by the purinergic agonist ATP activated predominantly KCNN4 K⁺ channels, but not ANO1. Niclosamide, an inhibitor of TMEM16A, blocked mucus production and mucus secretion in mice *in vivo* and *in vitro*. In contrast, the activator/potentiator of TMEM16A, Eact, and the potentiator of TMEM16A, brevenal, both induced acute mucus release from airway goblet cells in mice *in vivo*. Brevenal, a bioactive compound from the marine dinoflagellate *Karenia brevis*, strongly potentiated ATP-activated Cl⁻ currents, without directly increasing cytosolic Ca²⁺. Both Eact and brevenal induced acute airway contraction. Treatment of airway epithelial cells with niclosamide strongly inhibited expression of the central transcription factor for Th2 inflammation and goblet cell differentiation, SAM-pointed domain-containing ETS-like factor (SPDEF). Taken together, activators/potentiators of TMEM16A may induce preferentially airway mucus secretion and bronchoconstriction, which suggests the use of TMEM16A-inhibitors for the treatment of CF lung disease.

All animal experiments were approved by the local Ethics Committee and were conducted according to the guidelines of the American Physiologic Society and the German Law for the Welfare of Animals.

Acknowledgements :-

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SA11

Functional expression of TMEM16A in taste bud cells

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The calcium-activated chloride channels TMEM16A and TMEM16B play relevant roles in many physiological processes including neuronal excitability and regulation of Cl⁻ homeostasis. We examined the presence of calcium-activated chloride channels in taste cells of mouse vallate papillae. Taste buds contain three main types of taste cells named type I, II or III. Type II and type III cells express taste receptors and respond to tastants, while type I cells mainly have glial-like functions. The three cell types can be identified by the expression of specific proteins and by their electrophysiological fingerprint. Previous work has shown that both TMEM16A and TMEM16B were expressed in taste cells (Cherkashin *et al.*, 2016). We used *Tmem16a* and *Tmem16b* KO mice models as a control for the specificity of antibodies and found that only TMEM16A, but not TMEM16B, was expressed in taste bud cells. Moreover, TMEM16A largely colocalized with the inwardly rectifier K⁺ channel KCNJ1 at the apical portion of type I cells. By using whole-cell patch-clamp recordings in isolated cells from taste buds, we measured large currents activated by 1.5 μM Ca²⁺ in the intracellular solution in type I, but not in type II and III taste bud cells. Ion substitution experiments indicated that the current was mainly carried by anions. Calcium-activated chloride currents were blocked by the specific TMEM16A channel blocker Ani9. Moreover, in agreement with previous studies showing that type I taste cells exhibited large Ca²⁺-activated Cl⁻ currents when stimulated with P2Y receptor agonists (Kim *et al.* 2000; Cherkashin *et al.* (2016), we found that 50 μM ATP at the holding potential of -70 mV induced large inward currents in about 70% of type I taste cells. These currents were blocked by Ani9, indicating a possible role of TMEM16A in ATP-mediated signaling. ATP is released by type II cells in response to various tastants and reaches type I cells where it is hydrolyzed by ecto-ATPases but can also increase cytosolic Ca²⁺ by binding to P2Y receptors and indirectly activate TMEM16A channels causing a flux of Cl⁻ according to its electrochemical gradient.

Reference 1 :- Cherkashin AP, Kolesnikova AS, Tarasov MV, Romanov RA, Rogachevskaja OA, Bystrova MF & Kolesnikov SS (2016). Expression of calcium-activated chloride channels Ano1 and Ano2 in mouse taste cells. *Pflugers Arch* **468**, 305–319.

Reference 2 :- Kim YV, Bobkov YV & Kolesnikov SS (2000). Adenosine triphosphate mobilizes cytosolic calcium and modulates ionic currents in mouse taste receptor cells. *Neurosci Lett* **290**, 165–168.

SA12

Functional coupling between TRPV1 and ANO1 in sensory neurons requires ER Ca²⁺ release

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ANO1 (TMEM16A) is a widely expressed Ca²⁺ activated Cl⁻ channel (CaCC) with functions ranging from epithelial transport to sensory transmission. ANO1 is expressed in sensory neurons and involved in the process of detecting and relaying signals that arise after painful (noxious) stimulation. These neurons also express the Ca²⁺ permeable heat sensor TRPV1 with previous research suggesting that ANO1 and TRPV1 exist in a nanodomain arrangement where Ca²⁺ entering through TRPV1 is able to directly activate ANO1 due to their close proximity in dorsal root ganglion (DRG) neurons. We have previously shown that ANO1 activation is functionally coupled to Ca²⁺ release through inositol trisphosphate receptors (IP₃R) in DRG neurons, thus allowing the low Ca²⁺ sensitivity of ANO1 to be overcome. Interestingly, due to the ability of TRPV1 to induce Ca²⁺ release from the ER by activating phospholipase C (PLC), we hypothesised that TRPV1 may be able to activate ANO1 through IP₃R Ca²⁺ release as well. To this end, we developed a multi-wavelength live cell imaging approach to allow us to simultaneously monitor CaCC activity and Ca²⁺ dynamics in DRG and revealed that capsaicin activation of TRPV1 was able to induce CaCC activity. Furthermore, CaCC activity produced by capsaicin application was attenuated after depletion of the endoplasmic reticulum (ER) Ca²⁺ load, suggesting that ER Ca²⁺ release contributed to TRPV1-induced CaCC activation. To confirm that this effect was induced by plasmalemmal and not ER-localised TRPV1 channels, we used a cell impermeable TRPV1 activator- a derivative of double knot spider toxin- with an ER-Ca²⁺ sensor to demonstrate that ER depletion only occurs when membrane-localised TRPV1 are activated. To understand if there was a structural arrangement of channels that allowed this coupling to be facilitated in DRG neurons, we used in situ proximity ligation assay (PLA) to show that ANO1, TRPV1 and IP₃R receptors were often found in close proximity. This was also confirmed using superresolution stochastic optical reconstruction microscopy (STORM) which revealed that all 3 proteins were indeed found in close proximity. In summary, our findings demonstrate that functional coupling between ANO1 and TRPV1 in sensory neurons is facilitated by Ca²⁺ release through IP₃R with the channels found in a nanodomain structure to enable efficient ANO1 activation.

SA13

Real-time optical imaging of electrophysiology and tissue mechanics in beating hearts

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Optical imaging plays a crucial role in basic cardiovascular research. In this talk, I will discuss the latest developments in the field of cardiac optical mapping, and demonstrate that it is possible to image action potential and calcium wave phenomena on the surface of the beating heart in real-time. Using a mix of optical and numerical computer vision techniques, including, for instance, voltage-sensitive fluorescent dyes, ratiometric imaging and numerical motion tracking, it is possible to simultaneously measure mechanical tissue deformation, as well as electrophysiological wave phenomena at very high spatial and temporal resolutions. I will discuss some of the experimental requirements, e.g. illumination, optics, preparation of the tissue, and will review the state-of-the-art in numerical techniques and hardware requirements for performing the measurements in real-time at imaging speeds of 500fps. Further, I will discuss how the superposition of motion and fluorescence-related phenomena poses a tricky problem, which needs to be addressed when imaging and post-processing the data. In this context, I will discuss the origin of motion artifacts, the importance of a co-moving measurement, the effect of inhomogeneous illumination, and how cross-talk between mechanics and electrophysiology may negatively affect the measurement [1,2]. Most importantly, I will demonstrate that deep learning is very successful in solving some of these issues, as it can learn the complex relationship between motion, illumination and fluorescence-encoded electrophysiology, and can use this information to correctly disentangle the involved physical phenomena leading to reliable and accurate measurements. Using this powerful technique, it becomes possible to study electromechanical phenomena in great detail. For instance, we studied and were able to resolve rotor dynamics in fibrillating contracting hearts and found a strong correlation between tissue mechanics and electrophysiology [3]. Lastly, I will explain some of the limitations of cardiac optical mapping and potential future directions.

Reference 1 :- **J. Christoph**, S. Luther, Marker-free Tracking for Motion Artifact Compensation and Deformation Measurements in Optical Mapping Videos of Contracting Hearts, *Frontiers in Physiology*, 9:1483, doi: 10.3389/fphys.2018.01483, 2018

Reference 2 :- V. Kappadan, S. Telele, I. Uzelac, F. Fenton, U. Parlitz, S. Luther, **J. Christoph**, High-Resolution Optical Measurement of Cardiac Restitution, Contraction and Fibrillation Dynamics in Beating versus Blebbistatin-uncoupled Isolated Rabbit Hearts, *Frontiers in Physiology*, doi: 10.3389/fphys.2020.00464, 2020

Reference 3 :- **J. Christoph**, M. Chebbok, C. Richter, J. Schröder-Schetelig, P. Bittihn, S. Stein, I. Uzelac, G. Hasenfuß, F. Fenton, R. Gilmour Jr., S. Luther, Electromechanical Vortex Filaments during Cardiac Fibrillation, *Nature*, Vol. 555, p. 667-672, doi: 10.1038/nature26001, 2018

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SA14

Using light to study heterocellular contributions to cardiac electrophysiology

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The heart is composed of cardiomyocytes (CM) and non-myocytes (NM), the latter including stromal, endothelial, and immune cells. NM have long been suspected to electrically couple to CM in native myocardium [1]. However, only with the advent of optogenetics – a method to observe and manipulate cell-type specific electrophysiology with light – heterocellular interactions could be directly assessed in intact tissue [2,3]. Using optogenetic experiments electrotonic coupling was functionally confirmed for cardiac fibroblasts (FB) and macrophages (MΦ), and we are thus interested in exploring how FB and MΦ affect the electrical and mechanical activity during myocardial remodeling in response to heart disease and injury.

Methods: We used Cre-loxP recombination to selectively express fluorescent reporter proteins and optogenetic actuators in NM populations of murine hearts [4]. We optically cleared hearts using X-CLARITY and imaged them with super-resolution confocal microscopy to visualize fluorescently labelled NM populations. This allowed us to reconstruct 3D models of FB and MΦ *in situ*, and to assess their morphology, distribution, interconnectivity, and surface area. We characterized the electrophysiological properties of resident cardiac MΦ, using RNA sequencing, single-cell patch-clamp recordings and pharmacological interventions, showing functional expression of Cx43 and different voltage-gated K⁺ channels. Based on our structural and functional data, we developed a computational model describing cardiac MΦ electrophysiology, which we then used to quantitatively assess CM-MΦ coupling *in silico*. In on-going experiments, we utilize the light-gated cation channel channel-rhodopsin-2 and the voltage-sensitive fluorescent protein VSFP2.3 to study heterocellular coupling between CM, FB and MΦ, with a focus on NM effects on cardiac activity in developing scars following cardiac injury (ischemia-reperfusion injury and cryoablation).

Results: In healthy cleared ventricles, we found that FB networks consist of elongated, thin strands of interconnected cells, which appear to wrap around CM with finger-like nano-protrusions that may be related to tunneling nanotubes – as seen with electron microscopy in post-cryoinjury murine myocardium [2]. In the atria, on the other hand, FB display sheet-like morphologies. Quantitatively, the volume and surface area of 3D reconstructed FB does not differ statistically between atrial and ventricular walls (average surface area in right atrial and left ventricular myocardium are $2,130 \pm 280 \mu\text{m}^2$ [n=276 nuclei, N=3 hearts] and $1,770 \pm 190 \mu\text{m}^2$ [n=126 nuclei, N=3], respectively). Unlike FB, resident cardiac MΦ appear as solitary cells in intact ventricular myocardium with an average surface area of $1,160 \pm 80 \mu\text{m}^2$ (n=35 cells, N=3). In isolated and cultured cells, we found that passive electrophysiological properties of MΦ such as capacitance and membrane resistance scale with surface area (inverse relation for resistance). *In silico* models of CM-MΦ coupling illustrate that those parameters are directly linked to the electrical load of MΦ on coupled CM [5].

Conclusions: FB and tissue-resident MΦ exhibit surprisingly similar cell dimensions in intact tissue, although they differ in their distribution and electrophysiology. Upon injury, these cell populations undergo changes (local proliferation, recruitment, activation) leading to larger NM numbers in the scar and scar border zone, increasing the likelihood of electrotonic coupling. The functional effects of this are a matter of ongoing research.

Reference 1 :- [1] Kohl P, Gourdie RG. *J Mol Cell Cardiol* **2014**/70(100):37-46.

Reference 2 :- [2] Quinn TA et al. *Proc Natl Acad Sci* **2016**/113(51): 14852–7.

Reference 3 :- [3] Hulsmans M et al. *Cell* **2017**/169: 510-522.

Reference 4 :- [4] Fernandez MC et al. *Methods Mol Biol* **2021**/2191:287-307.

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SA15

Research tools to visualise and interrogate the glucagon-like peptide 1 receptor

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Motivation/problem statement: The glucagon-like peptide-1 receptor (GLP-1R) is expressed in various tissues such as the brain and pancreas. This class B G protein-coupled receptor (GPCR) is involved in the regulation of blood glucose levels and control of appetite. Despite being a major drug target for diabetes and obesity treatment, visualisation of the GLP-1R is limited to antibody detection, genetic manipulation or receptor-activating probes.

Methods/procedure/approach: Various fluorophores were fused to Exendin4(9-39) to produce LUXendins. These fluorescent antagonists bind the GLP-1R in live tissue, with specificity confirmed using a novel *Glp1r* knock-out mouse line and by co-localisation with GLP-1R antibody. A mouse line was also generated expressing SNAP-tagged GLP-1R using CRISPR/Cas9 genome editing. Isolated pancreatic islets of these mice were treated with fluorescent SNAP-tag label to visualise the GLP-1R in situ, followed by receptor activation and trafficking after addition of agonist.

Results: LUXendin645 (Cy5) intensely labelled the plasma membrane in live and fixed cells as well as pancreatic islets and brain. Most beta cells within an islet expressed the GLP-1R, whilst only ~5% of alpha cells were LUXendin+, as determined using *Ins1Cre;mTmG* reporter animals. STED imaging of

MIN6 beta-cells labelled with LUXendin651 (SiR) revealed that endogenous GLP1R are organised into nanodomains, and variable diffusion at the plasma membrane was observed using single-molecule light microscopy. Five other LUXendins were also synthesised and tested, spanning green-near infrared spectra, with all variants producing bright membrane labelling of GLP1R.

In SNAP_GLP1R knock-in mice, fluorescent SNAP-tag label co-localised with LUXendin and GLP-1R antibody. Thus, this mouse line can be used to visualise the GLP-1R in live tissue without stimulating the receptor. Addition of GLP-1R agonists allowed the monitoring of receptor activation and trafficking in situ. SNAP_GLP1R knock-in mice had normal body weight and responded to glucose like their wild type littermates.

Conclusion/implications: LUXendins can be used in live tissue and be fixed to label endogenous GLP-1R and allows to study the receptor using confocal, two-photon and super-resolution microscopy in situ or in vivo. While LUXendins act as antagonists, linking fluorophores or dyes to SNAP_GLP-1R of the knock-in mouse line does not interfere with receptor activity and allows labelling post-fixation.

All animal research complied with the Animals (Scientific Procedures) Act 1986 of the U.K. Approval was granted by the University of Birmingham's Animal Welfare and Ethical Review Body.

SA16

GLP-1 Receptor Agonists: Mechanisms Relevant for the Treatment of Obesity

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Glucagon-Like Peptide-1 receptor agonists (GLP-1RAs) have a broadly applicable biology and are approved for the treatment of diabetes as well as obesity. Physiologically, GLP-1 is short-acting, and a key element to treatment success is that pharmacological agents are long-acting. Liraglutide and semaglutide are fatty acid acylated long-acting GLP-1RAs that bind to serum albumin non-covalently via the fatty acid binding sites, and thereby have pharmacokinetic profiles which last for 24 hours/day. Liraglutide has been approved for the treatment of obesity since 2014, and recently also received a paediatric indication, whereas semaglutide has completed phase 3 clinical studies. Semaglutide has been documented to lead to significantly greater weight loss compared to other GLP-1RAs. GLP-1R expression in the pancreas and brain accounts for the respective improvements in glycemic control and body weight. Mechanistically, GLP-1RAs lower body weight through an effect to reduce energy intake. There is a reduction in feelings of hunger, increases in satiety and effects on the reward system resulting in reduced craving for food and improved food choices. Neural GLP-1Rs are the main targets for the weight loss mechanism. Whole brain imaging studies in rodents show that peripherally-administered GLP-1RAs have access to select sites in the brain, and also communicate to the brain through the circumventricular organs, of which many have GLP-1Rs. These agents do not cross the blood brain barrier passively, and their brain accumulation is dependent on

presence of GLP-1R. New data show that there are differences in the brain distribution of semaglutide compared to liraglutide. Both molecules also positively affect cardiovascular (CV) outcomes in individuals with type 2 diabetes, and an outcome study in obesity is ongoing. This ongoing study recruits 17,500 patients with obesity and is an event driven trial. In conclusion, the GLP-1 biology shows potential in obesity and semaglutide is undergoing testing for an effect to lower cardiovascular risk also in patients with obesity.

SA17

Central and peripheral glucagon-like peptide-1 systems independently suppresses eating

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Glucagon-like peptide-1 (GLP-1) acts as both an incretin hormone, and an anorexigenic neuropeptide, which has prompted the successful and ongoing development of GLP-1-based therapies for type 2 diabetes and obesity. Endogenous GLP-1 is produced by enteroendocrine cells in the gut, and preproglucagon (PPG) neurons in the brainstem, which are the defining populations of the peripheral and central GLP-1 systems, respectively. PPG neurons in the brainstem nucleus tractus solitarius (PPG^{NTS} neurons) are widely assumed to link the peripheral and central GLP-1 systems in a unified gut–brain satiation circuit. However, direct evidence for this hypothesis is lacking, and the necessary circuitry has not been demonstrated. We used transgenic mice expressing Cre-recombinase under the glucagon (i.e. PPG) or GLP-1 receptor (*Glp1r*) promoters, coupled with viral targeting of Cre-dependent tracing and effector tools, to selectively map and interrogate gut-brain connectivity between the central and peripheral GLP-1 systems. This allowed us to test whether PPG^{NTS} neurons have a role in physiological satiation, and whether endogenous or exogenous peripheral GLP-1 signalling drives central GLP-1-induced suppression of eating.

We report that PPG^{NTS} neurons encode satiation specifically during large meals, and have the capability for pharmacological activation to suppress eating without compensatory rebound hyperphagia or behavioural disruption. Activation of *Glp1r* vagal afferent neurons similarly suppressed intake, but conditioned a flavour avoidance, and circuit mapping approaches demonstrated that PPG^{NTS} neurons are not a major synaptic target of this vagal population. PPG^{NTS} neurons instead predominantly receive vagal input from oxytocin receptor-expressing vagal afferent neurons, and are necessary for peripheral oxytocin-induced eating suppression. Similarly, PPG^{NTS} neurons are at most a minor synaptic target of *Glp1r* neurons in the area postrema, suggesting that endocrine GLP-1 signalling from the periphery by this route does not require PPG^{NTS} neurons. Consistent with this observation, PPG^{NTS} neurons are not activated by peripheral administration of the *Glp1r* agonist semaglutide, nor are they required for semaglutide-induced suppression of eating or bodyweight loss. Furthermore, chemogenetic activation of PPG^{NTS} neurons concurrent with peripheral semaglutide administration suppresses eating more potently than semaglutide alone.

We therefore conclude that the unified peripheral to central GLP-1 satiation circuit hypothesis is not supported, but that the peripheral and central GLP-1 systems are instead components of functionally and anatomically independent eating control circuits. These findings provide a rationale for pharmacological activation of PPG^{NTS} neurons in combination with GLP-1 receptor agonists as an obesity treatment strategy.

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SA18

Elucidating the molecular mechanisms required for vagally-mediated physiological response to peripheral GLP1

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The vagus nerve is the primary neural mechanism for gut-brain communication. Vagal afferent neurons, located in the nodose ganglia, express numerous receptors that sense hormones released from the gut, and provide meal related information about the composition and quantity of food consumed to the brain. The gastrointestinal hormone, glucagon like peptide 1 (GLP1), released from enteroendocrine cells in response to a meal acts on GLP1 receptors located on vagal sensory nerve terminals in the gut to inhibit food intake, increase glucose clearance, and block gastric emptying. The mechanisms by which GLP1 produces these vagally-mediated physiological responses remains unknown. Recent evidence will be presented identifying a key role for the neuropeptide cocaine and amphetamine regulated transcript in vagal sensory neurons as a mediator of peripheral GLP1 signaling. Using an available single-cell RNA sequencing dataset obtained from mice nodose ganglia we characterized the extent of co-expression between *Glp1r* and several classic neuropeptides and identified the neuropeptide cocaine and amphetamine regulated transcript (CART) as having the highest level of co-expression with GLP1 receptor in nodose ganglia neurons. *In situ* hybridization demonstrates that CART and GLP1 receptor colocalization is conserved in rat nodose ganglia neurons. In rat primary cultures, GLP1 preferentially upregulates CART expression through a GLP1 receptor dependent mechanism. *In vivo*, viral mediated knockdown of CART in vagal sensory neurons blocks GLP1-induced satiation, gastric emptying, and alters circulating insulin levels. Similarly immunoneutralization of CART in the nucleus tractus solitarius, the site of vagal termination in the hindbrain, blunts GLP1-induced satiation and gastric emptying. Chemogenetic stimulation of CART expressing vagal sensory neurons in mice inhibits food intake, slows gastric emptying and alter glucose clearance in response to a meal. Together these data demonstrate that CART synthesis and release by vagal sensory neurons is necessary and sufficient to mediate the physiological response to peripheral GLP1.

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SA19

Microvascular actions of the dipeptidyl peptidase-IV inhibitor, vildagliptin, in obesity

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Diabetes and obesity are associated with vascular dysfunction (e.g. capillary rarefaction) and an increased risk of vascular complications of both large blood vessels (macrovasculature) (e.g. cardiovascular disease) and small vessels (microvasculature) (e.g. diabetic retinopathy). There is an unmet need for better preventative and therapeutic strategies for these vascular complications. One such avenue maybe incretin based medications, for example dipeptidyl peptidase-IV inhibitors (DPP-IVi) such as vildagliptin and sitagliptin, that are already licenced to aid glycaemic control in type 2 diabetes. DPP-IVi slow the breakdown of endogenous incretins (glucagon-like peptide-1 and glucose-dependent insulintropic polypeptide) thereby prolonging their insulin releasing action. There is interest in their non-glycaemic effects, particularly whether they have favourable effects on the cardiovascular system, and thus have beneficial effects in individuals at higher risk of vascular disease / dysfunction. The majority of previous research has focused on the macrovascular effects of incretin based medications and little is known about the potential microvascular effects of these therapies, particularly the DPP-IVi in humans, *in vivo*. This study examined whether short term DPP-IV inhibition with vildagliptin alters micro- and macrovascular function in obese men.

Study design: Crossover, randomised double-blind placebo-controlled study.

Method: 15 obese men were recruited (age range:28-72years). Following recruitment participants were randomised to vildagliptin (50mg twice daily) or placebo for 12 weeks. At the end of the treatment period, microvascular (dermal capillary density; maximum hyperaemia; endothelial (in)dependent microvascular function; microvascular filtration capacity; peak reactive hyperaemia) and macrovascular assessments (flow mediated dilatation of the brachial artery and applanation tonometry) were performed. Following a 4 week washout period the participant received the alternative treatment for 12 weeks. Vascular assessments were repeated within the last 2 weeks of treatment. The study was approved by local NHS Research Ethics Committee (REC number: 09/H0206/33). Written, informed consent was obtained from all participants.

Results: 14 participants completed the study. Active treatment resulted in a small but significant reduction in HbA1c (active mean $\pm$ standard deviation: 38.1 $\pm$ 4.8 vs placebo: 39.1 $\pm$ 4.1mmol/mol, $p=0.003$ paired T-test). Maximal dermal capillary density was increased with DPP-IV inhibition (active median (25th,75th centiles) 129 (118,158) mm² vs placebo: 122 (111,139) mm², $p=0.018$ Wilcoxon Signed Rank test). There was no significant change in other vascular assessments. Ongoing *in vitro* experiments are exploring this area further by examining the impact of DPP-IV inhibition on

proliferation, migration and angiogenic tube formation of human microvascular endothelial cells. Preliminary results suggest that sitagliptin (1 μ M) significantly increases endothelial cell proliferation as assessed by BrdU (DNA) incorporation ($331.6 \pm 10.4\%$ vs control (100%), $p < 0.01$ Mann-Whitney U test, $n = 9$) and DNA staining ($135.4 \pm 6.5\%$ vs control (100%), $p < 0.01$, $n = 6$).

Discussion: DPP-IV inhibition with vildagliptin, increased maximal dermal capillary density in obese men. Preliminary data also suggests that DPP-IV inhibition increases microvascular endothelial cell proliferation *in vitro*. Further work is required to explore these potential pro-angiogenic actions of DPP-IVi and their underlying mechanisms.

Acknowledgements :- This study was supported by an unrestricted educational grant from Novartis as an investigator led trial. The research was also supported by the National Institute for Health Research (NIHR) Exeter Clinical Research Facility. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

SA20

Imaging the glomerulus to understand leukocyte function during glomerulonephritis

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Glomerulonephritis is one of the leading causes of end-stage renal failure. In many forms of this condition, leukocytes recruited to the glomerulus play essential roles in initiating and mediating disease. In order to understand the mechanisms of leukocyte recruitment to the glomerulus and their behaviour within the glomerulus, we have used multiphoton intravital microscopy (MP-IVM) to image glomeruli of mice undergoing glomerular inflammatory responses. These studies have revealed previously unrecognised behaviour of neutrophils, monocytes and T cells during the development of antibody- and T cell-mediated models of glomerulonephritis. The major effect of glomerular inflammation is to induce immune cells to undergo prolonged intravascular retention within the glomerular microvasculature. From this location within glomerular capillaries, immune cells perform pro-inflammatory functions and undergo cell-cell interactions that are important to the initiation and progression of glomerular inflammation. In this presentation, alterations to immune cell behaviour occurring within the inflamed glomerular microvasculature will be discussed, highlighting examples of neutrophil, monocyte and CD4+ T cell activity.

SA21

IMAGING THE INJURED AND AGED CORONARY MICROCIRCULATION IN VIVO IN THE MURINE BEATING HEART

Juma El-Awaisi¹, Dean Kavanagh¹, Marco Rink², Christopher Weston², Neena Kalia¹

Aims/Objectives: Treatment of myocardial infarction (MI) focuses on the rapid re-establishment of regional myocardial perfusion following a blockage in one or more of the coronary arteries. Although the culprit artery can be re-perfused, a significant proportion of patients still incur extensive myocardial damage resulting in heart failure. This is partly due to reperfusion paradoxically leading to additional tissue damage, a condition called ischaemia-reperfusion (IR) injury that has been ascribed to inadequate coronary microcirculatory perfusion. Nevertheless, the degree of microvascular perfusion is unknown, limited by an inability to clinically image coronary microvessels. We have previously shown using intravital microscopy that myocardial IR injury induces thromboinflammation and reduces functional capillary density (FCD) in the adult mouse beating heart microcirculation *in vivo*. [1] The newly discovered and inflammatory cytokine, interleukin-36 (IL-36), could potentially mediate these disturbances. However, its role in myocardial IR injury is not known. This study firstly aimed to determine whether the coronary microcirculatory disturbances and infarct size post-IR injury was modified by age and gender. Secondly, we investigated whether an IL-36 receptor antagonist (IL-36Ra) could confer vasculoprotection and reduce infarct size

Methods: Adult (3-months) and aged (>18-months) male and female C57BL/6 mice (n=5/group) were anaesthetised using intraperitoneal administration of ketamine hydrochloride (100mg/kg) and medetomidine hydrochloride (100mg/kg). IR injury was induced by reversibly suture ligating the left anterior descending coronary artery for 45 minutes with reperfusion mediated for 2 hours. A custom designed 3D-printed stabiliser was attached to the left ventricle downstream of the ligation site to permit intravital imaging. In some studies, recombinant mouse IL-36Ra (15ug/mouse) was injected intra-arterially at 5 minutes pre-reperfusion and 60 minutes post-reperfusion. Beating heart coronary microcirculation was imaged in real-time intravitaly and also *ex vivo* using multiphoton microscopy. Infarct size was measured using dual TTC/Evans Blue staining. Experiments were conducted in accordance with the Animals (Scientific Procedures) Act of 1986 (Project licence: P552D4447).

Results: Significantly increased basal ($p<0.0001$) and IR injury-induced ($p<0.0001$) neutrophil recruitment, and greater decreases in FCD, was observed in aged mice compared to adults (ANOVA + Tukey's post hoc test). Neutrophils primarily adhered within coronary capillaries although in aged hearts remarkable venular adhesion was also identified. These events were mirrored in deeper myocardial layers when imaged using multiphoton microscopy. Interesting gender-dependent perturbations were noted with neutrophil recruitment dominating in IR injured female hearts whilst male hearts demonstrated a greater presence of occlusive platelet microthrombi. IL-36Ra significantly reduced inflammation ($p<0.0001$) and infarct size ($p<0.0001$) in both adult and aged mice.

Conclusion: Our novel findings of enhanced coronary microcirculatory perturbations associated with age may explain the poorer outcomes in elderly patients following MI. Furthermore, the cellular nature of the thromboinflammatory response may explain the gender-related differences in outcome

after MI. Importantly, we are the first to demonstrate that targeting IL-36 may be a potential novel therapy for treatment of myocardial IR injury.

Reference 1 :- Kavanagh D, Lokman A, Neag G, Colley A, Kalia N. Imaging the injured heart intravitaly and the vasculoprotection afforded by haematopoietic stem cells. *Cardiovascular Research* 2019; 115:1918-32.

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SA22

BACE1: A novel regulator of the microcirculation from head to toe.

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The current global epidemic of obesity and diabetes is driving an increased incidence of cardiometabolic disease. Microvascular abnormalities have been found to be an important underlying pathology of a number of cardiometabolic diseases. Indeed, microvascular dysfunction, the inability of the microcirculation to feed tissues adequately, is strongly associated with type 2 diabetes and obesity, atherosclerosis, stroke and dementia. Thus, targeting the mechanisms by which nutrient excess drives the induction of microvasculardysfunction could reduce cardiovascular disease prevalence.

Our work has revealed that a protein called BACE1, more commonly associated with Alzheimer's disease, plays an important role in type 2 diabetes and obesity. BACE1 activity is required for the production of β -amyloid peptides, from the amyloid precursor protein, which form the characteristic amyloid plaques found in Alzheimer's disease. There is growing evidence that Alzheimer's disease, diabetes and cardiovascular disease are intimately linked, with inflammation, oxidative stress and insulin resistance common features. BACE1 protein is expressed in a wide variety of tissues and cells, including vascular smooth muscle and endothelial cells. Expression level and activity are increased by chronic stress (e.g. oxidative, metabolic and inflammatory) and we have shown that high fat diet drives its transcription and translation.

We have shown that global deletion of BACE1 in mice enhances insulin sensitivity and protects against diabetes-induced endothelial dysfunction. Importantly, pharmacological inhibition of BACE1 can restore metabolic and vascular health in mouse models of disease. These effects are via modulation of BACE1 cleavage of both the amyloid precursor protein and the insulin receptor, which we recently identified as a novel substrate of BACE1. Furthermore, we have also demonstrated that

this role translates into human physiology, with BACE1 activity inversely correlated with endothelial and vascular dysfunction.

Therefore, our research focus is on determining whether BACE1 can be a novel drug target to provide an innovative therapy for type 2 diabetes and its vascular co-morbidities. With BACE1 inhibitors currently in clinical trials for AD, repurposing these drugs for cardiometabolic disease this could be a promising strategy.

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SA23

Prefrontal thalamocortical connectivity: cracking the circuitry of cognition
Prefrontal thalamocortical connectivity: cracking the circuitry of cognition

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Interactions between the thalamus and prefrontal cortex (PFC) play a critical role in cognitive function and arousal and are disrupted in neuropsychiatric disorders. The PFC is reciprocally connected with ventromedial (VM) and mediodorsal (MD) thalamus, both higher-order nuclei with distinct properties to the classically studied sensory relay nuclei. To understand the properties of the circuits linking PFC and thalamus we use anatomical tracing, electrophysiology, optogenetics, and 2-photon Ca²⁺ imaging, determining how VM and MD target specific cell types and subcellular compartments of mouse PFC. Focusing on cortical layer 1, we find thalamic nuclei target distinct sublayers, with VM engaging NDNF⁺ cells in L1a, and MD driving VIP⁺ cells in L1b. These separate populations of L1 interneurons participate in different inhibitory networks in superficial layers by targeting either PV⁺ or SOM⁺ interneurons. NDNF⁺ cells mediate a unique form of thalamus-evoked inhibition at PT cells, selectively blocking VM-evoked dendritic Ca²⁺ spikes. Together, our findings reveal how two thalamic nuclei differentially communicate with the PFC through distinct L1 micro-circuits and how inhibition is critical for controlling PFC output back to thalamus.

SA24

Cognitive switches and value-guided remapping in cortical circuits

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Adaptive decision-making enables animals to select appropriate goal-directed strategies based on the evaluation of recent stimulus/action-outcomes. The orbitofrontal cortex (OFC) play an important role in invoking rule-based strategies to enable flexible learning. However, the neural circuit mechanisms in the lateral OFC (lOFC) and its interactions with sensory cortical areas underlying such processes remain elusive. To investigate this, we trained head-fixed mice on a tactile-discrimination-based reversal-learning task and enforced them to relearn the task after a 'Go/No-go' rule-switch. Mice exhibited high performance during learning and re-learned the task upon rule-switch. To investigate how distinct neuronal subpopulations in lOFC respond during the task, we employed two-photon imaging through a rod-like GRIN lens. Longitudinal imaging of trial-by-trial Ca²⁺ responses from the same subsets of lOFC neurons in mice expressing GCaMP6 in layer 2/3 neurons revealed that OFC neurons are a key substrate encoding value prediction error. We also measured neuronal responses in the primary somatosensory cortex (S1). In S1, distinct neuronal subpopulations showed 'stimulus-selective' as well as 'outcome-selective' responses. The 'outcome-selective' neurons were differentially modulated by reward history and altered response selectivity upon reversal. Silencing lOFC following rule-switch impairs plastic changes in S1 neurons. Taken together, our experiments shed light on the long-range cortical circuit interactions underlying behavioural flexibility, indicating a crucial role of mouse OFC neurons in encoding predictive 'teaching signals' that drive adaptive changes in behaviour.

Reference 1 :- Banerjee et al. (2020) Value-guided remapping of sensory cortex by lateral orbitofrontal cortex. *Nature* 585: 245-250.

SA25

Evidence of neural interactions between the mediodorsal thalamus and orbitofrontal cortex during visuospatial discrimination learning in macaques

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Anatomical evidence in mammals shows that the mediodorsal thalamus (MD) is a key component within wider prefrontal thalamocortical networks. Accordingly, damage to, or disruption of MD function impairs specific types of complex learning and decision making in primates. Yet, the underlying mechanisms behind the influence of MD on the frontal cortex during these higher order cognitive processes remain unknown. In the current study, two male rhesus macaques were trained to perform a touchscreen-based visuo-spatial discrimination learning task prior to having recording chambers implanted above the MD and orbitofrontal cortex (OFC). All experimental procedures were approved and conducted under a United Kingdom Home Office Project Licence issued to Dr Mitchell. After recovery, we established the precise coordinates to target the MD and the OFC using magnetic resonance imaging (MRI). Once the animals had re-established their pre-procedure learning rates, we recorded task related neural activity from the MD and OFC using multi-contact probes that were

lowered before each testing session. MD and OFC units were modulated during specific phases of a trial, responding differently to the trial onset, when the visuo-spatial images first appeared on the touchscreen; when the monkey made a response to the touchscreen; and before or after the delivery of the smoothie reward for correct trials. We also observed some MD and OFC units progressively modulated their responses throughout the session, coinciding with improved cognitive performance. Finally, during correct trials, some MD units were phase-locked to OFC local field potentials, suggesting prefrontal thalamocortical interactions support learning. Our preliminary evidence highlights different, but complementary and interactive neural activity between the MD and OFC during learning of a visuo-spatial discrimination task.

SA26

Heterogeneity of function within primate ventromedial prefrontal cortex

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Neuroimaging studies have variably implicated the ventromedial prefrontal cortex (vmPFC) in emotion regulation, inhibitory control of fear, action control, memory and comparative reward evaluation. Reported reductions or increases in activity have been linked to the aetiology of depression and reversal of this dysregulation can be an effective treatment for refractory depression. However, this is a large, structurally heterogeneous region composed of a number of sub-regions including areas 25, 32, 24, 14 and 10. Perhaps surprisingly though, there have been few studies that have attempted to determine the extent to which this structural heterogeneity reflects functional heterogeneity. To address this issue, we have performed a series of investigations in the common marmoset, a new world monkey, combining temporary manipulations of distinct regions within the vmPFC with behavioural and physiological analysis of animal's reactivity to a wide range of rewarding and threatening contexts. Fluorodeoxyglucose PET imaging has been used in concert with these manipulations to determine circuit-wide changes. Focussing on the effects of inactivation and over-activation of caudal subcallosal region, area 25, results will be presented that highlight the specific role of this region in maintaining a negative affective state that, through apparently distinct downstream circuits, activates cardiovascular, endocrine and behavioural reactivity to threat; whilst blunting anticipatory and motivational appetitive arousal. These findings will be discussed in the context of the markedly different functional profile of neighbouring regions with respect to positive and negative emotion regulation, altogether providing important new insights into the functional heterogeneity within vmPFC.

Reference 1 :- Roberts, A.C., Clarke, H.F. (2019) Why we need non-human primates to study the role of ventromedial prefrontal cortex in the regulation of threat and reward elicited responses. *Proceedings of the National Academy of Sciences* 116:26297-26304.

Reference 2 :- Alexander L., Wood C.M., Gaskin, P.R.L., Sawiak S.J., Fryer T.D., Hong, Y.T., McIver L., Clarke, H.F., Roberts A.C. Over-activation of primate subgenual cingulate cortex enhances the cardiovascular, behavioural and neural responses to threat reactivity (2020) Nature Communications 11, 5386.

Reference 3 :- Alexander L., Gaskin P., Sawiak S.J., Fryer T., Hong Y.T., Cockcroft G., Clarke H.F., Roberts A.C. Fractionating anhedonia by over-activating area 25 of primate subgenual anterior cingulate cortex (2019) Neuron 101:307-320.

Acknowledgements :-

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SA27

Thyroid hormone transporters within the hypothalamus-pituitary-thyroid axis

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Thyroid hormone (TH) transporters facilitate the cellular transmembrane passage of TH and are, consequently, required for proper TH signaling and metabolism in all target cells. Their pathophysiological significance is best illustrated in patients with inactivating mutations in the highly specific TH transporter MCT8 since affected patients display a severe form of psychomotor retardation (so called Allen-Herndon-Dudley syndrome) due to tissue-specific changes in TH homeostasis. MCT8 deficient patients as well as Mct8 mouse mutants exhibit abnormal serum TH profile in combination with an altered activity of the hypothalamic-pituitary-thyroid (HPT) axis indicating that TH transporter deficiency compromises the HPT axis on all levels.

Here, I will briefly summarize recent studies of mice lacking Mct8 alone or in combination with other well-established TH transporters (Mct10 and Oatp1c1) as these studies shed light on many aspects and pathogenic events underlying global MCT8 deficiency. Moreover, development of conditional knock-out mice that allow a cell-specific inactivation of TH transporters in distinct cell types disclosed distinct cell-specific changes in TH signaling and sensing within the HPT axis. Altogether, our findings underscore the gate-keeper function of TH transporters as critical components in regulating local TH action.

SA28

Revealing a modular network of committed pituitary thyrotrophs in health and disease

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Thyroid hormone (TH) dysregulation is one of the most prevalent endocrine defects worldwide (3.8% of EU citizens). Regulation of TH output from the thyroid is dependent on secretion of thyroid-stimulating-hormone (TSH) from a small population of pituitary thyrotrophs in response to hypothalamic thyrotropin-releasing-hormone (TRH) and thyroid hormone feedback. Importantly, diagnosis of hypothyroidism is based on a single TSH measurement, thus dismissing the pulsatile property of TSH secretion and the non-linear TSH-TH relationship upon disease onset and subsequent recovery. Despite the undeniable implication of thyrotropes in the hypothalamus-pituitary-thyroid (HPT) axis regulation, little is known about the mechanisms underlying pulsatile and adaptive TSH secretion. The aim of this study is to decipher how thyrotropes form a very finely regulated and highly plastic functionally-organised cell population capable of adapting to TH demand. Pituitary-scale 3D imaging revealed an anatomical thyrotroph network that is established during late embryogenesis, when TSH β -positive cells begin forming homotypic anatomical network motifs, prior to an increase in TH demand during the neonate period. We found that, at weaning, concomitantly with a decrease in TH levels, TSH-expressing thyrotroph motifs disaggregate. Lineage-tracing experiments unveiled that thyrotroph cluster motifs persist after weaning in the form of intermingled thyrotroph sub-sets: a sub-population of TSH β -expressing (TSH^{high}) thyrotrophs and another subset of thyrotrophs that express low, if any, TSH β hormone subunit (TSH^{low}). Using *in vivo* calcium imaging in both freely-moving and anesthetized TSH β -crexR26fl-flGCaMP6f mice, we show that the surprisingly unified cell ensemble of TSH^{high} and TSH^{low} thyrotrophs generates large-scale intercellular waves of the universal second messenger Ca²⁺, which recur autonomously and independently of hypothalamic TRH inputs. This mode of intercellular communication is a network signature of this composite thyrotroph population, which persists in a mouse model of thyroid dysfunction, since the thyrotroph network-driven wave generator of Ca²⁺ oscillations is robust regardless of the significant increase in TSH cell mass upon hypothyroidism onset. Altogether, our findings redefine the role of pituitary thyrotrophs in HPT axis function. Their division into two sub-populations of cells organised as a single network may explain TSH pulsatility and the non-linear TSH-TH relationship. This shall provide a clearer understanding of the interactions between the pituitary thyrotrophs and the thyroid gland, thus allowing a refinement of current diagnosis and treatment of thyroid hormone defects.

SA29

Probiotics alter the antibiotic resistance gene reservoir along the human GI tract

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Antimicrobial resistance poses a substantial threat to human health. The gut microbiome is considered a reservoir (the gut “resistome”) for potential spread of resistance genes from commensals to pathogens. The impact of probiotics, commonly consumed by many in health or in conjunction to antibiotics administration, on the gut resistome remains elusive. Direct assessment of the gut resistome *in situ* along the gastrointestinal tract in healthy antibiotics-naïve humans supplemented with an 11-probiotic-strain preparation demonstrated that probiotics reduce the number of antibiotic resistance genes exclusively in the gut of colonization-permissive individuals. In mice and in a separate cohort of humans, a course of antibiotics resulted in expansion of the lower gastrointestinal (GI) tract resistome, which was mitigated by autologous fecal microbiome transplantation or during spontaneous recovery. In contrast, probiotics further exacerbated resistome expansion in the GI mucosa, by supporting the bloom of strains carrying vancomycin resistance genes, but not resistance genes encoded by the probiotic strains. Importantly, the aforementioned effects were not reflected in stool samples, highlighting the importance of direct sampling for analyzing the effect of probiotics and antibiotics on the gut resistome. Analyzing antibiotic resistance genes content in additional published clinical trials with probiotics further highlighted the importance of per-person metagenomics-based profiling of the gut resistome using direct sampling. Collectively, these findings suggest opposing person-specific and antibiotics-dependent effects of probiotics on the resistome, whose contribution to the spread of antimicrobial resistance genes along the human gastrointestinal tract merit further studies.

Acknowledgements :-

Emmanuel Montassier and Rafael Valdés-Mas contributed equally to this work.

Jotham Suez and Erin Elinav are joint last authors.

SA29

An update of key morphogenetic events of thyroid development in *Zebrafish*

Federica Marelli¹, Luca Persani^{1, 2}

The thyroid gland is a highly conserved organ in vertebrates that regulates a wide range of developmental processes and controls the homeostasis of fundamental physiological mechanisms. Defects during thyroid development can lead to irreversible intellectual disabilities and dwarfism if not timely diagnosed and treated. Therefore, the mechanisms underlying thyroid gland development have a central interest in biology, and zebrafish represents a valuable model to study thyroid physiology with significant advantages compared to the mammalian models (1). Zebrafish is an emerging powerful vertebrate model for thyroid research due to its external fertilization, transparency of developing embryos and rapid organ development. Moreover, the availability of molecular tools for the manipulation of zebrafish genome allows the generation of targeted-genetic mutants or transgenic lines that express fluorescent proteins into specific cell types. Thanks to this, we are now able to study the relative contribution of different genes during thyroid development, and track the dynamic changes in thyroid shape, size, and location in live embryos (2). During zebrafish embryogenesis, the acquisition of thyroid competence occurs between the 20-24 hours post fertilization (hpf), when a restricted group of pharyngeal endoderm cells start to express the thyroid transcription factors (TTFs) *pax2a*, *nkx2.4b* and *hhex*, forming the so-called thyroid anlage. Around 36-40 hpf, the thyroid anlage expands forming a placode that protrudes into the underlying mesenchyme and buds from the pharyngeal epithelium in a rostro-ventral direction, in close proximity to the developing heart tube. The first detectable thyroid follicular cell (TFC) is formed by 55 hpf and expresses all of the functional thyroid markers, such as thyroglobulin (*tg*), thyroid peroxidase (*tpo*), Na/I symporter (*slc5a5*), and thyroid stimulating hormone receptor (*tshr*) required for thyroid hormone synthesis. During the later stages of thyroid organogenesis (55-72 hpf), the TFCs proliferate and migrate posteriorly forming distinct follicular units scattered along the pharyngeal midline, moving close and along the ventral aorta (2). Among the various organs derived from foregut endoderm, the thyroid gland is unique because a complex crosstalk between intrinsic and extrinsic factors coming from surrounding tissues (e.g., cardiac mesoderm and pharyngeal vessels) is essential for proper thyroid organogenesis. In particular, the coordinate activity of different morphogens (Notch, Shh, Bmp, Fgf, and Wnt) during critical developmental windows of thyroid development have been recently described in zebrafish (3-5). During the symposium, we will present the recent findings produced in the various zebrafish experimental models with the aim to define a comprehensive picture of the morphogenetic events underlying zebrafish thyroid development.

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SA30

The gut-heart axis

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The gut-heart axis is a newly evolving field centred around the contribution of the gut microbiome to heart disease. This host-microbiome interaction has been a previously under investigated field in the pathophysiology of heart disease.

The identification of metabolites that require metabolism by gut microbes which then contribute to disease physiology in recent years has contributed to the interest in this field.

One metabolite, trimethylamine-N-oxide (TMAO), has been a key molecule of interest as it is known to be associated and potentially causal of heart disease, and importantly, requires the gut microbiome for its production.

My research group has investigated the clinical implications of TMAO and other related molecules in heart disease ranging from heart failure to myocardial infarction, and have characterised its properties including response to treatment, ethnic and geographic differences which will be discussed during the symposium.

Our work and those of others have advanced understanding in the contribution of the gut microbiome to heart disease (the gut-heart axis).

SA31

Muscle damage and reactive oxygen species

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Our interest in the role of reactive oxygen species (ROS) in skeletal muscle was ignited in the early 1980's by an upsurge in publicity about nutritional myopathies that were economically important in some farm animals, such as the so-called "White Muscle Disease" in cows and sheep. These disorders had been recognised to be caused by selenium or vitamin E deficiency. This prompted studies to determine common mechanisms that might underlie these disorders and potentially be relevant to human myopathies involving David Jones, Richard Edwards and myself in the muscle group based at University College, London [1, 2]. An understanding of the roles of these nutrients as antioxidants and the recognition that skeletal muscle generates free radicals and other ROS during contractile activity has stimulated research into potential physiological and pathophysiological roles of ROS in skeletal muscle. Superoxide is generated by NADPH oxidases during muscle contractile activity. It is rapidly converted to hydrogen peroxide (H₂O₂) which acts as a signalling molecule to stimulate multiple adaptations to contractile activity through redox-regulated signalling pathways. These physiological pathways appear to be modified by ageing leading to attenuation of some specific responses to muscle contractile activity and exercise in older humans and animals [3]. Our studies

have recently focussed on understanding how this attenuation of redox responses occurs in ageing and indicate that mitochondria plays a major role. In older humans and mice, muscle shows substantial evidence of motor unit remodelling and focal denervation which has been shown to be associated with a substantial increase in mitochondrial peroxide generation by denervated and neighbouring innervated muscle fibres [4], leading to a local disruption of redox signalling. How such changes contribute to the loss of skeletal muscle mass and function that accompanies ageing remains a subject of extensive study.

Reference 1 :- Vitamin E and skeletal muscle. Jackson MJ, Jones DA and Edwards RHT. *Ciba Foundation Symposium No. 101 'Biology of Vitamin E'* pp224- 239 Pub. Pitman, London (1983).

Reference 2 :- Lipid peroxidation of skeletal muscle - an in vitro study. **Jackson MJ**, Jones DA and Edwards, RHT. *Bioscience Reports*, 3, 609-619 (1983).

Reference 3 :- Free radical generation by skeletal muscle of young and old mice: Effect of contractile activity. Vasilaki A, Mansouri A, Van Remmen H, van der Meulen J, Larkin L, Richardson AG, McArdle A, Faulkner JA and **Jackson MJ** *Aging Cell* 5: 109-117 (2006).

Reference 4 :- Denervated muscle fibers induce mitochondrial peroxide generation in neighbouring innervated fibers: Role in aging. Pollock N, Staunton C, Vasilaki A, McArdle A and **Jackson MJ**. *Free Rad. Biol. Med.* 112: 84-92 (2017).

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SA32

Age-related muscle atrophy: A battle of nerves?

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The age-related loss of muscle mass and function may be explained by the combination of individual muscle fibre *atrophy* and fibre *loss*. Although the relative contribution of each remains largely unknown, the loss of fibres is associated with the loss of the innervating motor neurons, and is in part compensated for by an expansion of surviving motor units. This talk will address in vivo methods recently applied in the exploration of age-related loss and consequential remodelling of human motor units, and present data supporting the corresponding adaptation of neural input as a defining factor in neuromuscular decrements.

SA33

From cigarette smoking to burning mitochondria

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Although smoking-related diseases, such as chronic obstructive pulmonary disease and heart failure with preserved ejection fraction, are often accompanied by increased peripheral muscle fatigability, the extent to which this is directly caused by cigarette smoke per se is less well understood. Our earlier observation that smoking history did not correlate with fatigability in humans suggests an acute effect caused by carbon monoxide and/or other substances in smoke, hampering skeletal muscle oxygen delivery and mitochondrial function. Indeed, carbon monoxide inhalation acutely and negatively impacts on muscle fatigue. Acutely exposing permeabilized mouse skeletal muscle fibers with smoke extract indicated a clear reduction in the maximal oxidative phosphorylation capacity. These data raise the question whether smoking cessation can be accompanied by beneficial effects on skeletal and cardiac muscle function. Indeed, smoke cessation as short as one to two weeks is associated with improved skeletal muscle structure and mitochondrial function in mice and men. Particularly the diaphragm and cardiac muscle were sensitive to smoke exposure and cessation in mice. Markers of low-grade systemic inflammation reduced in ex-smokers, which was linked to cardiac infiltration of macrophages and fibrosis in mice. Cardiac metabolome analysis revealed altered metabolism, which was partially restored after smoke cessation. Overall, skeletal and cardiac muscle metabolism are acutely altered upon cigarette smoke, and the accompanying systemic inflammation can cause additional skeletal muscle alterations. Smoke cessation quickly restores skeletal muscle mitochondrial function and fatigue resistance.

SA34

Mental Health and the kynurenine pathway: Does exercise influence the activity of the kynurenine pathway?

Sophie Erhardt¹

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It is well accepted that physical exercise is associated with health benefits and reduces risks of psychiatric illness, diabetes, cancer and cardiovascular disease. Previous studies also show that psychiatric disorders, such as depression is associated with changes in peripheral and central concentrations of kynurenine metabolites of tryptophan degradation. Animal experiments indicate that exercise protects against depression by increased expression of skeletal muscle kynurenine aminotransferase (KAT), hereby promoting synthesis of the neuroprotectant kynurenine acid (KYNA)

over the neurotoxic quinolinic acid (QUIN). In a series of studies, we have investigated the effects of exercise on kynurenine metabolism in humans.

We found that KAT gene and protein expression increased in the muscles of endurance-trained subjects compared with untrained subjects. Furthermore, one hour after cycling 150-km plasma KYNA was found to be substantially increased (+63%) while the ratio QUIN/KYNA was found to be decreased (27%). Both KYNA and the QUIN/KYNA ratio returned to baseline within 24 h. In another group of subjects, we analyzed kynurenines in the plasma before and after performing a half-marathon. In this group plasma KYNA increased (+125%) 30 min after the race. In contrast, plasma concentrations of KYNA and QUIN, did not change following eccentric exercise (consisting of a series of 100 drop jumps).

We further investigated the effect of exercise on both peripheral and central concentrations of kynurenine pathway metabolites. Cerebrospinal fluid (CSF) concentrations of KYNA, 3-hydroxykynurenine and picolinic acid (PIC) increased after an acute exercise paradigm, while tryptophan and kynurenine remained unchanged. At the same time, plasma tryptophan and kynurenine levels decreased while KYNA, 3-HK, QUIN and PIC did not change. We further found that the most robust effect of exercise was detected in subjects performing acute bouts of exercise over four consecutive days, while changes induced by engaging in three times weekly training exercise over 4 weeks, led to very limited effects. We further observed that the correlation between plasma and CSF concentrations of most of the kynurenine metabolites was low, indicating limited equilibration through an intact blood brain barrier. The only exception was PIC, that correlated to a high degree between compartments both before and after the intervention

In summary, these results show that acute vigorous aerobic exercise intervention causes adaptations in kynurenine metabolism, while a training exercise protocol over a longer time frame may have more limited effects. Further studies are needed to address the durability of effects and also if other modalities of exercise, such as muscle strength training, may influence the results.

SA35

Physical activity for mental health: an update of the evidence

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While physical activity and exercise are well-established to be beneficial for physical health, the effects on mental health are also now becoming more apparent. Specifically, there is an increasingly large clinical and academic interest in how physical activity may be used to (i) reduce the risk of developing mental illness, and (ii) improve recovery and outcomes in those with diagnosed conditions. Within this, multiple national and international health bodies have begun to produce

guidelines around the role of physical activity in the promotion of mental well-being. However, the overall evidence for if and how to use physical activity interventions in the prevention and treatment of various mental illnesses is unclear. Therefore, this presentation will aim to provide an update on the current state of the evidence on the benefits of physical activity across a broad spectrum of mental health conditions, such as depression, anxiety, bipolar and psychotic disorders, and ADHD. Within this, the published 'top-tier evidence' (including meta-analyses, randomized controlled trials, and Mendelian randomization studies) will be discussed, particularly with regards to determining the causal effects of physical activity on mental health/illness. Following this, an update of key recent international guidelines on the topic will be presented, along with an exploration of the emergent literature around how physical activity and exercise can be best implemented for improving outcomes in the context of mental healthcare.

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SA36

Exercise and the microbiome-gut-brain axis: moving beyond tryptophan metabolism

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Background/objective:

Exercise exerts beneficial effects in depression with a number of mechanisms proposed. There is a substantial overlap between these biological pathways and those regulated by the gut microbiota via the gut-brain axis, including via the regulation of tryptophan metabolism. The physiological impact of exercise may also manifest as hormetic effects on gastrointestinal permeability, acting initially as an acute stressor that subsequently stimulates positive adaptation to support good mental health. Indeed, alterations in barrier function can impact the central nervous system via gut-brain axis signaling pathways including tryptophan metabolism. Evidence of an association between stress-related psychiatric disorders including depression, microbiota composition and increased intestinal permeability levels is accumulating. This study aims to assess the impact of exercise training intensity on gastrointestinal permeability and stress level in sedentary healthy controls, both acutely and chronically over a 12-week duration.

Methods:

Monthly fitness and psychometric assessments were performed throughout a 12-week exercise program in 35 sedentary healthy adults (3-sessions/week). Intestinal permeability was assessed in plasma samples, pre-exercise, and one-hour post-exercise, for each monthly visit using Lipopolysaccharide binding protein (LBP) and soluble cluster of differentiation 14 (sCD14) protein as biological markers (figure 1). Additionally, we investigated the dose-dependent nature of exercise upon measures of stress, anxiety, and depression.

Results:

Preliminary analyses show that a 12-week exercise program resulted in significant increases in fitness ($p < 0.05$) and performance ($p < 0.001$), with modest reductions in reported levels of stress ($p = .08$) and depression ($p = .06$). Acutely, intestinal permeability markers (LBP; sCD14) were unchanged one-hour post the monthly fitness assessment. Chronically, we observe a significant effect of training ($p < 0.05$) and significant training x exercise group interaction ($p < 0.05$) in LBP concentration levels, which were increased in the high-intensity group only.

Conclusions:

Recent clinical and preclinical research charting the impact of exercise on the microbiota-gut-brain axis as a potential mediator of the benefits for mood and cognition. Our preliminary data shows that 12-weeks exercise training has a modest impact on intestinal permeability. Further research is needed to understand the CNS implications of exercise-induced alterations in gastrointestinal physiology and gut-brain axis signaling, and to understand how this integrates with the beneficial effects of exercise in modulating stress and depression.

Ethical Standards:

Approval of the study protocol was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals and written informed consent was obtained from all subjects. The study was carried out in accordance with the Declaration of Helsinki. All experiments were in full accordance with the EU legislation (Directive 2010/63/EU).

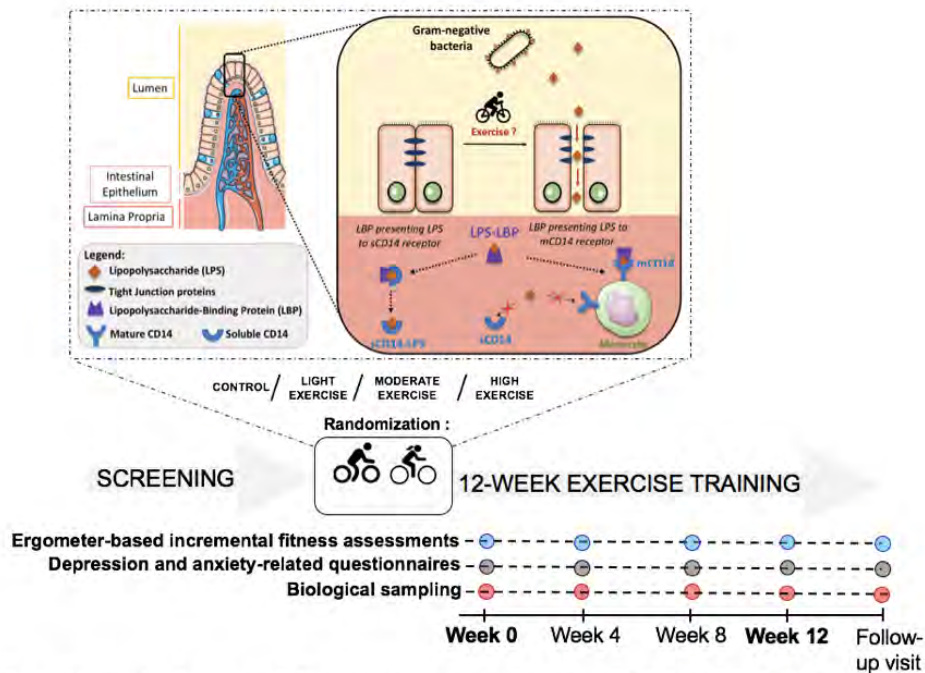


Figure 1. Altered intestinal permeability can be assessed using sCD14 and LPS-binding protein (LBP).

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Jason A. Martin is a co-first author in this work.

SA37

Physical activity, exercise, myokines and muscle-brain crosstalk

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Skeletal muscle secretes several hundred myokines that facilitate communication from muscle to other organs, such as, adipose tissue, pancreas, liver, gut, and brain. The biological roles of myokines include effects on e.g glucose and lipid metabolism, tumour metabolism, inflammation as well as brain function.

Exercise has many beneficial effects on brain health, contributing to decreased risks of dementia, depression and stress, and it has a role in restoring and maintaining cognitive function and metabolic control. The fact that exercise is sensed by the brain suggests that muscle-induced peripheral factors enable direct crosstalk between muscle and brain function. Muscle secretes myokines that contribute to the regulation of hippocampal function. Evidence is accumulating that the myokine cathepsin B passes through the blood-brain barrier to enhance brain-derived neurotrophic factor production and hence neurogenesis, memory and learning. Exercise increases neuronal gene expression of FNDC5 (which encodes the PGC1 α -dependent myokine FNDC5), which can likewise contribute to increased brain-derived neurotrophic factor levels. Serum levels of the prototype myokine, IL-6, increase with exercise and might contribute to the suppression of central mechanisms of feeding. Exercise also increases the PGC1 α -dependent muscular expression of kynurenine aminotransferase enzymes, which induces a beneficial shift in the balance between the neurotoxic kynurenine and the neuroprotective kynurenic acid, thereby reducing depression-like symptoms. Myokine signaling, other muscular factors and exercise-induced hepatokines and adipokines are implicated in mediating the exercise-induced beneficial impact on neurogenesis, cognitive function, appetite and metabolism, thus supporting the existence of a muscle-brain endocrine loop.

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SA38

Manipulation of cellular pathways by SARS-CoV-2 in the gastro-intestinal tract

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Exacerbated pro-inflammatory immune response contributes to COVID-19 pathology. However, despite the mounting evidence about SARS-CoV-2 infecting the human gut, little is known about the antiviral programs triggered in this organ. To address this gap, we performed single-cell transcriptomics of SARS-CoV-2-infected intestinal organoids. We identified a subpopulation of enterocytes as the prime target of SARS-CoV-2 and, interestingly, found the lack of positive correlation between susceptibility to infection and the expression of *ACE2*. Infected cells activated strong proinflammatory programs and produced interferon, while expression of interferon-stimulated genes was limited to bystander cells due to SARS-CoV-2 suppressing the autocrine action of interferon. These findings reveal that SARS-CoV-2 curtails the immune response and highlights the gut as a proinflammatory reservoir that should be considered to fully understand SARS-CoV-2 pathogenesis.

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SA39

Human organoid systems to study the pathogenesis, cell biology and evolution of SARS-CoV-2

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As obligate intracellular parasites, viruses rely on fine-tuned interactions with their hosts. Nevertheless, host switching is observed for viruses with a high degree of adaptive plasticity and a high mutation rate, such as certain coronaviruses. SARS-CoV-2 spilled over to humans possibly from bats and since this event has adapted to optimize its transmission between humans. However, traditionally, pathogenic viruses are studied in experimental animal models, in which specific virus-host interactions may not be modelled correctly, forcing viruses to adapt and limiting the use of these models. Studying SARS-CoV-2 pathogenesis and biology, as well as identifying potential treatments therefore benefits from the development of in vitro cell culture systems that closely mimic human physiology. We have established human organoid systems of the intestines, airways and alveoli to study the pathogenesis, cell biology and evolution of SARS-CoV-2. Combining these systems with CRISPR/Cas9 gene editing technology allows the identification of realistic antiviral drug targets. In addition, our findings unveil human organoids as powerful tools to phenotype SARS-CoV-2 variants-of-concern and identify correlates of fitness for SARS-CoV-2. Organoids are emerging as versatile tools to study SARS-CoV-2, laying a foundation for future pandemic responses.

SA40

Effects of SARS-CoV-2 infection on airway epithelial ion transport

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Introduction: SARS-CoV-2, the cause of COVID-19, belongs to the β -coronavirus subgroup that includes SARS-CoV-1 and MERS. SARS-CoV-2 utilizes the ACE2 receptor/TMPRSS2 protease complex expressed on the apical surfaces of respiratory epithelial cells as an entry mechanism. Removal of infectious agents from the airways is accomplished by mucociliary clearance (MCC), which requires a delicate balance between secretion and reabsorption of fluids. Ion channels such as the epithelial sodium channel (ENaC), the cystic fibrosis transmembrane conductance regulator (CFTR), and the calcium-activated chloride channel, TMEM16A, play key roles in regulating airway surface liquid homeostasis. SARS-CoV-2 encodes 4 major structural proteins: envelope (E), spike, membrane, and nucleocapsid proteins, which are required to produce a complete viral particle. CoV E proteins oligomerize to form viroporin channels that have been suggested to conduct cations and therefore may also affect airway hydration.

Objectives: To elucidate the impact of SARS-CoV-2 on ion transport in airway epithelia and to determine whether the SARS-CoV E protein can be used as a novel and effective target to treat COVID-19 as well as future SARS outbreaks.

Methods: We performed viral infections of primary airway epithelial cultures with CoV or CoV E. Because SARS-CoV-2 research requires BL3 facilities, the human CoV, NL63, which causes minor cold-like symptoms but shares the ACE2 entry mechanism with SARS-CoV-2, was used as a surrogate. We conducted measurements in Ussing chambers to determine ion channel function of CFTR, ENaC, and TMEM16A in the presence of CoV or SARS-CoV-2 E. In addition, we studied intracellular localization of CFTR, ENaC and SARS-CoV-2 E in primary airway cultures by immunofluorescence microscopy, and analyzed protein processing and expression by Western blotting. Furthermore, CoV E peptide studies in planar lipid bilayers by single-channel measurements were performed by fusion of membrane vesicles from HEK-293 expressing SARS-CoV-2 E or utilizing artificial lipid vesicles with reconstituted SARS-CoV-2 E peptides and conducting single-channel measurements.

Results: CoV infection substantially affected ion channel function in primary airway epithelia with a significant reduction in ENaC activity, while function of CFTR and TMEM16A, similar to responses observed after airway inflammation (1), were enhanced. Furthermore, we demonstrated that SARS-CoV-2 E conducts cations, such as Na^+ and K^+ and notably, that hexamethylene amiloride (HMA) inhibits CoV E channels and reduces the titer of CoV.

Conclusions: CFTR and ENaC were recently shown to be localized to secretory cells (2), where they regulate airway hydration required for MCC. Proper ENaC function requires proteolytic cleavage by the serine protease furin (3). We are currently exploring whether expression of viral spike protein, which contains the same furin cleavage sequence as ENaC, could interfere with ENaC processing. The

most likely host-infection sequence for SARS-CoV-2 begins with infection of ciliated cells in the nose, followed by viral replication and shedding. Subsequent spreading of viral particles from the nasal cavity by inhalation results in infection of bronchiolar ciliated cells and, ultimately, alveolar cells (4,5). Understanding the role of CoV-2 E may be key to developing therapies to inhibit viral propagation in nasal and bronchial epithelia, before SARS-CoV-2 spreads to alveoli.

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SA41

Assessment of SARS-CoV-2 transmission potential under experiment settings and in an acute healthcare setting

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Ferrets have been extensively characterised as models for SARS-CoV-2 transmission experiments, showing fairly robust infection and transmission. we assessed whether Spike mutations were selected for during experimental ferret infections in our laboratory, and, having detected a virus with a single Y453F mutation, we analysed the effect of this change on replication in ferrets, and in primary human airway epithelial cells. We confirm that these mutations likely adapt virus to mink/ferrets by enhancing entry into cells expressing mustelid ACE2. In addition, we assessed whether this single amino acid change altered neutralization of pseudovirus or live virus by antibodies in convalescent sera of individuals infected and recovered from COVID-19 during the first wave in the UK.

We performed a prospective cross-sectional observational study in a multi-site London hospital. Air and surface samples were collected from seven clinical areas, occupied by patients with COVID-19, and a public area of the hospital. Three or four 1.0 m³ air samples were collected in each area using an active air sampler. Surface samples were collected by swabbing items in the immediate vicinity of each air sample. SARS-CoV-2 was detected by RT-qPCR and viral culture; the limit of detection for culturing SARS-CoV-2 from surfaces was determined. Viral RNA was detected on 114/218 (52.3%) of surfaces and 14/31 (38.7%) air samples but no virus was cultured. The proportion of surface samples contaminated with viral RNA varied by item sampled and by clinical area. Viral RNA was detected on

surfaces and in air in public areas of the hospital but was more likely to be found in areas immediately occupied by COVID-19 patients than in other areas (67/105 (63.8%) vs. 29/64 (45.3%) (odds ratio 0.5, 95% confidence interval 0.2-0.9, $p=0.025$, Chi squared test)). The high PCR Ct value for all samples (>30) indicated that the virus would not be culturable. Our findings of extensive viral RNA contamination of surfaces and air across a range of acute healthcare settings in the absence of cultured virus underlines the potential risk from environmental contamination in managing COVID-19, and the need for effective use of PPE, physical distancing, and hand/surface hygiene.

SA42

Coordinated functions of STIM-Orai complexes fine tune calcium flux across nanojunctions of the ER/SR, plasma membrane and mitochondria

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In essentially all eukaryotic cells, activation of phospholipase C (PLC)-coupled receptors leads to inositol1,4,5-trisphosphate (IP_3)-dependent depletion of endoplasmic reticulum (ER) Ca^{2+} stores. ER Store depletion is sensed by Stromal-interaction molecule 1 (STIM1), which then activate Orai1 channels located in the plasma membrane (PM). STIM1/Orai1 coalesce within ER-PM junctions and generate Ca^{2+} nanodomains crucial for activation gene programs, including those driven by Calcineurin/nuclear factor for activated T-cells (NFAT) that regulate cell motility, growth, and metabolism. ER-mitochondria contact sites and functional PM-mitochondria interactions further shape receptor-evoked Ca^{2+} signaling to fine-tune cell function. In turn, mitochondrial Ca^{2+} uptake and extrusion, through the mitochondrial Ca^{2+} uniporter (MCU) and the $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX) respectively, are crucial for proper mitochondrial function and for coupling receptor stimulation to cellular bioenergetics. While the essential role of STIM1 and Orai1 in receptor evoked Ca^{2+} signaling is well understood, little is known about the physiological activation and the choreography of interactions between the five STIM1,2/Orai1,2,3 proteins natively expressed in all mammalian cells. Here we will discuss our recent findings revealing that coordinated functions of the five STIM1,2/Orai1,2,3, IP_3 receptors, MCU and NCLX at the ER-PM and ER-mitochondria contact sites translate the strength of agonist stimulation to precise levels of Ca^{2+} signaling and NFAT induction, ensuring the fidelity of complex mammalian Ca^{2+} signaling.

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SA43

ER-dependent scaling of site-specific calcium transients in astroglia

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Astroglia are an abundant and electrically non-excitable cell type in the brain, which can profoundly modify neuronal activity. For the latter, store-dependent calcium transients triggered by G-protein-coupled receptor are indispensable. Such calcium transients vary strongly in their spatial and temporal characteristics. They can be observed as fast, local elevations in small microdomains of the fine and fussy branches but also as large, slower waves propagating throughout a single branch, the whole astrocytic territory or even from cell to cell. While the functional relevance of these transients is well characterized, it is incompletely understood how the amplitude and waveform of these transients are controlled.

The IP₃ receptor type 2 is a key mediator of endoplasmic reticulum (ER)-dependent calcium elevations in astrocytes. The open probability of these receptors depends on the cytosolic calcium concentration. As the resting calcium concentration shows variations within and between astroglia, it could be responsible for site-specific scaling of calcium transients.

To explore this hypothesis in acute brain slices and in vivo, we used two-photon excitation microscopy and quantified calcium concentrations and their changes by analyzing the fluorescence lifetime or intensity of suitable indicators. Independent of the type of calcium transients (spontaneous, agonist- or behavior-induced) and recording technique, we observed a positive correlation between the maximum (peak) of the transient and a negative correlation between the scale (amplitude) and the local cytosolic calcium concentration. Mechanistically, the latter relationship is linked to store-dependent calcium entry into the cytosol. Importantly, deliberately increasing or decreasing the cytosolic resting calcium concentration altered calcium transient peaks and amplitudes accordingly, which indicates that the local resting calcium concentration dynamically controls the scale of local calcium transients.

In summary, our findings uncovered basic generic rules of calcium signal formation in astrocytes.

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SA44

Calcium signalling across the cell-wide web and its nuclear envelope invaginations: coordinating cellular process and gene expression by directing site-specific calcium flux

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A decade ago the panjunctional sarcoplasmic reticulum hypothesis was proposed (van Breemen *et al.*, 2013), that incorporated the idea that a network of tubules and quilts capable of auto-regulating their calcium content and determining junctional calcium concentration through loading and unloading at membrane-membrane nanojunctions. By this mechanism it was postulated that local calcium signals could be targeted to control a variety of cellular processes, from contraction and metabolism to transcriptional activities. In accordance with this, we recently identified a cell-wide network of distinct cytoplasmic nanocourses with the nucleus at its centre, demarcated by nanojunctions (<400 nm across) between sarcoplasmic reticulum (SR) and other organelles, that restrict calcium diffusion and thus provide for signal segregation in a manner facilitated by nanocourse-specific calcium pumps and release channels (Duan *et al.*, 2019). Ryanodine receptor subtype 1 (RyR1) supports relaxation of arterial myocytes by unloading calcium into peripheral nanocourses delimited by plasmalemma-SR junctions, fed by sarco/endoplasmic reticulum calcium ATPase 2b (SERCA2b). Conversely, stimulus-specified increases in calcium flux through RyR2/3 clusters selects for rapid propagation of calcium signals throughout deeper extraperinuclear nanocourses and thus myocyte contraction. Beyond these, nuclearenvelope invaginations and SERCA1 demarcate further diverse networks of cytoplasmic nanocourses that receive calcium signals through discrete RyR1 clusters that do not freely enter the nucleoplasm, yet impact gene expression through epigenetic marks segregated by their associated invaginations. Critically, this circuit is not hardwired and remodels for different outputs during cell proliferation.

Regardless of their functional subdivision, within cytoplasmic nanocourses all path lengths from calcium release site to targeted signalling complex are on the nanoscale. With picolitre volumes of cytoplasm lying within the boundaries of each nanocourse, relatively small net increases in local calcium flux (1-2 ions per picolitre) will be sufficient to raise the local concentration into the affinity ranges of most cytoplasmic calcium binding proteins (Fameli *et al.*, 2007; Fameli *et al.*, 2014). It is intriguing, therefore, that recent studies have established that unicellular organisms employ sequential computational logic to engage behaviour selection (Dexter *et al.*, 2019). Sequential logic can be actioned by progression through a series of circuit memory elements called flip-flops, which store a single bit (binary digit) of data, by switch and reset between 0 and 1. By analogy I propose that nanocourse-specific calcium binding proteins operate as local “switches”, their position

and reset directed, in part, by changes in local calcium flux. In this way coincident increases in calcium flux could be triggered in two distant parts of the cell-wide web at the same time (Duan *et al.*, 2019), enabling coordination of multiple cellular functions through either sequential, or parallel logic processing. This draws obvious parallels to mechanisms of conduction in carbon nanotubes, that transmit charge carriers through discrete conduction channels, enabling memory, logic and parallel processing. Thus, by analogy, our observations point to the incredible signalling potential that may be afforded by modulating “quantum calcium flux” on the nanoscale, in support of network activities within cells with the capacity to permit stimulus-dependent orchestration of the full panoply of diverse cellular processes.

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SA45

Molecular drivers of progressive renal injury in sickle cell disease

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Renal injury and progressive nephropathy is a major consequence of sickle cell disease (SCD) and is a cause of early mortality in this patient population. However, little is known about the molecular drivers that promote sickle cell nephropathy (SCN). Endothelin (ET) is a vasoconstrictor and pro-inflammatory peptide that is up-regulated in SCN. This presentation will provide preclinical data showing that specific blockade of the ET/ETA receptor pathway reduces the incidence of SCN. We will also provide data showing that endothelial-derived ET may specifically mediate SCN-induced immune cell activation, especially the Th17 pathway. Thus, we propose that the ET/ETA receptor pathway interacts with the activation of the Th17 pathway to promote SCN.

SA46

Clinical progression of proteinuric disease

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Urinalysis is an integral part of the assessment and management of individuals with kidney diseases. The finding of proteinuria in patients with kidney diseases is a poor prognostic sign for both for future renal function and cardiovascular disease. The role of proteinuria as an independent risk factor for renal function loss is now well recognized. The failing kidney is characterized histologically by tubulointerstitial inflammation, tubular cell apoptosis, tubular atrophy and fibrosis, and these changes correlate with the degree of proteinuria. Multiple clinical trials have indicated that antiproteinuric strategies are, in general, renoprotective. Such observations have led to the suggestion of a causal relationship between proteinuria and renal inflammation or scarring. Understanding the mechanisms underlying proteinuric nephropathy has attracted intense interest. It is now clear that the association between proteinuria and progression is causal and strong biological plausibility has been demonstrated. Targeting proteinuria is thus a rational target for clinical trial interventions.

SA47

A model of megalin trafficking in the proximal tubule

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The polarized epithelial cells that comprise the proximal tubule (PT) have a specialized apical endocytic pathway that allows for the high-capacity endocytosis necessary to recover essential nutrients and to maintain a protein-free urine. Impairments in this pathway result in tubular proteinuria, which can worsen to cause more severe kidney disease. Megalin and cubilin, multi-ligand receptors at the apical surface of the epithelial cells, bind proteins in the ultrafiltrate and internalize them via receptor-mediated endocytosis. Ligands are sorted from receptors in endocytic compartments, and the receptors are recycled back to the surface through structures called dense apical tubules (DATs). The molecular identities of the compartments involved in sorting and recycling in PT cells and the kinetics of megalin trafficking through them are unknown. Understanding megalin's endocytic trafficking itinerary and the key regulatory steps in this pathway is important for discerning the basis of proteinuric diseases and devising therapies. To address this, we identified endocytic compartment markers in a previously developed opossum kidney (OK) cell culture model that recapitulates morphologic and functional features of the PT *in vivo*. The fraction of total megalin colocalizing with each compartment marker was quantified by Mander's coefficient from deconvolved confocal images. Most megalin is localized primarily in Rab11-positive compartments at steady state while a much smaller fraction of megalin is localized in Rab4-positive structures. Surface biotinylation based assays revealed that only a small fraction of total megalin is present at the apical surface at steady state and that megalin is rapidly internalized from the surface. The half-life of surface megalin was also assessed. Our biochemical and quantitative colocalization data was used to construct and refine a kinetic model of megalin trafficking in the PT. These data suggest that recycling is the rate-limiting step in regulating the fraction of total megalin available at the surface. Together, our results provide valuable insight into the spatial organization of the endolysosomal

system and the itinerary and regulation of megalin traffic in the PT. Our kinetic model will be used to identify how genetic mutations and other conditions that cause tubular proteinuria disrupt traffic along the endocytic pathway.

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SA48

Megalín and cubilin function in proteinuric disease

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Introduction

An important role of the kidney proximal tubule is to reabsorb glomerularly filtered peptides and proteins. This occurs by receptor-mediated uptake by two large multiligand endocytic receptors, megalin and cubilin, which are highly enriched on the apical surface of proximal tubule epithelial cells. The process is highly efficient as reflected by the fact that in the healthy kidney, urine is practically devoid of protein. However, kidney disease with glomerular injury increases the load of filtered plasma proteins, in particular albumin, leading to proteinuria.

Objectives

Our objective is to understand the molecular basis of proximal tubule protein reabsorption and how it is regulated under normal and pathological conditions, with focus on the megalin and cubilin receptor system and its role in proteinuric disease.

Method

In our studies, we use a variety of molecular and cellular techniques as well transgenic animal models, to investigate the renal handling of filtered proteins by the megalin and cubilin receptors.

Results

Our research and that of co-workers has over the years established the essential role of the megalin and cubilin receptor system in modulating urinary protein excretion. Using a genetic model of glomerular kidney disease in combination with gene knockout of the receptors, we have further recently explored the implications of receptor dysfunction under nephrotic-range proteinuria. We

observed massive albumin uptake in proximal tubules under nephrotic proteinuria, which was efficiently blocked by megalin and cubilin receptor knockout. In addition, we found evidence of potential distinct roles of the cubilin and megalin receptor in the uptake of albumin at normal filtered levels and nephrotic levels of albumin.

Conclusion

Renal tubular reabsorption and rescue of filtered proteins, including albumin, is dependent on the megalin and cubilin receptors. Dysfunction of the receptors causes mild tubular proteinuria whereas glomerular kidney disease with increased filtration of plasma proteins overwhelms the receptors, causing nephrotic range proteinuria accompanied by massive protein uptake in the proximal tubules. These findings have implications for the pathophysiology and diagnosis of proteinuric renal diseases.

Ethics

All animal experiments were performed in accordance with the EU Animal Welfare Act for humane treatment of vertebrate animals and licenses issued by the Danish Animal Experiments Inspectorate.

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SA49

Novel insights into the role of intestinal NHE3 as a therapeutic target

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The sodium-hydrogen exchanger isoform 3 (NHE3) is abundantly expressed in the gastrointestinal tract and plays an essential role in sodium/fluid absorption and acid-base homeostasis. However, understanding the precise role of intestinal NHE3 has been challenging due to the lack of a suitable animal model. To circumvent this problem, we generated a tamoxifen-inducible intestinal epithelial cell-specific NHE3 knockout mouse model (NHE3IEC-KO). Before tamoxifen administration, the phenotype and blood parameters of NHE3IEC-KO were unremarkable compared with control mice. After tamoxifen administration, NHE3IEC-KO mice have undetectable levels of NHE3 in the intestine. NHE3IEC-KO mice develop watery, alkaline diarrhea in combination with a dilated small intestine, cecum and colon. They have higher fluid intake compared to controls due to persistent diarrhea. The mortality rate in NHE3IEC-KO is ~25% after 3 weeks. We found that NHE3IEC-KO mice exhibit metabolic acidosis, lower blood bicarbonate levels, hyponatremia and hyperkalemia associated with drastically elevated plasma aldosterone levels. These results demonstrate that intestinal NHE3 has a

significant contribution to acid-base, Na⁺ and volume homeostasis, and lack of intestinal NHE3 has consequences on intestinal structural integrity. Based on the physiological role of NHE3, pharmacological inhibition of intestinal NHE3 is an interesting treatment strategy. In fact, nonabsorbable NHE3 inhibitors have been developed, and preclinical as well as clinical trials indicate possible pharmacological use in fluid overload, hypertension, chronic kidney disease, hyperphosphatemia, and constipation.

Acknowledgements :- This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases [grant number 1R01DK110621 (to T.R.)]; American Heart Association Transformational Research Award [grant number 19TPA34850116 (to T.R.)]; the LeDucq Foundation (to R.A.F.); the Danish Medical Research Council; the Novo Nordisk Foundation; American Heart Association predoctoral fellowship [grant number 18PRE33990236 (to J.X.)]; and L.T. by a postdoctoral fellowship [grant number 19POST34400026].

SA50

Transporters feat. the lung: What do we learn from current understandings.

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This talk will provide an overview of recent knowledge on the (patho)physiological and pharmacological functions of organic cation transporters in the lung. The organic cation transporters (OCT) 1, 2 and 3 and novel organic cation transporters (OCTN) 1 and 2 of the solute carrier 22 (SLC22) family are involved in the various cellular transport mechanisms of endogenous compounds such as neurotransmitters and L-carnitine and further play an important role in the interaction with inhaled drugs such as beta2-agonists and anticholinergic drugs. They may be associated with the development of chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). In particular OCTN2 may be a potential target to enhance the pulmonary delivery of inhaled drugs for better therapeutical responsiveness in patients. The talk will further elucidate this and discuss future options of OCT/N molecular targets for therapeutic approaches.

SA51

Npt2a as a target for treating hyperphosphatemia with and without kidney disease

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The precise regulation of the body's phosphate (P_i) homeostasis is a critical task. Treatment of hyperphosphatemia, which becomes inevitable in later stages of chronic kidney disease (CKD), is limited to dietary P_i restriction and oral P_i binders. Two transport proteins mediate renal P_i reabsorption, the sodium-phosphate cotransporters Npt2a and Npt2c. The former mediates the majority of renal P_i reabsorption (70-80%), which is a hormonally regulated process and requires parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). Currently, no renal P_i cotransporter is a pharmacological target. Studies were performed *in vitro* and *in vivo* employing a novel Npt2a inhibitor (Npt2a-I, PF-06869206). In opossum kidney (OK) cells, a model of the proximal tubule, the Npt2a-I caused a dose-dependent decrease of Na^+ -dependent P_i uptake (IC_{50} : $\sim 1.4 \mu\text{mol/L}$). Michaelis-Menten kinetics in OK cells identified an ~ 2.4 -fold higher K_m for P_i in response to Npt2a inhibition with no significant change in apparent V_{max} . *In vivo*, the Npt2a-I induced a dose-dependent increase in urinary P_i excretion in wild-type mice (ED_{50} : $\sim 23 \text{ mg/kg}$), a finding completely absent in Npt2a^{-/-} mice. The observed decrease in plasma P_i in WT mice is also absent in Npt2a^{-/-} mice. Surprisingly the Npt2a-I-induced increase in urinary Na^+ excretion was unaffected in Npt2a^{-/-} mice, a response possibly mediated by an off-target acute inhibitory effect of the Npt2a inhibitor on open probability of the epithelial Na^+ channel (ENaC) in the cortical collecting duct. The effects on urinary P_i excretion and plasma P_i were also observed in a 5/6 nephrectomy (Nx) model but were somewhat attenuated. In addition, Sham and 5/6 Nx mice show a similar decrease in PTH in response to Npt2 inhibition. In summary, Npt2a inhibition is a possible treatment option in conditions where hyperphosphatemia is present, e.g., in severe CKD, acute tumor lysis syndrome, rhabdomyolysis, hemolysis, hyperthermia, profound catabolic stress, or acute leukemia.

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SA52

Pathophysiological significance of the prostaglandin transporter SLCO2A1 in lung inflammation

Takeo Nakanishi¹

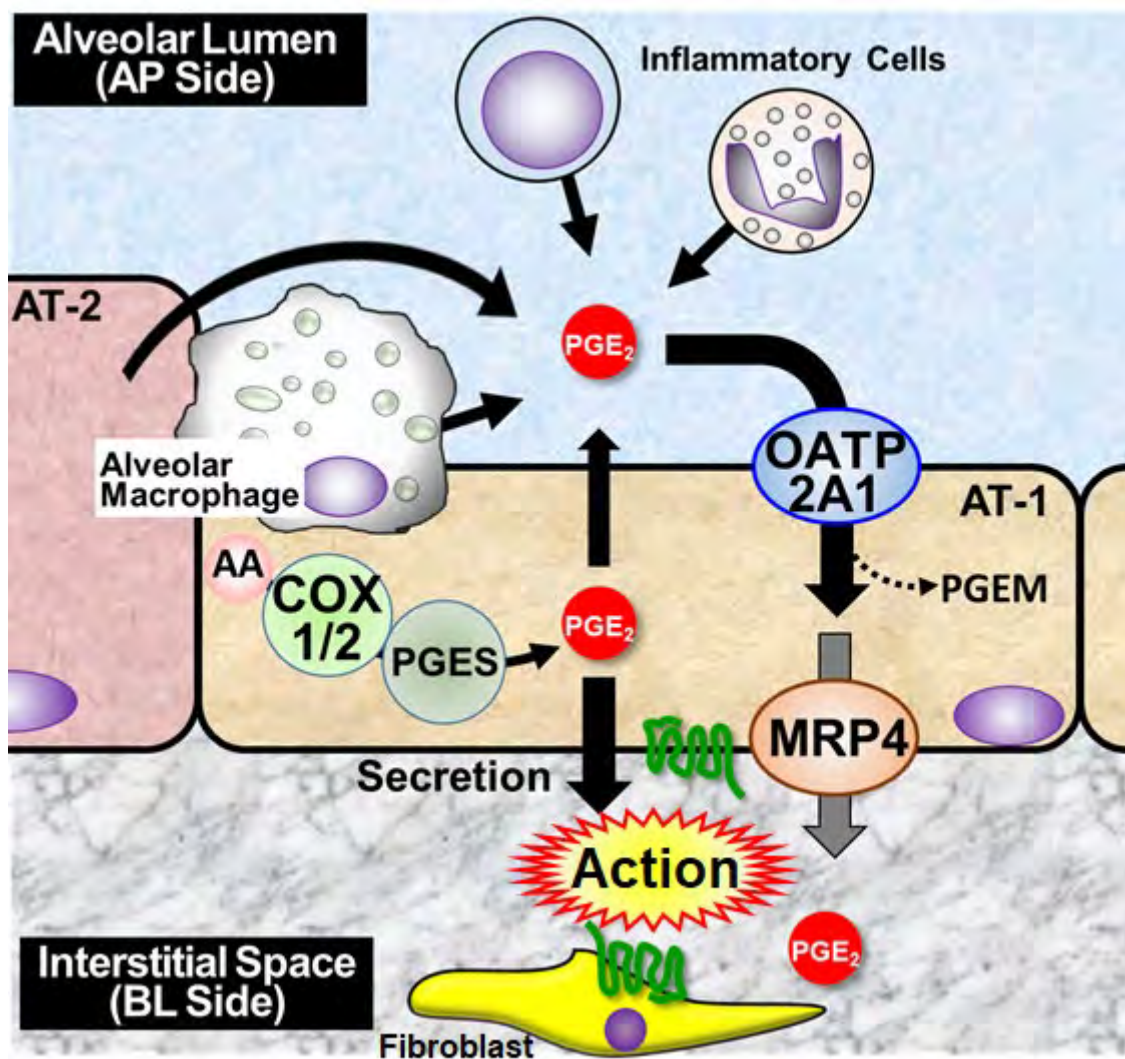
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Solute carrier organic anion transporter family member 2A1 (SLCO2A1) is a plasma membrane transporter consisting of 12 transmembrane domains and mediates cellular uptake of major prostaglandins (PGE₂, PGF_{2α}, and PGD₂) and, to a lesser extent, thromboxanes (e.g., TxB₂). Recent whole-exome analyses demonstrated that recessive inheritance of *SLCO2A1* mutations causes primary hypertrophic osteoarthropathy (PHO) and chronic enteropathy associated with *SLCO2A1* (CEAS), suggesting that impaired function of *SLCO2A1* is associated with a disorder of PG metabolism (1). In the lungs, there is significant evidence that the anti-fibrotic action of PGE₂ results in the increased sensitivity of fibroblasts to apoptosis. Although the importance of PG biosynthesis and signaling is well established, much less is known about the contribution of transporters to PGE₂ action in the respiratory regions. Our laboratory has been investigating the role of *SLCO2A1* in

actions of PGE₂ in the lungs since we observed more severe pulmonary fibrosis in bleomycin-treated *Slco2a1*-deficient mice (*Slco2a1*^{-/-}) (2). This study aimed to understand the pathophysiological significance of SLCO2A1 in pulmonary inflammation.

We conducted immunohistochemistry and transport studies using type 1 alveolar epithelial cell-like (AT1-L) cells transdifferentiated from type 2 alveolar epithelial cells prepared from rats and mice. Anti-Slco2a1 immunoreactivity was detected mainly in the alveolar epithelium. *Slco2a1* deficiency remarkably reduced cellular uptake of PGE₂ by AT1-L cells and increased PGE₂ in bronchoalveolar lavage (BAL) fluid of mice. Transcellular transport of PGE₂ across the monolayer of rat AT1-L cells from the alveolar lumen (AP side) to the interstitial space (BL side) was greater than that in the BL-to-AP direction (3). Permeation coefficient of PGE₂ was significantly reduced in the presence of SLCO2A1 inhibitors and, to a lesser extent, ceefourin-1 (an MRP4 inhibitor), suggesting that SLCO2A1 transports PGE₂ from alveolar lumen to stromal tissues in cooperation with MRP4 (Figure 1).

We further investigated the impact of SLCO2A1 on lung inflammation. BAL fluid cell analysis indicated more severe inflammation occurred in *Slco2a1*^{-/-} on day 5 after BLM intratracheal instillation, and *Slco2a1* deletion increased mRNA expression of pro-inflammatory cytokines (Tnf- α and Il-1 β) and chemokine (Ccl5) in BAL cells (4). Male *Slco2a1*^{-/-} exhibited significantly higher amounts of released Il-1 β and PGE₂ concentration in BAL fluid than female *Slco2a1*^{-/-}. Thus, *Slco2a1*-deficient male mice are likely more susceptible to airway inflammation. This observation is consistent with our recent report of NLRP3 inflammasome activation caused by increased peripheral PGE₂ level in dextran sulfate sodium-induced colitis mouse model (5). Finally, we found that cigarette smoke extracts (CSE) significantly reduced SLCO2A1-mediated PGE₂ uptake; therefore, SLCO2A1 inhibition by xenobiotics is considered a new rationale for lung toxicity. In conclusion, excess amount of PGE₂ due to impaired function of SLCO2A1 in the alveolar fluid may contribute to activation of inflammasomes in alveolar macrophages, resulting in aggravated lung inflammation. These results suggest that SLCO2A1 regulating PGE₂ biodistribution in the respiratory region is essential for lung homeostasis.



Reference 1 :- Nakanishi T et al, *Pharmacol Ther*, 223:107803, 2021

Reference 2 :- Nakanishi T et al, *PLOS ONE*, 10:e0123895, 2015

Reference 3 :- Nakanishi T et al, *J Pharmacol Exp Ther*, 368:317, 2019

Reference 4 :- Nakanishi T et al, *Toxicol Appl Pharmacol*, 405:115201, 2020

Reference 5 :- Nakata R et al, *Sci Rep*, 10:4883, 2020

Acknowledgements :- Grants-in-Aid for Scientific Research (KAKENHI 20K07018) from the Japan Society for the Promotion of Science, and a grant from the Smoking Research Foundation (Tokyo, Japan)

SA53

Probing photoreceptor physiology and pathophysiology in vivo using electrophysiology

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The electroretinogram (ERG) represents the summed electrical response of the retina to light stimuli. It can be recorded from human subjects non-invasively *in vivo* using electrodes placed around the eye. In response to flashes of light of a range of strengths, an initial negative-going wave is observed (the a-wave), followed by a positive-going wave (the b-wave). Stimulus protocols can be employed to estimate dark-adapted rod-driven and cone-driven response components. Application of mathematical models to the a-wave allow parameters relating to retinal current flows to be extracted from the human eye. This can be applied to probe retinal function in health and disease. Examples will be given in this talk. The ERG can also be used to track kinetics of retinal light and dark adaptation, including by monitoring the b-wave elicited by dim flashes delivered during and following exposure to different backgrounds (Cameron et al., 2006). We used a similar protocol to track dark adaptation in healthy volunteers and patients with selected diseases. Participants' pupils were pharmacologically dilated. ERGs were recorded using conductive fibre electrodes in response to the dim white flashes (c.0.02 scotopic cd m⁻² s) delivered following the extinction of a white background (30 photopic and c.85 scotopic cd m⁻²), after steady state exposure to this background. Flash series were repeated every 2 min for 20-60 min, and b-wave amplitudes (normalised to their final level) were plotted against time in the dark. Participants gave informed consent; the study had local ethics committee approval and complied with the tenets of the Declaration of Helsinki. The following participants underwent recordings: 7 healthy volunteers (aged 21-82 years); a patient (aged 50) with early-onset widespread retinal drusen; two patients (ages 42, 49), with molecularly confirmed Sorsby Fundus Dystrophy (SFD); a 70 year old with Vitamin A deficiency (VAD), both after treatment and at varying levels of deficiency. Responses were initially of low amplitude but then recovered gradually in the dark. In healthy participants (and the participant with early-onset drusen), recovery kinetics were similar: amplitudes reached the final level at c.20 min (and half the final level by c.10 min). The VAD patient showed normal recovery following treatment, but no recovery when markedly deficient, and a slowed recovery when mildly deficient. The SFD patients displayed recoveries similar to that found in the VAD patient when mildly deficient. Although abnormalities in SFD on clinical examination are largely in the macula, these findings confirm delayed recovery affecting the retina as a whole, consistent with "ocular vitamin A deficiency" in SFD. The protocol can be useful in investigating conditions in which photoreceptor dark adaptation might be impaired.

Reference 1 :- Cameron AM, Mahroo OA, Lamb TD (2006). Dark adaptation of human rod bipolar cells measured from the b-wave of the scotopic electroretinogram. *J Physiol.* **575**(Pt 2), 507-526.

Acknowledgements :- Fight for Sight UK and Birdshot Uveitis Society (24BU141); Fight for Sight UK (1409/10); TwinsUK receives support from the Wellcome Trust and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London; Moorfields Eye Charity (R190037A).

SA54

Photoreceptor physiology: Cellular and molecular basis of rod and cone phototransduction

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Phototransduction in rod and cone photoreceptors utilises a G-protein cascade that is well understood at a cellular and molecular level. Rhodopsin (or its cone equivalent) is a typical G-protein-coupled receptor and, when activated by light, enzymatically activates the G-protein, transducin. Transducin in turn activates a cyclic-nucleotide phosphodiesterase (PDE6), increasing the hydrolysis of cGMP, and thereby causing closure of cyclic nucleotide-gated channels in the cell's plasma membrane. The activation phase of the light response in rods has been modelled accurately at a molecular level. An additional half-dozen proteins are involved in shut-off of the light response and light adaptation.

Remarkably, the homologous cascades in rods and cones use different isoforms of most of the main players. This duality can be traced back to the two rounds of whole-genome duplication (WGD) that occurred roughly 500 million years ago in a chordate ancestor of all jawed vertebrates, or in some cases to more ancient duplications (Lamb, 2020). For transducin α , for the PDE6 catalytic and regulatory units, and for the CNGC α and β subunits, this rod versus cone distinction arose at the first round (1R) of WGD. The HGNC names of the respective rod / cone isoforms of these genes are: *GNAT1* / *GNAT2*; *PDE6A+PDE6B* / *PDE6C*; *PDE6G* / *PDE6H*; *CNGA1* / *CNGA3* and *CNGB1* / *CNGB3*. For the visual pigments, three cone-type opsins with different spectral sensitivities had already evolved prior to 1R, along with pinopsin, which appears to have been the ancestral scotopic visual opsin; rhodopsin did not appear as a distinct isoform until 1R.

Likewise, most of the other proteins involved in response recovery and light adaptation exhibit distinct rod/cone isoforms, and they too arose through a combination of ancient (pre-1R) individual gene duplications and the first round of WGD.

The existence of these multiple isoforms underlies some marked differences in rod/cone manifestations in a variety of monogenic retinal disorders.

Recently, it has become apparent that the dimeric nature of the rod PDE6a/b plays an important role in enabling the rod to operate at very low intensities. Activation of the two catalytic subunits exhibits significant cooperativity, in that a single bound transducin elicits only a small fraction of the activity elicited when two transducins bind. This cooperativity provides immunity against spontaneous thermal activation of transducin, because appreciable PDE6 activity occurs only upon a coordinated burst of transducin activation. Such a noise-reduction mechanism appears crucial in enabling the rod to respond reliably to a single photon. However, this benefit comes at the cost of a short delay (of ~5 ms); this is probably immaterial to rods, but would be a severe disadvantage if it were to occur in cones which mediate responses at high temporal frequencies. Analysis of the dimeric PDE6 activation scheme provides a quantitative explanation for rod recovery from intense

flashes, and for the occurrence of an increase in the rod's 'dominant time constant' at high intensities.

Reference 1 :- Lamb TD (2020). Prog. Ret. Eye Res. 76, 100823.

SA55

Monogenic retinal disease: mechanisms and the landscape of novel therapies

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Inherited retinal diseases associated with single gene disorders affect about 1 in 4,000 people and although individually rare, as a group are the commonest cause of severe visual impairment in the working age population. The final common pathway to blindness is impaired function or death of photoreceptors. There is considerable phenotypic and genetic heterogeneity with variable visual prognosis. Some disorders present in infancy with severe visual impairment whilst in other conditions the first symptoms appear in adult life. In most cases the disease is confined to the eye, but the underlying genetic mutation(s) may result in syndromic disorders with additional involvement of other organs.

There have been a number of scientific advances that have had a major impact on the field in recent years. These include improved retinal imaging, including the ability to image individual photoreceptor cells in the human eye, next generation sequencing which has allowed a precise molecular diagnosis in the majority of patients and animal and iPSC models of disease which can be used to explore potential therapeutic approaches. Recently the first gene replacement therapy for an infantile onset retinal dystrophy has been approved by the FDA and is in current clinical practice. A number of other gene based clinical trials are underway.

This brief talk will discuss how precise molecular diagnosis and deep phenotyping has led to improved understanding of molecular pathogenesis and informed the development of clinical trials of novel therapies.

SA56

Restoring vision after photoreceptor degeneration using optogenetic techniques

Samantha De Silva^{1, 2, 3}

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Inherited retinal degenerations such as retinitis pigmentosa (RP) affect approximately 1 in 3000 people and are the leading cause of blindness in working age adults in England and Wales. In these, typically monogenic, conditions there is progressive degeneration of photoreceptors, however inner retinal neurons such as bipolar cells and ganglion cells remain largely structurally intact, even in end-stage disease.

Optogenetics is a method of neuromodulation that has wide applicability in neuroscience, and utilises light to activate neurons that have been engineered to ectopically express a light-sensitive protein. Given this naturally occurs in the eye when light triggers phototransduction in rods and cones, an intuitive application of optogenetic techniques would be to induce light sensitivity in remaining retinal cells and restore vision in end-stage retinal degenerations.

Developing an effective optogenetic approach requires consideration of multiple factors including the light-sensitive protein that is used, the method of gene delivery and the target cell for expression. A range of photosensitive proteins have been investigated including microbial opsins e.g. channelrhodopsin and halorhodopsin; mammalian opsins e.g. rhodopsin, cone opsin and melanopsin, and engineered ion channels. These vary in sensitivity, kinetics of their response and wavelength of light causing peak stimulation.

Gene therapy using adeno-associated viral vectors (AAV) has been extensively investigated for gene transfer to the retina, with the first gene therapy now licensed for the treatment of a rare retinal disorder. Modification of surface amino acid residues on AAV vectors, the promoter driving gene expression and administration via subretinal or intravitreal injection, all impact vector efficacy and which retinal cell type expresses the light sensitive protein. This in turn influences potential visual output: for example transducing bipolar cells, the most distal remaining neuron in visual circuitry in end-stage RP, may preserve processing of signals within the retina, but thus far these cells have proven most difficult to target.

In this talk, we will explore different optogenetic approaches with a focus on human melanopsin gene therapy (De Silva et al, 2017*), and the current clinical trials now underway to evaluate optogenetic gene therapy in reversing visual loss in end-stage retinitis pigmentosa.

Reference 1 :- *De Silva SR. et al, (2017), Proceedings of the National Academy of Sciences, 114, 11211 - 11216

Acknowledgements :- The Wellcome Trust (094448/Z/10/Z)

Placental endocrine insufficiency programs atypical behaviour in mothers and their offspringDavid Harrison¹, Hugo Creeth¹, Hannah Tyson¹, Raquel Boque Sastre¹, Anthony Isles¹, Rosalind John¹¹Cardiff University, Cardiff, United Kingdom

Fetal growth restriction (FGR) is a major threat to human health impacting neonatal survival and increasing the risk of behavioural and metabolic disorders later in life, a phenomenon described as fetal programming or developmental origins of disease. Higher than normal placental expression of the imprinted gene *PHLDA2* has been reported in a number of studies of FGR, fetal death and low birth weight. We modelled this specific alteration and demonstrated that two-fold increased expression of *Phlda2* is sufficient to reduce birth weight by 10% followed by rapid postnatal catch-up (1). We further showed that *Phlda2* functions to negatively regulate the endocrine compartment of the placenta (2) and, through this function, influences the behaviour of wild type mothers towards their offspring (3). Being born small and being exposed to suboptimal maternal care are both risk factors for behavioural disorders in human populations. We therefore undertook a behavioural and molecular characterisation of *Phlda2* transgenic and non-transgenic littermates alongside concurrently bred fully wild type cohort. Offspring exhibited increased anxiety-like behaviours, deficits in cognition and atypical social behaviours alongside changes in the transcriptional signatures of key brain regions with males relatively more impacted than female (4). Our work establishes, for the first time, the experimental paradigm that placental endocrine insufficiency programs atypical behaviour in both mothers and their offspring. Importantly, we have evidence that this mechanism may contribute to the co-occurrence of low birth weight and perinatal mood disorders in human populations.

Reference 1 :- Isolating the role of elevated *Phlda2* in asymmetric late fetal growth restriction. Tunster, SJ, Van De Pette, M, and **John, RM**. *Dis Model Mech*. 2014 Oct;7(10):1185-91.

Reference 2 :- The imprinted *Phlda2* gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. Tunster SJ, Creeth HD, **John RM**. *Dev Biol*. 2016 Jan 1;409(1):251-60.

Reference 3 :- Maternal care boosted by paternal imprinting in mammals. Creeth, HDJ, McNamara, GI, Tunster, SJ, Boque-Sastre, R, Allen, B, Sumption, L, Eddy, JB, Isles, AR and **John, RM**. *PLOS Biology*. 2018 Jul 31;16(7):e2006599.

Reference 4 :- Placental endocrine insufficiency programs anxiety, deficits in cognition and atypical social behaviour in mouse offspring. Harrison, DJ, Creeth, HDJ, Tyson, HR, Boque-Sastre, R, Hunter, S, Dwyer, D, Isles, AR and **John, RM**. *Human Molecular Genetics*, in press

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SA58

Development of the gut microbiome in early life

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Following birth, neonates are rapidly colonized by microbes, which play important roles in health and disease. The gut contains the largest density of microorganisms, termed the gut microbiome, which plays fundamental roles in protection from pathogens, immune system training, and the breakdown of dietary compounds. Our research has shown that over the first year of life the infant gut microbiome is highly dynamic, providing a window of opportunity in which to seed a potentially beneficial microbiome to reduce the risk of early- and later-life disease risk. For instance, in a recent study of term infants, we showed that birth mode and breastfeeding are the most important variables for shaping the early life microbiome, which are directly correlated to an increased risk of obesity, allergy, asthma, and other disorders later in life. This, early life host-microbiome crosstalk and immune development is hypothesised to have important roles in long-term health.

Our research has made important advances in the role of the preterm infant microbiome in health and disease. Unlike infants born at term, extremely preterm infants (<32 weeks gestation) have immature intestinal architecture and an underdeveloped immune system. They are also less likely to be vaginally delivered and breastfed, and they receive limited exposure to microbes during the first months of life, leading to a reduction in potentially beneficial bacteria. Because the preterm gut can become leaky, translocation of microbes into the bloodstream and/or intestinal cell death represent major problems in this vulnerable population. However, evidence from my group and others has shown that certain types of bacteria, such as *Bifidobacterium*, may increase gut and immune maturation. The latest work from my group further shows specific components of human milk are linked to colonisation by *Bifidobacterium* which together contribute toward the health or disease of an infant.

While such associations provide insights, it is not possible to determine cause or effect. To advance this work, we have recently developed a novel model derived from primary human intestinal organoids that accurately recapitulates physiologically relevant oxygen conditions. This allows the co-culture of intestinal organoids with enteric anaerobic bacteria (i.e., oxygen sensitive bacteria that reside in the gut lumen). A better understanding of the interaction between bacteria and infant gut epithelial cells holds incredibly exciting possibilities to better predict, diagnose, and manipulate the microbiome of preterm infants at risk of disease.

PL01

Teaching physiology: Past present and future

James Clark¹

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Educating our expanding undergraduate student cohorts in whole body physiology is complex, and to do this effectively can sometimes be a challenge. I was awarded the Otto Hutter Physiology Teaching Prize in 2019 (pre-COVID) and my oral presentation at Physiology 2020 was *going* to be looking at the role of online teaching in physiology education. However, the recent global COVID-19 Pandemic has hit physiology education hard with restrictions on face-to-face on-campus teaching and instructors have had to be more and more imaginative to delivery high quality online teaching. In this presentation, therefore we will explore the challenges of physiology education with a specific focus on practical skills and human physiology looking back at our traditional approach, how we responded to the COVID-Pandemic and the ways we might be educating our students in the socially distanced future within our Institutions.

PL02

Hidden clocks: circadian rhythms and the vulnerable fetus.

Laura Bennet¹

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Despite marking our age from the day of birth, our physiological journey starts 9 months before; assuming we went to full term gestation. During this time we build our existence upon the merging of two cells, growing an entire human being, the environment it lives in; the amniotic sac, and the organ of gas, nutrient and waste exchange; the placenta. An undeniably fantastic bioengineering feat, and one which provides the physiological foundation for our post-natal development to adulthood. However, our physiological journey from conception to birth can face adverse challenges, such as hypoxia and inflammation, leading to impaired development of organs and their functional control and injury, all of which has consequences for our post-natal physiology. Further, such adverse events may cause the fetus to be vulnerable to further challenges to its environment leading to death before birth, as is seen with stillbirth. To develop methods of detecting the at-risk-fetus to inform clinical decision making and targeting of perinatal treatments requires a greater understanding of the maturational changes in fetal physiology and pathophysiology. This includes the role of a key regulator of our own biology; circadian rhythms.

This talk will discuss how a preterm fetus can survive a significant hypoxic insult and continue to develop to full maturity, but with evolving brain injury. It will examine how injury and/or impaired brain development may make the fetus more vulnerable to further episodes of hypoxia. The talk will

then explore how we can utilise information about the temporal changes in fetal physiology and behaviour after hypoxia to develop potential clinical biomarkers to detect an adverse event and determine phases of injury. This is vital for facilitating implementation of targeted therapies and inform clinical decision making. Here, time of day is an important factor as the fetus has robust circadian and ultradian rhythms, but their nature and functional role are poorly understood. The talk will present new data about fetal cardiovascular and neurophysiological circadian and ultradian rhythms. It will detail how they change with maturation and are altered by hypoxia and inflammation. The talk will highlight the importance of the time of day when utilising fetal diagnostic and prognostic biomarkers and the implications for the timing of routine clinical assessments. Finally, the talk will end with reflection upon the challenges we face to improve our understanding about the first 9 months of our physiological journey if we are to optimise our physiological development from birth to adulthood.

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The Health Research Council of New Zealand

PL03

Regulation of blood flow at the capillary level in health and disease

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It is often assumed that local increases of tissue blood flow are mediated by relaxation of arteriolar smooth muscle but in many tissues, including the brain, heart, kidney and pancreas, capillary control of blood flow by contractile pericytes also occurs. In the brain, most of the adjustable resistance of the intra-cerebral vasculature is located in capillaries. I will demonstrate that neuronal activity mainly increases cerebral blood flow by dilating capillaries via pericytes, that this involves signalling via astrocytes, and that dilation of capillaries and of arterioles are mediated by different messengers. In the brain, heart and kidney, ischaemia leads to pericytes constricting, producing a long-lasting decrease of blood flow after ischaemia. Constriction of cerebral capillaries by pericytes also occurs at an early stage of Alzheimer's Disease, when it is expected to amplify the production of amyloid beta. The SARS-CoV-2 virus causing Covid-19 binds to ACE2 on cerebral pericytes and amplifies angiotensin II - evoked pericyte constriction by decreasing ACE2 function. Thus, awareness of the possibility of pericyte-mediated capillary constriction reveals new therapeutic targets to increase blood flow in numerous pathologies.

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PL04

The gut endocrine axis in the control of metabolism

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The gut endocrine system comprises a collection of enteroendocrine cells scattered throughout the intestinal epithelium, producing hormones that signal locally within the gut and distantly at tissues such as the brain and pancreas. In the field of diabetes and obesity, the best studied gut hormone is glucagon-like peptide-1 (GLP-1), which has been exploited therapeutically for the treatment of type 2 diabetes and obesity through the development of GLP-1 receptor agonists and DPP4 inhibitors.

Our research is focussed on gaining a molecular understanding the enteroendocrine system and its involvement in the control of metabolism and food intake. Technical advances now make it possible to apply live single cell recording and transcriptomic techniques to human enteroendocrine cells using intestinal organoid models engineered by CRISPR-Cas9 to express fluorescent sensors in specific cell types of interest. Mirroring previous findings from mouse models, we have shown that human GLP-1 secreting cells utilise a variety of signalling pathways for nutrient detection, including electrogenic glucose uptake and activation of specific G-protein coupled receptors by free fatty acids and amino acids.

Overall, we aim that our research will identify new drugs for type 2 diabetes and obesity that act by targeting gut endocrine cells, thus mimicking the gut endocrine consequences of bariatric surgery.

PL05

Local Iron Control- Mechanisms, Importance and Implications

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Our knowledge of iron homeostasis has grown enormously since the discovery of hepcidin at the turn of the century. Hepcidin was discovered as a liver-derived hormone that controls circulating iron levels by binding and degrading the iron exporter ferroportin in the gut and spleen, respective sites of iron absorption and recycling. Until recently, the consensus was that iron levels within tissues were a function of circulating iron availability and controlled primarily at the point of uptake. Recent work from our laboratory has changed that consensus. We have discovered regulated iron export as an essential mechanism of local iron control in the heart, pulmonary vasculature, renal tubules and fetal liver¹⁻⁵. These tissues produce their own hepcidin for cell-autonomous regulation of ferroportin-

mediated iron export. Disruption of local this iron control in a manner that increases or decreases iron levels in these tissues is sufficient to impair their physiological function, even against a background of normal circulating iron levels. Importantly, we found that the pathways mediating the pathophysiological effects of disrupted local iron control differ from tissue to tissue, revealing new, unexpected and diverse functions for local iron control in systems physiology. These discoveries change our understanding of iron disorders from the simplistic notions of “too little” or “too much” iron, to that of sophisticated tissue-level iron control. They also highlight local iron-regulated pathways as new therapeutic targets in certain pathophysiology. Our aim now is to translate these discoveries into better clinical management of iron disorders, particularly iron deficiency of chronic disease.

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PL06

Short Palate and Nasal Epithelial Clone 1 (SPLUNC1) is an Allosteric Regulator of Cation Channels

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Plasma membrane cation channels play critical roles in lung health: The epithelial Na⁺ channel (ENaC) is expressed in pulmonary epithelia where it regulates fluid homeostasis and mucus clearance. The Orai1 Ca²⁺ channel is ubiquitously expressed and controls gene expression and inflammation, to list but two of its functions. SPLUNC1 (gene name BPIFA1) is a 256 amino acid long protein that is secreted from airway surface epithelia and glands into the lung lining fluid. We were initially interested in identifying secreted proteins that could regulate ENaC in airway epithelia. Using

a proteomic screen, we identified SPLUNC1 as a protein that could interact with ENaC. Subsequent studies confirmed that SPLUNC1 bound to and internalized ENaC to reduce Na⁺ and fluid absorption¹. Fluorescence resonance energy transfer (FRET) studies demonstrated that extracellular SPLUNC1 binding to ENaC caused intracellular changes in ENaC (i.e. allostery). We also found that SPLUNC1 binding caused the ubiquitin ligase NEDD4-2 to ubiquitinate ENaC and send it to lysosomes for degradation. Indeed, immobilized ENaC concatamers, or expression of a dominant negative form of NEDD4-2 were no longer internalized². We found that a region in human SPLUNC1's N-terminus, historically called the S18 region, was responsible for the ENaC-mediated inhibition, and peptides that corresponded to this region fully replicated this inhibition. Murine SPLUNC1 does not express the S18 region and cannot regulate ENaC. However, we noticed that SPLUNC1^{-/-} mice exhibited both spontaneous airway hyperreactivity and increased ex vivo airway smooth muscle contraction, which could be rescued with recombinant SPLUNC1³. Further study indicated that SPLUNC1 inhibited Orai1-dependent, store operated Ca²⁺ entry in both human and murine tissues. However, while ENaC inhibition was mediated by SPLUNC1's N-terminus, Orai1 inhibition was mediated by SPLUNC1's C-terminus. Surprisingly, SPLUNC1's C-terminus induced NEDD4-2-dependent ubiquitination, internalization and lysosomal degradation of Orai1, without affecting ENaC. Cystic fibrosis is an inherited disease where mutations in the CFTR anion channel lead to chronic airway infection and inflammation. We recently found that CF patients with reduced SPLUNC1 levels undergo more frequent acute exacerbations and have greater rates of hospitalization⁴. Similarly, reduced SPLUNC1 levels correlate with increased inflammation and reduced lung function in asthma patients⁵. These data suggest that restoring SPLUNC1 function would benefit both asthma and CF patients. We have developed proteolytically-stable peptides based on SPLUNC1's C-terminus, and found that they exhibit potent anti-inflammatory actions in asthma, acute infection and CF murine models, indicating that they are suitable for development as anti-inflammatory compounds. Work in this area is ongoing in the Tarran lab.

Funded by the NIH and the CF Foundation.

RT has equity in Eldec Pharmaceuticals.

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PL07

A Colourful Experience

Semir Zeki¹

I will describe my discovery of the colour centre, area V4, in the primate brain, damage to which in the human brain leads to the syndrome of cerebral achromatopsia, or colourless vision. V4 was full of surprises, which challenged what we thought we knew about colour theory. As we explored it, it became increasingly difficult to deal with that discovery exclusively in terms of the classical colour theories of trichromacy and opponency, neither of which accounted adequately for the perceptual constancy of colour categories.

To explore V4 further we needed a new theoretical and methodological approach, using a more natural experimental paradigm than the traditional reduction screen, in which the colour of a 'point', or patch, isolated from its surrounds is manipulated by varying its constituent amounts of long, middle and short wave light. This was especially so since many V4 cells are selective for *hues* rather than to lights of specific wavelength. There thus appeared to be a major electrophysiological disconnect between the perceived colour and the stimulating wavelengths that was not evident when using old methods which relied on the colour of isolated 'points'. What was required was a clear electrophysiological demarcation, not made before, between colour and wavelength. The Land Colour Mondrian, a relatively simple abstract multi-coloured composition with no recognisable shapes, provided the methodological platform needed to explore this physiological distinction. The use of Land's paradigm allowed me to demonstrate that wavelength selective cells are not necessarily colour selective and that hue selective cells respond to a patch of their preferred hue regardless of the precise wavelength- energy composition of the light reflected from it alone, thus demonstrating a physiological, single-cell, counterpart to perceptual colour categorisation constancy. Moreover, I found that wavelength opponent cells can be made to give an "ON" or "OFF" response to a patch of any hue of the Mondrian display placed in their receptive fields, by simply adjusting the wavelength composition of the light reflected from the patch, without changing its colour category.

More recent use of the Colour Mondrian in perceptual experiments has shown that the colour of the after-image produced by viewing a patch in a Mondrian context is, like the colour of the patch itself, independent of the precise-wavelength energy composition of the light reflected from it (e.g. the after image of a green surface that is reflecting more red than green light is magenta, just like the after-image of a green surface that is reflecting more green than red light). The use of theoretical and experimental approaches that are better adapted to studies of cortical involvement in colour vision, has thus revealed new and unsuspected ways of how the brain constructs colours.

In short, one of the most venerable and foundational fields of natural philosophy and modern science remains a pioneering field, as open-ended and as important as before for our understanding of how we obtain knowledge of our world through colour.

SA01

Changes in cellular Ca^{2+} and Na^+ regulation during the progression towards heart failure

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This symposium talk aims to give an overview of the role played by the cardiac sodium channel in the decline in function of the heart as it begins to fail. The following summary will provide some background for the talk.

In adapting to disease and loss of tissue the heart shows great phenotypic plasticity that involves changes to its structure, composition and electrophysiology. In parallel, global cardiovascular adaptations occur involving stimulation of the sympathetic nervous system, release of natriuretic peptides and activation of the renin-angiotensin-aldosterone pathway. These adaptations provide compensation for the initial decline in cardiac function but, for reasons not understood, do not provide continued benefit in the longer term. The heart begins to fail in its task to produce sufficient cardiac output to meet the body's requirements for oxygen and nutrients. Heart failure is a chronic and progressive condition with very poor prognosis. Approximately 60% of people diagnosed with heart failure are dead within five years and, although exact percentages depend on the type of failure, up to half of those will die from disturbances of rhythm.

At the cellular level compensation responses involve a spectrum of changes to structure, electrophysiology and Ca^{2+} homeostasis. There is an increase in late Na^+ current and a decrease in Na^+/K^+ ATPase current. The cardiac myocytes gain Na^+ and this alters the balance of Ca^{2+} flux mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchange that limits early contractile impairment. Action potential duration prolongs as a result of the increase in late Na^+ current and changes in the expression and function of other ion channels and transporters, notably those carrying K^+ . Cytosolic Na^+ perturbations can disturb Ca^{2+} -dependent energy metabolism and reactive oxygen production in mitochondria reducing energy supply.

The normal spatial arrangements of Ca^{2+} -handling proteins are essential for efficient excitation–contraction (EC) coupling and the stability of this amplification mechanism. There is a reduction in T-tubule density in myocytes from failing hearts leading to an increased spatial separation of the junctional SR from the T-tubule membrane. These structural changes are thought to underlie temporal delays in EC coupling and an increase in spontaneous Ca^{2+} -release events (Ca^{2+} sparks).

It is likely that these structural and electrophysiological responses occur at the expense of (1) increasing the likelihood of arrhythmogenesis, (2) activating hypertrophic and apoptotic signalling pathways and (3) decreasing the efficiency of EC coupling. The combination of action potential prolongation, altered Na^+ regulation, inconsistent Ca^{2+} release from SR and a less stable ventricular membrane potential provides a setting for triggered arrhythmias initiated either by early or delayed afterdepolarizations. The cardiac sodium channel plays an important role in the passage of the heart from function to dysfunction.

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SA02

Cardiac sodium channel (dys)function and arrhythmias: novel mechanisms and therapeutic targets

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The cardiac sodium channel Nav1.5, encoded by the *SCN5A* gene, is a key regulator of cardiac conduction, and sodium channel dysfunction may lead to cardiac arrhythmias and sudden cardiac death. Mutations in *SCN5A* are associated with a myriad of clinical syndromes, including Brugada syndrome, Long QT syndrome type 3, cardiac conduction disease, and overlap syndromes. The underlying mechanisms include a decrease in peak sodium current leading to conduction abnormalities and/or an increase in late sodium current causing repolarization disturbances. Yet, some Nav1.5-related disturbances, in particular structural abnormalities, may not be directly or solely explained on the basis of defective Nav1.5 expression or biophysics. An emerging concept that may explain the large disease spectrum associated with *SCN5A* mutations centres around the diversity, complexity and multifunctionality of the Nav1.5 complex. For instance, alterations in Nav1.5 may affect processes that are independent of its canonical ion-conducting role, including intracellular homeostasis. In addition, subcellular pools of Nav1.5-based sodium channels within the cardiomyocyte have been demonstrated, in particular at the intercalated disc and lateral membrane regions, where they associate with specific interacting proteins and display distinct functional properties. The trafficking pathways through which sodium channels reach their various subcellular destinations within the cardiomyocyte are now coming into focus. This lecture will address these recent insights and discuss the potential implications for the development of novel therapeutic strategies.

SA03

Structural and functional roles of the β 3-subunit on the cardiac sodium channel: implications for arrhythmogenesis.

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The cardiac sodium channel, Nav1.5, drives the rising phase of the cardiac action potential. It comprises an ion-conducting α -subunit together with one or more regulatory β -subunits of which there are four main isoforms, $\beta 1$ - $\beta 4$. The $\beta 3$ -subunit is thought to influence Nav1.5 trafficking and gating; its mutations or knock-outs are associated with a variety of cardiac arrhythmic conditions including Brugada Syndrome (BrS; 1, 2). Available cryo-EM structures suggest multiple points of interaction in α - β subunit combinations. Previous evidence highlights a particularly important role for the extracellular immunoglobulin (Ig) domain of $\beta 3$ in functional modulation of Nav1.5 (3).

We have utilized a combination of electrophysiological, biochemical and cell-biological techniques to evaluate the functional behaviour of the Nav1.5- $\beta 3$ -subunit complex *in vivo* (4, 5). We show that the $\beta 3$ -subunit can form monomers, dimers and trimers within the plasma membrane independently of its binding of the Nav1.5 α -subunit. Using super-resolution stochastic optical reconstruction microscopy (STORM), we show that the Nav1.5 α -subunit assembles into localised, higher-order, two-dimensional clusters within the plasma membrane both in the presence and absence of the $\beta 3$ -subunit. Although the $\beta 3$ -subunit did not affect the average number of α -subunits within these clusters, it significantly increased the cluster radii (KS test, $P = 4 \times 10^{-9}$), suggesting that orientation and cluster packing of the Nav1.5 α -subunits is sensitive to the presence of the $\beta 3$ -subunit. Whole-cell patch-clamp experiments identified a depolarising shift of steady-state inactivation in the presence of $\beta 3$ as well as an accelerated recovery from inactivation which would act to enhance channel availability. Following on from this we have used site-directed mutagenesis to investigate two unusual mutations in structurally distinct regions of the $\beta 3$ -subunit. One mutation is a novel in-frame threonine deletion within the extracellular Ig domain recently identified in a BrS patient in the absence of any other causative mutations. Whole cell patch-clamp experiments highlighted a reduction in peak current compared to wild-type $\beta 3$ and a loss of the depolarising shift of steady-state inactivation. Yet this mutation did not abolish the Nav1.5- $\beta 3$ interaction. The second mutation is targeted at an unusually positioned and highly conserved glutamic acid residue within the transmembrane domain of the $\beta 3$ -subunit. This mutation selectively abrogated the acceleration of recovery from inactivation that is normally exerted by wild-type $\beta 3$.

Our work highlights multiple structural and functional interactions between Nav1.5 and $\beta 3$ -subunits in which the latter plays crucial roles, both in regulating normal Nav1.5 function and in the higher-order organisation of Nav1.5 within the plasma membrane. Future experiments will investigate the stoichiometry of these interactions and how mutations affect the oligomeric structures.

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SA04

Structural insight to sodium channel function

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Voltage-gated sodium channels enable the translocation of sodium ions across cell membranes and play crucial roles in electrical signalling by initiating the action potential. In humans, sodium channel mutations give rise to a number of cardiovascular diseases, hence they are important targets for pharmaceutical drugs. The NavMs channel has been shown to be a good model for human channels, based on its high levels of sequence, structural and functional similarities to the human Nav1.5 isoform. Complexes of NavMs have been studied using crystallography, cryo-electron microscopy, and biophysical techniques including circular dichroism spectroscopy, bioinformatics, and molecular dynamics calculations, enabling identification of binding sites, molecular interactions, and the functional effects of both on-target and off-target drugs which bind to sodium channels. These studies can provide crucial information for the development of new pharmaceuticals and for the understanding of side-effects of currently-prescribed drugs.

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SA05

Overcoming amenorrhea in athletes with high training loads

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Menstrual function can be affected in female athletes with high training loads, ultimately resulting in functional hypothalamic amenorrhea. Can athletes overcome the effect of high training loads while maintaining a busy training and competitive schedule?

Since early reports of high prevalence of amenorrhea in athletes in the 1980s, decades of research support the idea that it is not training load *per se* that induces menstrual dysfunction. Instead, the aetiology of this condition may be the decreased energy available to sustain normal physiological function, what has also been referred to as 'low energy availability'.

To recover from the chronic effects of the energetic stress induced by low energy availability, it is theorised that athletes have to decrease training load, increase energy intake or both. However, due to the nature of competitive sports, elite athletes are often reluctant to decrease their training load so as to maintain a competitive advantage. Also, because access to elite athletes is very limited to researchers, there are no strictly controlled laboratory-based studies in elite population looking at recovery from low energy availability.

While decades of research have helped developing an understanding of the aetiology of menstrual disturbances in athletes, there is limited research on how to best recover from this condition and a definite answer to this question remains elusive. This invited talk will provide an overview of this topic and present evidence from a recent case study documenting recovery of menses of an amenorrheic elite road cyclist during a 5-year follow up after an episode of significant weight gain, while maintaining —and increasing— a high training load, physical capacity, and remaining competitive at an international level.

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Reference 4 :- Areta JL (2020). Case Study: Resumption of Eumenorrhea in Parallel With High Training Load After 4 Years of Menstrual Dysfunction: A 5-Year Follow-Up of an Elite Female Cyclist. *International Journal of Sport Nutrition and Exercise Metabolism* **30**, 229–234.

The next step in female research; endocrine and methodological considerations for research in eumenorrheic females

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Data on eumenorrheic females is scarce and is often low to moderate quality, which is due to a number of reasons: (i) there is a shortage of specialist knowledge on female physiology, in particular endocrinology, in the sport and exercise science community; (ii) until recently, there has been a reluctance to include female participants in research studies, due to the numerous adaptations, to experimental design, needed to incorporate female specific considerations, such as the menstrual cycle; (iii) there is no consensus on the terminology and methodological approaches needed to produce high-quality studies. These issues have undoubtedly slowed the pursuit of knowledge in this field of research. The purpose of this talk is to detail the specific considerations needed when employing eumenorrheic women as participants in sport and exercise science-based research (Elliott-Sale et al., 2021). These considerations relate to participant selection criteria and adaptations for experimental design. This talk is intended to promote more high-quality research in eumenorrheic females.

Reference 1 :- Elliott-Sale KJ et al. (2021). Sports Med 51(5), 843-861

SA07

Oral contraceptive use and substrate metabolism during endurance exercise in women.

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While women's participation in leisure physical activities and competitive sports has progressively increased over the last century, many studies continue to exclude women, to overlook woman-specific features, or to extrapolate data obtained in men to women.

In recent decades, several research groups have stressed this weakness and called for a better consideration of women and of their hormonal specificities (Elliott-sale et al. 2021). Specifically, many adult women of childbearing age use hormonal agents that support menstrual regularity and provide birth control, with oral contraceptives (OC) being one of the most popular form. Due to their specific nature and concentrations, synthetic ovarian hormones contained in OC could influence physiological responses during exercise.

Substrate metabolism during endurance exercise is a key factor of health management but also of sport performance. Carbohydrates and fat are the two major fuels used by muscles for energy production during endurance exercise, and it is acknowledged that substrate partitioning depends on several inter- and intra-individual factors, including exercise intensity and duration, body

composition, training and nutritional status, physical activity level and sex. In addition, due to their specific characteristics, the exogenous hormones contained in OC are likely to influence substrate metabolism during exercise in women.

By analysing the available literature, we will try here to discuss the effect of OC use on substrate metabolism during endurance exercise.

Several studies conducted among women using different types of OC showed that substrate oxidation during moderate prolonged exercise was similar to what is observed in non-OC users (Bonen et al. 1991; Casazza et al. 2004; Isacco et al. 2012). Interestingly, some studies specifically reported increased lipolysis or decreased glucose flux without any change in substrate oxidation rates during endurance exercise in women using OC (Casazza et al. 2004; Isacco et al. 2012 and 2014). In addition, we previously reported that although OC use increases lipid mobilization, this effect was blunted by the lipolytic activity that occurs during endurance exercise (Isacco et al. 2014).

Results on the effect of synthetic ovarian hormones contained in OC on substrate metabolism during endurance exercise remain however unclear to date. While there are important health and performance implications regarding the use of OC among women, further studies are today needed to better understand this increased lipolytic activity without any substantial (or detectable) effect on substrate use during endurance exercise in OC users.

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Reference 2 :- Casazza GA et al. (2004). J Appl Physiol 97, 302-309.

Reference 3 :- Elliott-Sale KJ et al. (2021). Sports Med 51, 843-861.

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SA08

The influence of the menstrual cycle on substrate metabolism during exercise; applications for female athletes

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The fluctuating oestrogen and progesterone concentrations across the menstrual cycle in eumenorrhoeic women can alter substrate utilisation during exercise. Menstrual phase comparative studies (in the late 1990s to early 2000s) employed stable-isotopic tracers to study carbohydrate, fat and protein metabolism during exercise and provide the grounding for our current knowledge in the field. More recently, the complexed molecular signalling pathways of oestrogen and progesterone are being uncovered and lends clearer understanding to explain mechanisms and potential magnitude of menstrual phase effect on energy metabolism during exercise. Multiple pathways of genomic and rapid non-genomic effects are described that vary by receptor isoform and tissue, thus

explaining menstrual phase-specific differences in hepatic glucose production, whole body glucose uptake, tissue specific lipid storage and fat oxidation, and protein turnover during exercise (with added implications during fasted or carbohydrate-restricted training). For example, oestrogen suppresses, while progesterone either suppresses (in euglycaemic, insulin responsive state) or enhances (in glucose-deficient state) hepatic gluconeogenesis. This may drive the decrease in plasma glucose kinetics during exercise frequently reported in menstrual phases or conditions of elevated compared with suppressed ovarian hormones, despite oestrogen's capacity to increase exercise-stimulated glucose uptake and GLUT4 expression in an oestrogen receptor isoform-specific manner. Instead, oestrogen enhances long chain fatty acid uptake and oxidation in skeletal muscle and directs lipid availability away from adipose and toward skeletal muscle (while progesterone may partially antagonise some of these effects) explaining frequently reported increases in fat oxidation during exercise in late follicular or mid-luteal menstrual phases when oestrogen concentration is elevated. Oestrogen opposes, although progesterone's effect to promote protein catabolism appears to dominate during exercise in the luteal phase possibly aligning with the ovarian hormones' influences over gluconeogenesis and the need to provide gluconeogenic substrate during prolonged exercise. Furthermore, the chemical structure and properties of oestrogen affords cellular protection against the side-effects of metabolic flux by promoting plasma membrane stability, curbing free radical or lipid peroxidative damage and consequently modulating post-exercise inflammatory and exercise-induced damage responses or recovery time that is therefore menstrual phase specific. Thorough consideration of the current trends produces a sensible pattern of effect between menstrual phases that has practical application for future research design aiming to include eumenorrhoeic women and for athletes to optimise training or performance during racing.

SA09

Structural and functional characterization of the lipid scramblase TMEM16F

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The lipid scramblase TMEM16F catalyses the exposure of phosphatidylserine in platelets to initiate blood coagulation. TMEM16F is a member of the TMEM16 family comprising calcium-activated lipid scramblases and ion channels. We have combined cryo-EM with patch-clamp electrophysiology and liposomal assays to characterize the dual function of TMEM16F as a lipid scramblase and an ion channel. Cryo-EM analyses of the protein in the absence and presence of calcium reveal rearrangements induced by ligand binding. Functional characterization shows that both processes are activated by the same mechanism, but appear to be catalysed by distinct protein conformations.

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Acknowledgements :-

SA10

The pros and cons of targeting TMEM16A in Cystic Fibrosis

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Recent work suggests activation of the Ca²⁺ activated chloride ion channel TMEM16A (anoctamin 1) as a genotype-independent (mutation-agnostic) treatment for cystic fibrosis, and also for other inflammatory airway diseases. This strategy is based on data obtained in studies performed almost exclusively *in vitro*, or under non-inflammatory conditions *in vivo*. It is assumed that activation of TMEM16A will induce airway electrolyte secretion, thereby compensating for the defect in CFTR-mediated Cl⁻ secretion. This should then lead to reduced airway mucus plugging and improved mucociliary clearance. However, in healthy lungs of human and mouse, TMEM16A is only weakly expressed, while in airways of patients with asthma and cystic fibrosis (CF), and in lungs of asthmatic mice, TMEM16A is upregulated particularly in glands and airway smooth muscle cells. Activation/potentialiation of TMEM16A in these cells may enhance mucus plugging and induce bronchoconstriction, which would favor a therapeutic use of inhibitors of TMEM16A, rather than activators. We analyzed expression of TMEM16A at different locations in human and mouse airways. We find upregulation of TMEM16A in lungs of people with CF or in asthmatic lungs, particularly in submucosal glands. However, in CF submucosal cells, stimulation by the purinergic agonist ATP activated predominantly KCNN4 K⁺ channels, but not ANO1. Niclosamide, an inhibitor of TMEM16A, blocked mucus production and mucus secretion in mice *in vivo* and *in vitro*. In contrast, the activator/potentiator of TMEM16A, Eact, and the potentiator of TMEM16A, brevenal, both induced acute mucus release from airway goblet cells in mice *in vivo*. Brevenal, a bioactive compound from the marine dinoflagellate *Karenia brevis*, strongly potentiated ATP-activated Cl⁻ currents, without directly increasing cytosolic Ca²⁺. Both Eact and brevenal induced acute airway contraction. Treatment of airway epithelial cells with niclosamide strongly inhibited expression of the central transcription factor for Th2 inflammation and goblet cell differentiation, SAM-pointed domain-containing ETS-like factor (SPDEF). Taken together, activators/potentiators of TMEM16A may induce preferentially airway mucus secretion and bronchoconstriction, which suggests the use of TMEM16A-inhibitors for the treatment of CF lung disease.

All animal experiments were approved by the local Ethics Committee and were conducted according to the guidelines of the American Physiologic Society and the German Law for the Welfare of Animals.

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SA11

Functional expression of TMEM16A in taste bud cells

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The calcium-activated chloride channels TMEM16A and TMEM16B play relevant roles in many physiological processes including neuronal excitability and regulation of Cl⁻ homeostasis. We examined the presence of calcium-activated chloride channels in taste cells of mouse vallate papillae. Taste buds contain three main types of taste cells named type I, II or III. Type II and type III cells express taste receptors and respond to tastants, while type I cells mainly have glial-like functions. The three cell types can be identified by the expression of specific proteins and by their electrophysiological fingerprint. Previous work has shown that both TMEM16A and TMEM16B were expressed in taste cells (Cherkashin *et al.*, 2016). We used *Tmem16a* and *Tmem16b* KO mice models as a control for the specificity of antibodies and found that only TMEM16A, but not TMEM16B, was expressed in taste bud cells. Moreover, TMEM16A largely colocalized with the inwardly rectifier K⁺ channel KCNJ1 at the apical portion of type I cells. By using whole-cell patch-clamp recordings in isolated cells from taste buds, we measured large currents activated by 1.5 μM Ca²⁺ in the intracellular solution in type I, but not in type II and III taste bud cells. Ion substitution experiments indicated that the current was mainly carried by anions. Calcium-activated chloride currents were blocked by the specific TMEM16A channel blocker Ani9. Moreover, in agreement with previous studies showing that type I taste cells exhibited large Ca²⁺-activated Cl⁻ currents when stimulated with P2Y receptor agonists (Kim *et al.* 2000; Cherkashin *et al.* (2016), we found that 50 μM ATP at the holding potential of -70 mV induced large inward currents in about 70% of type I taste cells. These currents were blocked by Ani9, indicating a possible role of TMEM16A in ATP-mediated signaling. ATP is released by type II cells in response to various tastants and reaches type I cells where it is hydrolyzed by ecto-ATPases but can also increase cytosolic Ca²⁺ by binding to P2Y receptors and indirectly activate TMEM16A channels causing a flux of Cl⁻ according to its electrochemical gradient.

Reference 1 :- Cherkashin AP, Kolesnikova AS, Tarasov MV, Romanov RA, Rogachevskaja OA, Bystrova MF & Kolesnikov SS (2016). Expression of calcium-activated chloride channels Ano1 and Ano2 in mouse taste cells. *Pflugers Arch* **468**, 305–319.

Reference 2 :- Kim YV, Bobkov YV & Kolesnikov SS (2000). Adenosine triphosphate mobilizes cytosolic calcium and modulates ionic currents in mouse taste receptor cells. *Neurosci Lett* **290**, 165–168.

SA12

Functional coupling between TRPV1 and ANO1 in sensory neurons requires ER Ca²⁺ release

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ANO1 (TMEM16A) is a widely expressed Ca²⁺ activated Cl⁻ channel (CaCC) with functions ranging from epithelial transport to sensory transmission. ANO1 is expressed in sensory neurons and involved in the process of detecting and relaying signals that arise after painful (noxious) stimulation. These neurons also express the Ca²⁺ permeable heat sensor TRPV1 with previous research suggesting that ANO1 and TRPV1 exist in a nanodomain arrangement where Ca²⁺ entering through TRPV1 is able to directly activate ANO1 due to their close proximity in dorsal root ganglion (DRG) neurons. We have previously shown that ANO1 activation is functionally coupled to Ca²⁺ release through inositol trisphosphate receptors (IP₃R) in DRG neurons, thus allowing the low Ca²⁺ sensitivity of ANO1 to be overcome. Interestingly, due to the ability of TRPV1 to induce Ca²⁺ release from the ER by activating phospholipase C (PLC), we hypothesised that TRPV1 may be able to activate ANO1 through IP₃R Ca²⁺ release as well. To this end, we developed a multi-wavelength live cell imaging approach to allow us to simultaneously monitor CaCC activity and Ca²⁺ dynamics in DRG and revealed that capsaicin activation of TRPV1 was able to induce CaCC activity. Furthermore, CaCC activity produced by capsaicin application was attenuated after depletion of the endoplasmic reticulum (ER) Ca²⁺ load, suggesting that ER Ca²⁺ release contributed to TRPV1-induced CaCC activation. To confirm that this effect was induced by plasmalemmal and not ER-localised TRPV1 channels, we used a cell impermeable TRPV1 activator- a derivative of double knot spider toxin- with an ER-Ca²⁺ sensor to demonstrate that ER depletion only occurs when membrane-localised TRPV1 are activated. To understand if there was a structural arrangement of channels that allowed this coupling to be facilitated in DRG neurons, we used in situ proximity ligation assay (PLA) to show that ANO1, TRPV1 and IP₃R receptors were often found in close proximity. This was also confirmed using superresolution stochastic optical reconstruction microscopy (STORM) which revealed that all 3 proteins were indeed found in close proximity. In summary, our findings demonstrate that functional coupling between ANO1 and TRPV1 in sensory neurons is facilitated by Ca²⁺ release through IP₃R with the channels found in a nanodomain structure to enable efficient ANO1 activation.

SA13

Real-time optical imaging of electrophysiology and tissue mechanics in beating hearts

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Optical imaging plays a crucial role in basic cardiovascular research. In this talk, I will discuss the latest developments in the field of cardiac optical mapping, and demonstrate that it is possible to image action potential and calcium wave phenomena on the surface of the beating heart in real-time. Using a mix of optical and numerical computer vision techniques, including, for instance, voltage-sensitive fluorescent dyes, ratiometric imaging and numerical motion tracking, it is possible to simultaneously measure mechanical tissue deformation, as well as electrophysiological wave phenomena at very high spatial and temporal resolutions. I will discuss some of the experimental requirements, e.g. illumination, optics, preparation of the tissue, and will review the state-of-the-art in numerical techniques and hardware requirements for performing the measurements in real-time at imaging speeds of 500fps. Further, I will discuss how the superposition of motion and fluorescence-related phenomena poses a tricky problem, which needs to be addressed when imaging and post-processing the data. In this context, I will discuss the origin of motion artifacts, the importance of a co-moving measurement, the effect of inhomogeneous illumination, and how cross-talk between mechanics and electrophysiology may negatively affect the measurement [1,2]. Most importantly, I will demonstrate that deep learning is very successful in solving some of these issues, as it can learn the complex relationship between motion, illumination and fluorescence-encoded electrophysiology, and can use this information to correctly disentangle the involved physical phenomena leading to reliable and accurate measurements. Using this powerful technique, it becomes possible to study electromechanical phenomena in great detail. For instance, we studied and were able to resolve rotor dynamics in fibrillating contracting hearts and found a strong correlation between tissue mechanics and electrophysiology [3]. Lastly, I will explain some of the limitations of cardiac optical mapping and potential future directions.

Reference 1 :- **J. Christoph**, S. Luther, Marker-free Tracking for Motion Artifact Compensation and Deformation Measurements in Optical Mapping Videos of Contracting Hearts, *Frontiers in Physiology*, 9:1483, doi: 10.3389/fphys.2018.01483, 2018

Reference 2 :- V. Kappadan, S. Telele, I. Uzelac, F. Fenton, U. Parlitz, S. Luther, **J. Christoph**, High-Resolution Optical Measurement of Cardiac Restitution, Contraction and Fibrillation Dynamics in Beating versus Blebbistatin-uncoupled Isolated Rabbit Hearts, *Frontiers in Physiology*, doi: 10.3389/fphys.2020.00464, 2020

Reference 3 :- **J. Christoph**, M. Chebbok, C. Richter, J. Schröder-Schetelig, P. Bittihn, S. Stein, I. Uzelac, G. Hasenfuß, F. Fenton, R. Gilmour Jr., S. Luther, Electromechanical Vortex Filaments during Cardiac Fibrillation, *Nature*, Vol. 555, p. 667-672, doi: 10.1038/nature26001, 2018

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SA14

Using light to study heterocellular contributions to cardiac electrophysiology

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The heart is composed of cardiomyocytes (CM) and non-myocytes (NM), the latter including stromal, endothelial, and immune cells. NM have long been suspected to electrically couple to CM in native myocardium [1]. However, only with the advent of optogenetics – a method to observe and manipulate cell-type specific electrophysiology with light – heterocellular interactions could be directly assessed in intact tissue [2,3]. Using optogenetic experiments electrotonic coupling was functionally confirmed for cardiac fibroblasts (FB) and macrophages (MΦ), and we are thus interested in exploring how FB and MΦ affect the electrical and mechanical activity during myocardial remodeling in response to heart disease and injury.

Methods: We used Cre-loxP recombination to selectively express fluorescent reporter proteins and optogenetic actuators in NM populations of murine hearts [4]. We optically cleared hearts using X-CLARITY and imaged them with super-resolution confocal microscopy to visualize fluorescently labelled NM populations. This allowed us to reconstruct 3D models of FB and MΦ *in situ*, and to assess their morphology, distribution, interconnectivity, and surface area. We characterized the electrophysiological properties of resident cardiac MΦ, using RNA sequencing, single-cell patch-clamp recordings and pharmacological interventions, showing functional expression of Cx43 and different voltage-gated K⁺ channels. Based on our structural and functional data, we developed a computational model describing cardiac MΦ electrophysiology, which we then used to quantitatively assess CM-MΦ coupling *in silico*. In on-going experiments, we utilize the light-gated cation channel channel-rhodopsin-2 and the voltage-sensitive fluorescent protein VSFP2.3 to study heterocellular coupling between CM, FB and MΦ, with a focus on NM effects on cardiac activity in developing scars following cardiac injury (ischemia-reperfusion injury and cryoablation).

Results: In healthy cleared ventricles, we found that FB networks consist of elongated, thin strands of interconnected cells, which appear to wrap around CM with finger-like nano-protrusions that may be related to tunneling nanotubes – as seen with electron microscopy in post-cryoinjury murine myocardium [2]. In the atria, on the other hand, FB display sheet-like morphologies. Quantitatively, the volume and surface area of 3D reconstructed FB does not differ statistically between atrial and ventricular walls (average surface area in right atrial and left ventricular myocardium are $2,130 \pm 280 \mu\text{m}^2$ [n=276 nuclei, N=3 hearts] and $1,770 \pm 190 \mu\text{m}^2$ [n=126 nuclei, N=3], respectively). Unlike FB, resident cardiac MΦ appear as solitary cells in intact ventricular myocardium with an average surface area of $1,160 \pm 80 \mu\text{m}^2$ (n=35 cells, N=3). In isolated and cultured cells, we found that passive electrophysiological properties of MΦ such as capacitance and membrane resistance scale with surface area (inverse relation for resistance). *In silico* models of CM-MΦ coupling illustrate that those parameters are directly linked to the electrical load of MΦ on coupled CM [5].

Conclusions: FB and tissue-resident MΦ exhibit surprisingly similar cell dimensions in intact tissue, although they differ in their distribution and electrophysiology. Upon injury, these cell populations undergo changes (local proliferation, recruitment, activation) leading to larger NM numbers in the scar and scar border zone, increasing the likelihood of electrotonic coupling. The functional effects of this are a matter of ongoing research.

Reference 1 :- [1] Kohl P, Gourdie RG. *J Mol Cell Cardiol* **2014**/70(100):37-46.

Reference 2 :- [2] Quinn TA et al. *Proc Natl Acad Sci* **2016**/113(51): 14852–7.

Reference 3 :- [3] Hulsmans M et al. *Cell* **2017**/169: 510-522.

Reference 4 :- [4] Fernandez MC et al. *Methods Mol Biol* **2021**/2191:287-307.

Reference 5 :- [5] Simon-Chica A et al. *Cardiovasc Res* **2021**/doi: 10.1093/cvr/cvab126.

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SA15

Research tools to visualise and interrogate the glucagon-like peptide 1 receptor

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Motivation/problem statement: The glucagon-like peptide-1 receptor (GLP-1R) is expressed in various tissues such as the brain and pancreas. This class B G protein-coupled receptor (GPCR) is involved in the regulation of blood glucose levels and control of appetite. Despite being a major drug target for diabetes and obesity treatment, visualisation of the GLP-1R is limited to antibody detection, genetic manipulation or receptor-activating probes.

Methods/procedure/approach: Various fluorophores were fused to Exendin4(9-39) to produce LUXendins. These fluorescent antagonists bind the GLP-1R in live tissue, with specificity confirmed using a novel *Glp1r* knock-out mouse line and by co-localisation with GLP-1R antibody. A mouse line was also generated expressing SNAP-tagged GLP-1R using CRISPR/Cas9 genome editing. Isolated pancreatic islets of these mice were treated with fluorescent SNAP-tag label to visualise the GLP-1R in situ, followed by receptor activation and trafficking after addition of agonist.

Results: LUXendin645 (Cy5) intensely labelled the plasma membrane in live and fixed cells as well as pancreatic islets and brain. Most beta cells within an islet expressed the GLP-1R, whilst only ~5% of alpha cells were LUXendin+, as determined using *Ins1Cre;mTmG* reporter animals. STED imaging of

MIN6 beta-cells labelled with LUXendin651 (SiR) revealed that endogenous GLP1R are organised into nanodomains, and variable diffusion at the plasma membrane was observed using single-molecule light microscopy. Five other LUXendins were also synthesised and tested, spanning green-near infrared spectra, with all variants producing bright membrane labelling of GLP1R.

In SNAP_GLP1R knock-in mice, fluorescent SNAP-tag label co-localised with LUXendin and GLP-1R antibody. Thus, this mouse line can be used to visualise the GLP-1R in live tissue without stimulating the receptor. Addition of GLP-1R agonists allowed the monitoring of receptor activation and trafficking in situ. SNAP_GLP1R knock-in mice had normal body weight and responded to glucose like their wild type littermates.

Conclusion/implications: LUXendins can be used in live tissue and be fixed to label endogenous GLP-1R and allows to study the receptor using confocal, two-photon and super-resolution microscopy in situ or in vivo. While LUXendins act as antagonists, linking fluorophores or dyes to SNAP_GLP-1R of the knock-in mouse line does not interfere with receptor activity and allows labelling post-fixation.

All animal research complied with the Animals (Scientific Procedures) Act 1986 of the U.K. Approval was granted by the University of Birmingham's Animal Welfare and Ethical Review Body.

SA16

GLP-1 Receptor Agonists: Mechanisms Relevant for the Treatment of Obesity

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Glucagon-Like Peptide-1 receptor agonists (GLP-1RAs) have a broadly applicable biology and are approved for the treatment of diabetes as well as obesity. Physiologically, GLP-1 is short-acting, and a key element to treatment success is that pharmacological agents are long-acting. Liraglutide and semaglutide are fatty acid acylated long-acting GLP-1RAs that bind to serum albumin non-covalently via the fatty acid binding sites, and thereby have pharmacokinetic profiles which last for 24 hours/day. Liraglutide has been approved for the treatment of obesity since 2014, and recently also received a paediatric indication, whereas semaglutide has completed phase 3 clinical studies. Semaglutide has been documented to lead to significantly greater weight loss compared to other GLP-1RAs. GLP-1R expression in the pancreas and brain accounts for the respective improvements in glycemic control and body weight. Mechanistically, GLP-1RAs lower body weight through an effect to reduce energy intake. There is a reduction in feelings of hunger, increases in satiety and effects on the reward system resulting in reduced craving for food and improved food choices. Neural GLP-1Rs are the main targets for the weight loss mechanism. Whole brain imaging studies in rodents show that peripherally-administered GLP-1RAs have access to select sites in the brain, and also communicate to the brain through the circumventricular organs, of which many have GLP-1Rs. These agents do not cross the blood brain barrier passively, and their brain accumulation is dependent on

presence of GLP-1R. New data show that there are differences in the brain distribution of semaglutide compared to liraglutide. Both molecules also positively affect cardiovascular (CV) outcomes in individuals with type 2 diabetes, and an outcome study in obesity is ongoing. This ongoing study recruits 17,500 patients with obesity and is an event driven trial. In conclusion, the GLP-1 biology shows potential in obesity and semaglutide is undergoing testing for an effect to lower cardiovascular risk also in patients with obesity.

SA17

Central and peripheral glucagon-like peptide-1 systems independently suppresses eating

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Glucagon-like peptide-1 (GLP-1) acts as both an incretin hormone, and an anorexigenic neuropeptide, which has prompted the successful and ongoing development of GLP-1-based therapies for type 2 diabetes and obesity. Endogenous GLP-1 is produced by enteroendocrine cells in the gut, and preproglucagon (PPG) neurons in the brainstem, which are the defining populations of the peripheral and central GLP-1 systems, respectively. PPG neurons in the brainstem nucleus tractus solitarius (PPG^{NTS} neurons) are widely assumed to link the peripheral and central GLP-1 systems in a unified gut–brain satiation circuit. However, direct evidence for this hypothesis is lacking, and the necessary circuitry has not been demonstrated. We used transgenic mice expressing Cre-recombinase under the glucagon (i.e. PPG) or GLP-1 receptor (*Glp1r*) promoters, coupled with viral targeting of Cre-dependent tracing and effector tools, to selectively map and interrogate gut-brain connectivity between the central and peripheral GLP-1 systems. This allowed us to test whether PPG^{NTS} neurons have a role in physiological satiation, and whether endogenous or exogenous peripheral GLP-1 signalling drives central GLP-1-induced suppression of eating.

We report that PPG^{NTS} neurons encode satiation specifically during large meals, and have the capability for pharmacological activation to suppress eating without compensatory rebound hyperphagia or behavioural disruption. Activation of *Glp1r* vagal afferent neurons similarly suppressed intake, but conditioned a flavour avoidance, and circuit mapping approaches demonstrated that PPG^{NTS} neurons are not a major synaptic target of this vagal population. PPG^{NTS} neurons instead predominantly receive vagal input from oxytocin receptor-expressing vagal afferent neurons, and are necessary for peripheral oxytocin-induced eating suppression. Similarly, PPG^{NTS} neurons are at most a minor synaptic target of *Glp1r* neurons in the area postrema, suggesting that endocrine GLP-1 signalling from the periphery by this route does not require PPG^{NTS} neurons. Consistent with this observation, PPG^{NTS} neurons are not activated by peripheral administration of the *Glp1r* agonist semaglutide, nor are they required for semaglutide-induced suppression of eating or bodyweight loss. Furthermore, chemogenetic activation of PPG^{NTS} neurons concurrent with peripheral semaglutide administration suppresses eating more potently than semaglutide alone.

We therefore conclude that the unified peripheral to central GLP-1 satiation circuit hypothesis is not supported, but that the peripheral and central GLP-1 systems are instead components of functionally and anatomically independent eating control circuits. These findings provide a rationale for pharmacological activation of PPG^{NTS} neurons in combination with GLP-1 receptor agonists as an obesity treatment strategy.

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SA18

Elucidating the molecular mechanisms required for vagally-mediated physiological response to peripheral GLP1

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The vagus nerve is the primary neural mechanism for gut-brain communication. Vagal afferent neurons, located in the nodose ganglia, express numerous receptors that sense hormones released from the gut, and provide meal related information about the composition and quantity of food consumed to the brain. The gastrointestinal hormone, glucagon like peptide 1 (GLP1), released from enteroendocrine cells in response to a meal acts on GLP1 receptors located on vagal sensory nerve terminals in the gut to inhibit food intake, increase glucose clearance, and block gastric emptying. The mechanisms by which GLP1 produces these vagally-mediated physiological responses remains unknown. Recent evidence will be presented identifying a key role for the neuropeptide cocaine and amphetamine regulated transcript in vagal sensory neurons as a mediator of peripheral GLP1 signaling. Using an available single-cell RNA sequencing dataset obtained from mice nodose ganglia we characterized the extent of co-expression between *Glp1r* and several classic neuropeptides and identified the neuropeptide cocaine and amphetamine regulated transcript (CART) as having the highest level of co-expression with GLP1 receptor in nodose ganglia neurons. *In situ* hybridization demonstrates that CART and GLP1 receptor colocalization is conserved in rat nodose ganglia neurons. In rat primary cultures, GLP1 preferentially upregulates CART expression through a GLP1 receptor dependent mechanism. *In vivo*, viral mediated knockdown of CART in vagal sensory neurons blocks GLP1-induced satiation, gastric emptying, and alters circulating insulin levels. Similarly immunoneutralization of CART in the nucleus tractus solitarius, the site of vagal termination in the hindbrain, blunts GLP1-induced satiation and gastric emptying. Chemogenetic stimulation of CART expressing vagal sensory neurons in mice inhibits food intake, slows gastric emptying and alter glucose clearance in response to a meal. Together these data demonstrate that CART synthesis and release by vagal sensory neurons is necessary and sufficient to mediate the physiological response to peripheral GLP1.

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SA19

Microvascular actions of the dipeptidyl peptidase-IV inhibitor, vildagliptin, in obesity

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Diabetes and obesity are associated with vascular dysfunction (e.g. capillary rarefaction) and an increased risk of vascular complications of both large blood vessels (macrovasculature) (e.g. cardiovascular disease) and small vessels (microvasculature) (e.g. diabetic retinopathy). There is an unmet need for better preventative and therapeutic strategies for these vascular complications. One such avenue maybe incretin based medications, for example dipeptidyl peptidase-IV inhibitors (DPP-IVi) such as vildagliptin and sitagliptin, that are already licenced to aid glycaemic control in type 2 diabetes. DPP-IVi slow the breakdown of endogenous incretins (glucagon-like peptide-1 and glucose-dependent insulintropic polypeptide) thereby prolonging their insulin releasing action. There is interest in their non-glycaemic effects, particularly whether they have favourable effects on the cardiovascular system, and thus have beneficial effects in individuals at higher risk of vascular disease / dysfunction. The majority of previous research has focused on the macrovascular effects of incretin based medications and little is known about the potential microvascular effects of these therapies, particularly the DPP-IVi in humans, *in vivo*. This study examined whether short term DPP-IV inhibition with vildagliptin alters micro- and macrovascular function in obese men.

Study design: Crossover, randomised double-blind placebo-controlled study.

Method: 15 obese men were recruited (age range:28-72years). Following recruitment participants were randomised to vildagliptin (50mg twice daily) or placebo for 12 weeks. At the end of the treatment period, microvascular (dermal capillary density; maximum hyperaemia; endothelial (in)dependent microvascular function; microvascular filtration capacity; peak reactive hyperaemia) and macrovascular assessments (flow mediated dilatation of the brachial artery and applanation tonometry) were performed. Following a 4 week washout period the participant received the alternative treatment for 12 weeks. Vascular assessments were repeated within the last 2 weeks of treatment. The study was approved by local NHS Research Ethics Committee (REC number: 09/H0206/33). Written, informed consent was obtained from all participants.

Results: 14 participants completed the study. Active treatment resulted in a small but significant reduction in HbA1c (active mean $\pm$ standard deviation: 38.1 $\pm$ 4.8 vs placebo: 39.1 $\pm$ 4.1mmol/mol, $p=0.003$ paired T-test). Maximal dermal capillary density was increased with DPP-IV inhibition (active median (25th,75th centiles) 129 (118,158) mm² vs placebo: 122 (111,139) mm², $p=0.018$ Wilcoxon Signed Rank test). There was no significant change in other vascular assessments. Ongoing *in vitro* experiments are exploring this area further by examining the impact of DPP-IV inhibition on

proliferation, migration and angiogenic tube formation of human microvascular endothelial cells. Preliminary results suggest that sitagliptin (1 μ M) significantly increases endothelial cell proliferation as assessed by BrdU (DNA) incorporation ($331.6 \pm 10.4\%$ vs control (100%), $p < 0.01$ Mann-Whitney U test, $n = 9$) and DNA staining ($135.4 \pm 6.5\%$ vs control (100%), $p < 0.01$, $n = 6$).

Discussion: DPP-IV inhibition with vildagliptin, increased maximal dermal capillary density in obese men. Preliminary data also suggests that DPP-IV inhibition increases microvascular endothelial cell proliferation *in vitro*. Further work is required to explore these potential pro-angiogenic actions of DPP-IVi and their underlying mechanisms.

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SA20

Imaging the glomerulus to understand leukocyte function during glomerulonephritis

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Glomerulonephritis is one of the leading causes of end-stage renal failure. In many forms of this condition, leukocytes recruited to the glomerulus play essential roles in initiating and mediating disease. In order to understand the mechanisms of leukocyte recruitment to the glomerulus and their behaviour within the glomerulus, we have used multiphoton intravital microscopy (MP-IVM) to image glomeruli of mice undergoing glomerular inflammatory responses. These studies have revealed previously unrecognised behaviour of neutrophils, monocytes and T cells during the development of antibody- and T cell-mediated models of glomerulonephritis. The major effect of glomerular inflammation is to induce immune cells to undergo prolonged intravascular retention within the glomerular microvasculature. From this location within glomerular capillaries, immune cells perform pro-inflammatory functions and undergo cell-cell interactions that are important to the initiation and progression of glomerular inflammation. In this presentation, alterations to immune cell behaviour occurring within the inflamed glomerular microvasculature will be discussed, highlighting examples of neutrophil, monocyte and CD4+ T cell activity.

SA21

IMAGING THE INJURED AND AGED CORONARY MICROCIRCULATION IN VIVO IN THE MURINE BEATING HEART

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Aims/Objectives: Treatment of myocardial infarction (MI) focuses on the rapid re-establishment of regional myocardial perfusion following a blockage in one or more of the coronary arteries. Although the culprit artery can be re-perfused, a significant proportion of patients still incur extensive myocardial damage resulting in heart failure. This is partly due to reperfusion paradoxically leading to additional tissue damage, a condition called ischaemia-reperfusion (IR) injury that has been ascribed to inadequate coronary microcirculatory perfusion. Nevertheless, the degree of microvascular perfusion is unknown, limited by an inability to clinically image coronary microvessels. We have previously shown using intravital microscopy that myocardial IR injury induces thromboinflammation and reduces functional capillary density (FCD) in the adult mouse beating heart microcirculation *in vivo*. [1] The newly discovered and inflammatory cytokine, interleukin-36 (IL-36), could potentially mediate these disturbances. However, its role in myocardial IR injury is not known. This study firstly aimed to determine whether the coronary microcirculatory disturbances and infarct size post-IR injury was modified by age and gender. Secondly, we investigated whether an IL-36 receptor antagonist (IL-36Ra) could confer vasculoprotection and reduce infarct size

Methods: Adult (3-months) and aged (>18-months) male and female C57BL/6 mice (n=5/group) were anaesthetised using intraperitoneal administration of ketamine hydrochloride (100mg/kg) and medetomidine hydrochloride (100mg/kg). IR injury was induced by reversibly suture ligating the left anterior descending coronary artery for 45 minutes with reperfusion mediated for 2 hours. A custom designed 3D-printed stabiliser was attached to the left ventricle downstream of the ligation site to permit intravital imaging. In some studies, recombinant mouse IL-36Ra (15ug/mouse) was injected intra-arterially at 5 minutes pre-reperfusion and 60 minutes post-reperfusion. Beating heart coronary microcirculation was imaged in real-time intravitaly and also *ex vivo* using multiphoton microscopy. Infarct size was measured using dual TTC/Evans Blue staining. Experiments were conducted in accordance with the Animals (Scientific Procedures) Act of 1986 (Project licence: P552D4447).

Results: Significantly increased basal ($p<0.0001$) and IR injury-induced ($p<0.0001$) neutrophil recruitment, and greater decreases in FCD, was observed in aged mice compared to adults (ANOVA + Tukey's post hoc test). Neutrophils primarily adhered within coronary capillaries although in aged hearts remarkable venular adhesion was also identified. These events were mirrored in deeper myocardial layers when imaged using multiphoton microscopy. Interesting gender-dependent perturbations were noted with neutrophil recruitment dominating in IR injured female hearts whilst male hearts demonstrated a greater presence of occlusive platelet microthrombi. IL-36Ra significantly reduced inflammation ($p<0.0001$) and infarct size ($p<0.0001$) in both adult and aged mice.

Conclusion: Our novel findings of enhanced coronary microcirculatory perturbations associated with age may explain the poorer outcomes in elderly patients following MI. Furthermore, the cellular nature of the thromboinflammatory response may explain the gender-related differences in outcome

after MI. Importantly, we are the first to demonstrate that targeting IL-36 may be a potential novel therapy for treatment of myocardial IR injury.

Reference 1 :- Kavanagh D, Lokman A, Neag G, Colley A, Kalia N. Imaging the injured heart intravitaly and the vasculoprotection afforded by haematopoietic stem cells. *Cardiovascular Research* 2019; 115:1918-32.

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SA22

BACE1: A novel regulator of the microcirculation from head to toe.

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The current global epidemic of obesity and diabetes is driving an increased incidence of cardiometabolic disease. Microvascular abnormalities have been found to be an important underlying pathology of a number of cardiometabolic diseases. Indeed, microvascular dysfunction, the inability of the microcirculation to feed tissues adequately, is strongly associated with type 2 diabetes and obesity, atherosclerosis, stroke and dementia. Thus, targeting the mechanisms by which nutrient excess drives the induction of microvasculardysfunction could reduce cardiovascular disease prevalence.

Our work has revealed that a protein called BACE1, more commonly associated with Alzheimer's disease, plays an important role in type 2 diabetes and obesity. BACE1 activity is required for the production of β -amyloid peptides, from the amyloid precursor protein, which form the characteristic amyloid plaques found in Alzheimer's disease. There is growing evidence that Alzheimer's disease, diabetes and cardiovascular disease are intimately linked, with inflammation, oxidative stress and insulin resistance common features. BACE1 protein is expressed in a wide variety of tissues and cells, including vascular smooth muscle and endothelial cells. Expression level and activity are increased by chronic stress (e.g. oxidative, metabolic and inflammatory) and we have shown that high fat diet drives its transcription and translation.

We have shown that global deletion of BACE1 in mice enhances insulin sensitivity and protects against diabetes-induced endothelial dysfunction. Importantly, pharmacological inhibition of BACE1 can restore metabolic and vascular health in mouse models of disease. These effects are via modulation of BACE1 cleavage of both the amyloid precursor protein and the insulin receptor, which we recently identified as a novel substrate of BACE1. Furthermore, we have also demonstrated that

this role translates into human physiology, with BACE1 activity inversely correlated with endothelial and vascular dysfunction.

Therefore, our research focus is on determining whether BACE1 can be a novel drug target to provide an innovative therapy for type 2 diabetes and its vascular co-morbidities. With BACE1 inhibitors currently in clinical trials for AD, repurposing these drugs for cardiometabolic disease this could be a promising strategy.

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SA23

Prefrontal thalamocortical connectivity: cracking the circuitry of cognition
Prefrontal thalamocortical connectivity: cracking the circuitry of cognition

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Interactions between the thalamus and prefrontal cortex (PFC) play a critical role in cognitive function and arousal and are disrupted in neuropsychiatric disorders. The PFC is reciprocally connected with ventromedial (VM) and mediodorsal (MD) thalamus, both higher-order nuclei with distinct properties to the classically studied sensory relay nuclei. To understand the properties of the circuits linking PFC and thalamus we use anatomical tracing, electrophysiology, optogenetics, and 2-photon Ca²⁺ imaging, determining how VM and MD target specific cell types and subcellular compartments of mouse PFC. Focusing on cortical layer 1, we find thalamic nuclei target distinct sublayers, with VM engaging NDNF⁺ cells in L1a, and MD driving VIP⁺ cells in L1b. These separate populations of L1 interneurons participate in different inhibitory networks in superficial layers by targeting either PV⁺ or SOM⁺ interneurons. NDNF⁺ cells mediate a unique form of thalamus-evoked inhibition at PT cells, selectively blocking VM-evoked dendritic Ca²⁺ spikes. Together, our findings reveal how two thalamic nuclei differentially communicate with the PFC through distinct L1 micro-circuits and how inhibition is critical for controlling PFC output back to thalamus.

SA24

Cognitive switches and value-guided remapping in cortical circuits

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Adaptive decision-making enables animals to select appropriate goal-directed strategies based on the evaluation of recent stimulus/action-outcomes. The orbitofrontal cortex (OFC) play an important role in invoking rule-based strategies to enable flexible learning. However, the neural circuit mechanisms in the lateral OFC (lOFC) and its interactions with sensory cortical areas underlying such processes remain elusive. To investigate this, we trained head-fixed mice on a tactile-discrimination-based reversal-learning task and enforced them to relearn the task after a 'Go/No-go' rule-switch. Mice exhibited high performance during learning and re-learned the task upon rule-switch. To investigate how distinct neuronal subpopulations in lOFC respond during the task, we employed two-photon imaging through a rod-like GRIN lens. Longitudinal imaging of trial-by-trial Ca²⁺ responses from the same subsets of lOFC neurons in mice expressing GCaMP6 in layer 2/3 neurons revealed that OFC neurons are a key substrate encoding value prediction error. We also measured neuronal responses in the primary somatosensory cortex (S1). In S1, distinct neuronal subpopulations showed 'stimulus-selective' as well as 'outcome-selective' responses. The 'outcome-selective' neurons were differentially modulated by reward history and altered response selectivity upon reversal. Silencing lOFC following rule-switch impairs plastic changes in S1 neurons. Taken together, our experiments shed light on the long-range cortical circuit interactions underlying behavioural flexibility, indicating a crucial role of mouse OFC neurons in encoding predictive 'teaching signals' that drive adaptive changes in behaviour.

Reference 1 :- Banerjee et al. (2020) Value-guided remapping of sensory cortex by lateral orbitofrontal cortex. Nature 585: 245-250.

SA25

Evidence of neural interactions between the mediodorsal thalamus and orbitofrontal cortex during visuospatial discrimination learning in macaques

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Anatomical evidence in mammals shows that the mediodorsal thalamus (MD) is a key component within wider prefrontal thalamocortical networks. Accordingly, damage to, or disruption of MD function impairs specific types of complex learning and decision making in primates. Yet, the underlying mechanisms behind the influence of MD on the frontal cortex during these higher order cognitive processes remain unknown. In the current study, two male rhesus macaques were trained to perform a touchscreen-based visuo-spatial discrimination learning task prior to having recording chambers implanted above the MD and orbitofrontal cortex (OFC). All experimental procedures were approved and conducted under a United Kingdom Home Office Project Licence issued to Dr Mitchell. After recovery, we established the precise coordinates to target the MD and the OFC using magnetic resonance imaging (MRI). Once the animals had re-established their pre-procedure learning rates, we recorded task related neural activity from the MD and OFC using multi-contact probes that were

lowered before each testing session. MD and OFC units were modulated during specific phases of a trial, responding differently to the trial onset, when the visuo-spatial images first appeared on the touchscreen; when the monkey made a response to the touchscreen; and before or after the delivery of the smoothie reward for correct trials. We also observed some MD and OFC units progressively modulated their responses throughout the session, coinciding with improved cognitive performance. Finally, during correct trials, some MD units were phase-locked to OFC local field potentials, suggesting prefrontal thalamocortical interactions support learning. Our preliminary evidence highlights different, but complementary and interactive neural activity between the MD and OFC during learning of a visuo-spatial discrimination task.

SA26

Heterogeneity of function within primate ventromedial prefrontal cortex

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Neuroimaging studies have variably implicated the ventromedial prefrontal cortex (vmPFC) in emotion regulation, inhibitory control of fear, action control, memory and comparative reward evaluation. Reported reductions or increases in activity have been linked to the aetiology of depression and reversal of this dysregulation can be an effective treatment for refractory depression. However, this is a large, structurally heterogeneous region composed of a number of sub-regions including areas 25, 32, 24, 14 and 10. Perhaps surprisingly though, there have been few studies that have attempted to determine the extent to which this structural heterogeneity reflects functional heterogeneity. To address this issue, we have performed a series of investigations in the common marmoset, a new world monkey, combining temporary manipulations of distinct regions within the vmPFC with behavioural and physiological analysis of animal's reactivity to a wide range of rewarding and threatening contexts. Fluorodeoxyglucose PET imaging has been used in concert with these manipulations to determine circuit-wide changes. Focussing on the effects of inactivation and over-activation of caudal subcallosal region, area 25, results will be presented that highlight the specific role of this region in maintaining a negative affective state that, through apparently distinct downstream circuits, activates cardiovascular, endocrine and behavioural reactivity to threat; whilst blunting anticipatory and motivational appetitive arousal. These findings will be discussed in the context of the markedly different functional profile of neighbouring regions with respect to positive and negative emotion regulation, altogether providing important new insights into the functional heterogeneity within vmPFC.

Reference 1 :- Roberts, A.C., Clarke, H.F. (2019) Why we need non-human primates to study the role of ventromedial prefrontal cortex in the regulation of threat and reward elicited responses. *Proceedings of the National Academy of Sciences* 116:26297-26304.

Reference 2 :- Alexander L., Wood C.M., Gaskin, P.R.L., Sawiak S.J., Fryer T.D., Hong, Y.T., McIver L., Clarke, H.F., Roberts A.C. Over-activation of primate subgenual cingulate cortex enhances the cardiovascular, behavioural and neural responses to threat reactivity (2020) Nature Communications 11, 5386.

Reference 3 :- Alexander L., Gaskin P., Sawiak S.J., Fryer T., Hong Y.T., Cockcroft G., Clarke H.F., Roberts A.C. Fractionating anhedonia by over-activating area 25 of primate subgenual anterior cingulate cortex (2019) Neuron 101:307-320.

Acknowledgements :-

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SA27

Thyroid hormone transporters within the hypothalamus-pituitary-thyroid axis

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Thyroid hormone (TH) transporters facilitate the cellular transmembrane passage of TH and are, consequently, required for proper TH signaling and metabolism in all target cells. Their pathophysiological significance is best illustrated in patients with inactivating mutations in the highly specific TH transporter MCT8 since affected patients display a severe form of psychomotor retardation (so called Allen-Herndon-Dudley syndrome) due to tissue-specific changes in TH homeostasis. MCT8 deficient patients as well as Mct8 mouse mutants exhibit abnormal serum TH profile in combination with an altered activity of the hypothalamic-pituitary-thyroid (HPT) axis indicating that TH transporter deficiency compromises the HPT axis on all levels.

Here, I will briefly summarize recent studies of mice lacking Mct8 alone or in combination with other well-established TH transporters (Mct10 and Oatp1c1) as these studies shed light on many aspects and pathogenic events underlying global MCT8 deficiency. Moreover, development of conditional knock-out mice that allow a cell-specific inactivation of TH transporters in distinct cell types disclosed distinct cell-specific changes in TH signaling and sensing within the HPT axis. Altogether, our findings underscore the gate-keeper function of TH transporters as critical components in regulating local TH action.

SA28

Revealing a modular network of committed pituitary thyrotrophs in health and disease

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Thyroid hormone (TH) dysregulation is one of the most prevalent endocrine defects worldwide (3.8% of EU citizens). Regulation of TH output from the thyroid is dependent on secretion of thyroid-stimulating-hormone (TSH) from a small population of pituitary thyrotrophs in response to hypothalamic thyrotropin-releasing-hormone (TRH) and thyroid hormone feedback. Importantly, diagnosis of hypothyroidism is based on a single TSH measurement, thus dismissing the pulsatile property of TSH secretion and the non-linear TSH-TH relationship upon disease onset and subsequent recovery. Despite the undeniable implication of thyrotrophs in the hypothalamus-pituitary-thyroid (HPT) axis regulation, little is known about the mechanisms underlying pulsatile and adaptive TSH secretion. The aim of this study is to decipher how thyrotrophs form a very finely regulated and highly plastic functionally-organised cell population capable of adapting to TH demand. Pituitary-scale 3D imaging revealed an anatomical thyrotroph network that is established during late embryogenesis, when TSH β -positive cells begin forming homotypic anatomical network motifs, prior to an increase in TH demand during the neonate period. We found that, at weaning, concomitantly with a decrease in TH levels, TSH-expressing thyrotroph motifs disaggregate. Lineage-tracing experiments unveiled that thyrotroph cluster motifs persist after weaning in the form of intermingled thyrotroph sub-sets: a sub-population of TSH β -expressing (TSH^{high}) thyrotrophs and another subset of thyrotrophs that express low, if any, TSH β hormone subunit (TSH^{low}). Using *in vivo* calcium imaging in both freely-moving and anesthetized TSH β -crexR26fl-flGCaMP6f mice, we show that the surprisingly unified cell ensemble of TSH^{high} and TSH^{low} thyrotrophs generates large-scale intercellular waves of the universal second messenger Ca²⁺, which recur autonomously and independently of hypothalamic TRH inputs. This mode of intercellular communication is a network signature of this composite thyrotroph population, which persists in a mouse model of thyroid dysfunction, since the thyrotroph network-driven wave generator of Ca²⁺ oscillations is robust regardless of the significant increase in TSH cell mass upon hypothyroidism onset. Altogether, our findings redefine the role of pituitary thyrotrophs in HPT axis function. Their division into two sub-populations of cells organised as a single network may explain TSH pulsatility and the non-linear TSH-TH relationship. This shall provide a clearer understanding of the interactions between the pituitary thyrotrophs and the thyroid gland, thus allowing a refinement of current diagnosis and treatment of thyroid hormone defects.

SA29

Probiotics alter the antibiotic resistance gene reservoir along the human GI tract

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Antimicrobial resistance poses a substantial threat to human health. The gut microbiome is considered a reservoir (the gut “resistome”) for potential spread of resistance genes from commensals to pathogens. The impact of probiotics, commonly consumed by many in health or in conjunction to antibiotics administration, on the gut resistome remains elusive. Direct assessment of the gut resistome *in situ* along the gastrointestinal tract in healthy antibiotics-naïve humans supplemented with an 11-probiotic-strain preparation demonstrated that probiotics reduce the number of antibiotic resistance genes exclusively in the gut of colonization-permissive individuals. In mice and in a separate cohort of humans, a course of antibiotics resulted in expansion of the lower gastrointestinal (GI) tract resistome, which was mitigated by autologous fecal microbiome transplantation or during spontaneous recovery. In contrast, probiotics further exacerbated resistome expansion in the GI mucosa, by supporting the bloom of strains carrying vancomycin resistance genes, but not resistance genes encoded by the probiotic strains. Importantly, the aforementioned effects were not reflected in stool samples, highlighting the importance of direct sampling for analyzing the effect of probiotics and antibiotics on the gut resistome. Analyzing antibiotic resistance genes content in additional published clinical trials with probiotics further highlighted the importance of per-person metagenomics-based profiling of the gut resistome using direct sampling. Collectively, these findings suggest opposing person-specific and antibiotics-dependent effects of probiotics on the resistome, whose contribution to the spread of antimicrobial resistance genes along the human gastrointestinal tract merit further studies.

Acknowledgements :-

Emmanuel Montassier and Rafael Valdés-Mas contributed equally to this work.

Jotham Suez and Erin Elinav are joint last authors.

SA29

An update of key morphogenetic events of thyroid development in *Zebrafish*

Federica Marelli¹, Luca Persani^{1, 2}

The thyroid gland is a highly conserved organ in vertebrates that regulates a wide range of developmental processes and controls the homeostasis of fundamental physiological mechanisms. Defects during thyroid development can lead to irreversible intellectual disabilities and dwarfism if not timely diagnosed and treated. Therefore, the mechanisms underlying thyroid gland development have a central interest in biology, and zebrafish represents a valuable model to study thyroid physiology with significant advantages compared to the mammalian models (1). Zebrafish is an emerging powerful vertebrate model for thyroid research due to its external fertilization, transparency of developing embryos and rapid organ development. Moreover, the availability of molecular tools for the manipulation of zebrafish genome allows the generation of targeted-genetic mutants or transgenic lines that express fluorescent proteins into specific cell types. Thanks to this, we are now able to study the relative contribution of different genes during thyroid development, and track the dynamic changes in thyroid shape, size, and location in live embryos (2). During zebrafish embryogenesis, the acquisition of thyroid competence occurs between the 20-24 hours post fertilization (hpf), when a restricted group of pharyngeal endoderm cells start to express the thyroid transcription factors (TTFs) *pax2a*, *nkx2.4b* and *hhex*, forming the so-called thyroid anlage. Around 36-40 hpf, the thyroid anlage expands forming a placode that protrudes into the underlying mesenchyme and buds from the pharyngeal epithelium in a rostro-ventral direction, in close proximity to the developing heart tube. The first detectable thyroid follicular cell (TFC) is formed by 55 hpf and expresses all of the functional thyroid markers, such as thyroglobulin (*tg*), thyroid peroxidase (*tpo*), Na/I symporter (*slc5a5*), and thyroid stimulating hormone receptor (*tshr*) required for thyroid hormone synthesis. During the later stages of thyroid organogenesis (55-72 hpf), the TFCs proliferate and migrate posteriorly forming distinct follicular units scattered along the pharyngeal midline, moving close and along the ventral aorta (2). Among the various organs derived from foregut endoderm, the thyroid gland is unique because a complex crosstalk between intrinsic and extrinsic factors coming from surrounding tissues (e.g., cardiac mesoderm and pharyngeal vessels) is essential for proper thyroid organogenesis. In particular, the coordinate activity of different morphogens (Notch, Shh, Bmp, Fgf, and Wnt) during critical developmental windows of thyroid development have been recently described in zebrafish (3-5). During the symposium, we will present the recent findings produced in the various zebrafish experimental models with the aim to define a comprehensive picture of the morphogenetic events underlying zebrafish thyroid development.

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SA30

The gut-heart axis

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The gut-heart axis is a newly evolving field centred around the contribution of the gut microbiome to heart disease. This host-microbiome interaction has been a previously under investigated field in the pathophysiology of heart disease.

The identification of metabolites that require metabolism by gut microbes which then contribute to disease physiology in recent years has contributed to the interest in this field.

One metabolite, trimethylamine-N-oxide (TMAO), has been a key molecule of interest as it is known to be associated and potentially causal of heart disease, and importantly, requires the gut microbiome for its production.

My research group has investigated the clinical implications of TMAO and other related molecules in heart disease ranging from heart failure to myocardial infarction, and have characterised its properties including response to treatment, ethnic and geographic differences which will be discussed during the symposium.

Our work and those of others have advanced understanding in the contribution of the gut microbiome to heart disease (the gut-heart axis).

SA31

Muscle damage and reactive oxygen species

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Our interest in the role of reactive oxygen species (ROS) in skeletal muscle was ignited in the early 1980's by an upsurge in publicity about nutritional myopathies that were economically important in some farm animals, such as the so-called "White Muscle Disease" in cows and sheep. These disorders had been recognised to be caused by selenium or vitamin E deficiency. This prompted studies to determine common mechanisms that might underlie these disorders and potentially be relevant to human myopathies involving David Jones, Richard Edwards and myself in the muscle group based at University College, London [1, 2]. An understanding of the roles of these nutrients as antioxidants and the recognition that skeletal muscle generates free radicals and other ROS during contractile activity has stimulated research into potential physiological and pathophysiological roles of ROS in skeletal muscle. Superoxide is generated by NADPH oxidases during muscle contractile activity. It is rapidly converted to hydrogen peroxide (H₂O₂) which acts as a signalling molecule to stimulate multiple adaptations to contractile activity through redox-regulated signalling pathways. These physiological pathways appear to be modified by ageing leading to attenuation of some specific responses to muscle contractile activity and exercise in older humans and animals [3]. Our studies

have recently focussed on understanding how this attenuation of redox responses occurs in ageing and indicate that mitochondria plays a major role. In older humans and mice, muscle shows substantial evidence of motor unit remodelling and focal denervation which has been shown to be associated with a substantial increase in mitochondrial peroxide generation by denervated and neighbouring innervated muscle fibres [4], leading to a local disruption of redox signalling. How such changes contribute to the loss of skeletal muscle mass and function that accompanies ageing remains a subject of extensive study.

Reference 1 :- Vitamin E and skeletal muscle. Jackson MJ, Jones DA and Edwards RHT. *Ciba Foundation Symposium No. 101 'Biology of Vitamin E'* pp224- 239 Pub. Pitman, London (1983).

Reference 2 :- Lipid peroxidation of skeletal muscle - an in vitro study. **Jackson MJ**, Jones DA and Edwards, RHT. *Bioscience Reports*, 3, 609-619 (1983).

Reference 3 :- Free radical generation by skeletal muscle of young and old mice: Effect of contractile activity. Vasilaki A, Mansouri A, Van Remmen H, van der Meulen J, Larkin L, Richardson AG, McArdle A, Faulkner JA and **Jackson MJ** *Aging Cell* 5: 109-117 (2006).

Reference 4 :- Denervated muscle fibers induce mitochondrial peroxide generation in neighbouring innervated fibers: Role in aging. Pollock N, Staunton C, Vasilaki A, McArdle A and **Jackson MJ**. *Free Rad. Biol. Med.* 112: 84-92 (2017).

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SA32

Age-related muscle atrophy: A battle of nerves?

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The age-related loss of muscle mass and function may be explained by the combination of individual muscle fibre *atrophy* and fibre *loss*. Although the relative contribution of each remains largely unknown, the loss of fibres is associated with the loss of the innervating motor neurons, and is in part compensated for by an expansion of surviving motor units. This talk will address in vivo methods recently applied in the exploration of age-related loss and consequential remodelling of human motor units, and present data supporting the corresponding adaptation of neural input as a defining factor in neuromuscular decrements.

SA33

From cigarette smoking to burning mitochondria

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Although smoking-related diseases, such as chronic obstructive pulmonary disease and heart failure with preserved ejection fraction, are often accompanied by increased peripheral muscle fatigability, the extent to which this is directly caused by cigarette smoke per se is less well understood. Our earlier observation that smoking history did not correlate with fatigability in humans suggests an acute effect caused by carbon monoxide and/or other substances in smoke, hampering skeletal muscle oxygen delivery and mitochondrial function. Indeed, carbon monoxide inhalation acutely and negatively impacts on muscle fatigue. Acutely exposing permeabilized mouse skeletal muscle fibers with smoke extract indicated a clear reduction in the maximal oxidative phosphorylation capacity. These data raise the question whether smoking cessation can be accompanied by beneficial effects on skeletal and cardiac muscle function. Indeed, smoke cessation as short as one to two weeks is associated with improved skeletal muscle structure and mitochondrial function in mice and men. Particularly the diaphragm and cardiac muscle were sensitive to smoke exposure and cessation in mice. Markers of low-grade systemic inflammation reduced in ex-smokers, which was linked to cardiac infiltration of macrophages and fibrosis in mice. Cardiac metabolome analysis revealed altered metabolism, which was partially restored after smoke cessation. Overall, skeletal and cardiac muscle metabolism are acutely altered upon cigarette smoke, and the accompanying systemic inflammation can cause additional skeletal muscle alterations. Smoke cessation quickly restores skeletal muscle mitochondrial function and fatigue resistance.

SA34

Mental Health and the kynurenine pathway: Does exercise influence the activity of the kynurenine pathway?

Sophie Erhardt¹

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It is well accepted that physical exercise is associated with health benefits and reduces risks of psychiatric illness, diabetes, cancer and cardiovascular disease. Previous studies also show that psychiatric disorders, such as depression is associated with changes in peripheral and central concentrations of kynurenine metabolites of tryptophan degradation. Animal experiments indicate that exercise protects against depression by increased expression of skeletal muscle kynurenine aminotransferase (KAT), hereby promoting synthesis of the neuroprotectant kynurenine acid (KYNA)

over the neurotoxic quinolinic acid (QUIN). In a series of studies, we have investigated the effects of exercise on kynurenine metabolism in humans.

We found that KAT gene and protein expression increased in the muscles of endurance-trained subjects compared with untrained subjects. Furthermore, one hour after cycling 150-km plasma KYNA was found to be substantially increased (+63%) while the ratio QUIN/KYNA was found to be decreased (27%). Both KYNA and the QUIN/KYNA ratio returned to baseline within 24 h. In another group of subjects, we analyzed kynurenines in the plasma before and after performing a half-marathon. In this group plasma KYNA increased (+125%) 30 min after the race. In contrast, plasma concentrations of KYNA and QUIN, did not change following eccentric exercise (consisting of a series of 100 drop jumps).

We further investigated the effect of exercise on both peripheral and central concentrations of kynurenine pathway metabolites. Cerebrospinal fluid (CSF) concentrations of KYNA, 3-hydroxykynurenine and picolinic acid (PIC) increased after an acute exercise paradigm, while tryptophan and kynurenine remained unchanged. At the same time, plasma tryptophan and kynurenine levels decreased while KYNA, 3-HK, QUIN and PIC did not change. We further found that the most robust effect of exercise was detected in subjects performing acute bouts of exercise over four consecutive days, while changes induced by engaging in three times weekly training exercise over 4 weeks, led to very limited effects. We further observed that the correlation between plasma and CSF concentrations of most of the kynurenine metabolites was low, indicating limited equilibration through an intact blood brain barrier. The only exception was PIC, that correlated to a high degree between compartments both before and after the intervention

In summary, these results show that acute vigorous aerobic exercise intervention causes adaptations in kynurenine metabolism, while a training exercise protocol over a longer time frame may have more limited effects. Further studies are needed to address the durability of effects and also if other modalities of exercise, such as muscle strength training, may influence the results.

SA35

Physical activity for mental health: an update of the evidence

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While physical activity and exercise are well-established to be beneficial for physical health, the effects on mental health are also now becoming more apparent. Specifically, there is an increasingly large clinical and academic interest in how physical activity may be used to (i) reduce the risk of developing mental illness, and (ii) improve recovery and outcomes in those with diagnosed conditions. Within this, multiple national and international health bodies have begun to produce

guidelines around the role of physical activity in the promotion of mental well-being. However, the overall evidence for if and how to use physical activity interventions in the prevention and treatment of various mental illnesses is unclear. Therefore, this presentation will aim to provide an update on the current state of the evidence on the benefits of physical activity across a broad spectrum of mental health conditions, such as depression, anxiety, bipolar and psychotic disorders, and ADHD. Within this, the published 'top-tier evidence' (including meta-analyses, randomized controlled trials, and Mendelian randomization studies) will be discussed, particularly with regards to determining the causal effects of physical activity on mental health/illness. Following this, an update of key recent international guidelines on the topic will be presented, along with an exploration of the emergent literature around how physical activity and exercise can be best implemented for improving outcomes in the context of mental healthcare.

Reference 1 :- Firth J, Solmi M, Wootton RE, Vancampfort D, et al., (2020). A meta-review of "lifestyle psychiatry": the role of exercise, smoking, diet and sleep in the prevention and treatment of mental disorders. *World Psychiatry*, 19 (3): 360-380.

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SA36

Exercise and the microbiome-gut-brain axis: moving beyond tryptophan metabolism

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Background/objective:

Exercise exerts beneficial effects in depression with a number of mechanisms proposed. There is a substantial overlap between these biological pathways and those regulated by the gut microbiota via the gut-brain axis, including via the regulation of tryptophan metabolism. The physiological impact of exercise may also manifest as hormetic effects on gastrointestinal permeability, acting initially as an acute stressor that subsequently stimulates positive adaptation to support good mental health. Indeed, alterations in barrier function can impact the central nervous system via gut-brain axis signaling pathways including tryptophan metabolism. Evidence of an association between stress-related psychiatric disorders including depression, microbiota composition and increased intestinal permeability levels is accumulating. This study aims to assess the impact of exercise training intensity on gastrointestinal permeability and stress level in sedentary healthy controls, both acutely and chronically over a 12-week duration.

Methods:

Monthly fitness and psychometric assessments were performed throughout a 12-week exercise program in 35 sedentary healthy adults (3-sessions/week). Intestinal permeability was assessed in plasma samples, pre-exercise, and one-hour post-exercise, for each monthly visit using Lipopolysaccharide binding protein (LBP) and soluble cluster of differentiation 14 (sCD14) protein as biological markers (figure 1). Additionally, we investigated the dose-dependent nature of exercise upon measures of stress, anxiety, and depression.

Results:

Preliminary analyses show that a 12-week exercise program resulted in significant increases in fitness ($p < 0.05$) and performance ($p < 0.001$), with modest reductions in reported levels of stress ($p = .08$) and depression ($p = .06$). Acutely, intestinal permeability markers (LBP; sCD14) were unchanged one-hour post the monthly fitness assessment. Chronically, we observe a significant effect of training ($p < 0.05$) and significant training x exercise group interaction ($p < 0.05$) in LBP concentration levels, which were increased in the high-intensity group only.

Conclusions:

Recent clinical and preclinical research charting the impact of exercise on the microbiota-gut-brain axis as a potential mediator of the benefits for mood and cognition. Our preliminary data shows that 12-weeks exercise training has a modest impact on intestinal permeability. Further research is needed to understand the CNS implications of exercise-induced alterations in gastrointestinal physiology and gut-brain axis signaling, and to understand how this integrates with the beneficial effects of exercise in modulating stress and depression.

Ethical Standards:

Approval of the study protocol was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals and written informed consent was obtained from all subjects. The study was carried out in accordance with the Declaration of Helsinki. All experiments were in full accordance with the EU legislation (Directive 2010/63/EU).

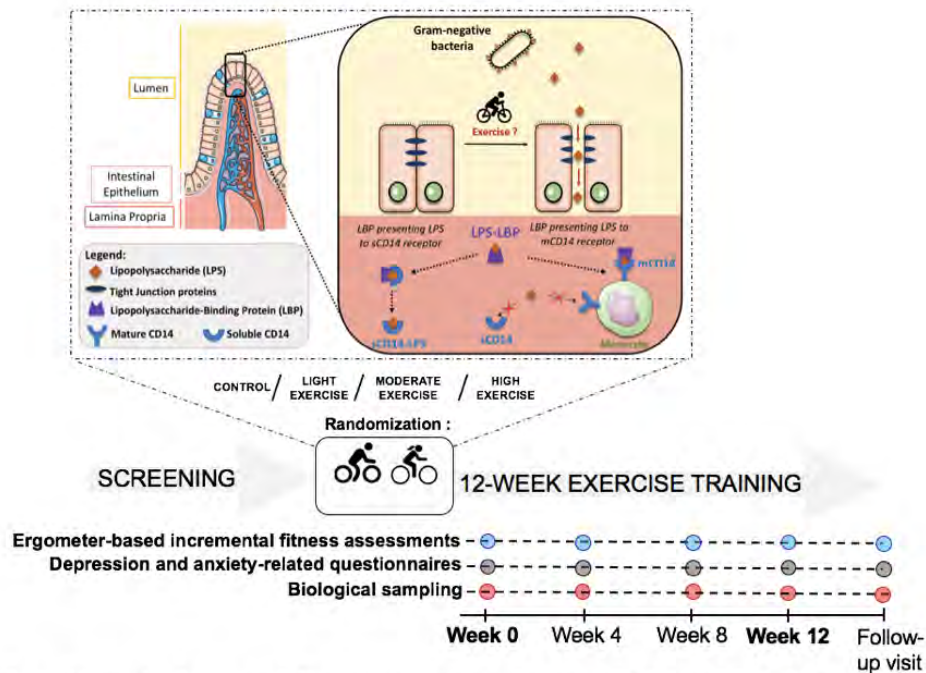


Figure 1. Altered intestinal permeability can be assessed using sCD14 and LPS-binding protein (LBP).

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Jason A. Martin is a co-first author in this work.

SA37

Physical activity, exercise, myokines and muscle-brain crosstalk

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Skeletal muscle secretes several hundred myokines that facilitate communication from muscle to other organs, such as, adipose tissue, pancreas, liver, gut, and brain. The biological roles of myokines include effects on e.g glucose and lipid metabolism, tumour metabolism, inflammation as well as brain function.

Exercise has many beneficial effects on brain health, contributing to decreased risks of dementia, depression and stress, and it has a role in restoring and maintaining cognitive function and metabolic control. The fact that exercise is sensed by the brain suggests that muscle-induced peripheral factors enable direct crosstalk between muscle and brain function. Muscle secretes myokines that contribute to the regulation of hippocampal function. Evidence is accumulating that the myokine cathepsin B passes through the blood-brain barrier to enhance brain-derived neurotrophic factor production and hence neurogenesis, memory and learning. Exercise increases neuronal gene expression of FNDC5 (which encodes the PGC1 α -dependent myokine FNDC5), which can likewise contribute to increased brain-derived neurotrophic factor levels. Serum levels of the prototype myokine, IL-6, increase with exercise and might contribute to the suppression of central mechanisms of feeding. Exercise also increases the PGC1 α -dependent muscular expression of kynurenine aminotransferase enzymes, which induces a beneficial shift in the balance between the neurotoxic kynurenine and the neuroprotective kynurenic acid, thereby reducing depression-like symptoms. Myokine signaling, other muscular factors and exercise-induced hepatokines and adipokines are implicated in mediating the exercise-induced beneficial impact on neurogenesis, cognitive function, appetite and metabolism, thus supporting the existence of a muscle-brain endocrine loop.

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SA38

Manipulation of cellular pathways by SARS-CoV-2 in the gastro-intestinal tract

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Exacerbated pro-inflammatory immune response contributes to COVID-19 pathology. However, despite the mounting evidence about SARS-CoV-2 infecting the human gut, little is known about the antiviral programs triggered in this organ. To address this gap, we performed single-cell transcriptomics of SARS-CoV-2-infected intestinal organoids. We identified a subpopulation of enterocytes as the prime target of SARS-CoV-2 and, interestingly, found the lack of positive correlation between susceptibility to infection and the expression of *ACE2*. Infected cells activated strong proinflammatory programs and produced interferon, while expression of interferon-stimulated genes was limited to bystander cells due to SARS-CoV-2 suppressing the autocrine action of interferon. These findings reveal that SARS-CoV-2 curtails the immune response and highlights the gut as a proinflammatory reservoir that should be considered to fully understand SARS-CoV-2 pathogenesis.

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SA39

Human organoid systems to study the pathogenesis, cell biology and evolution of SARS-CoV-2

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As obligate intracellular parasites, viruses rely on fine-tuned interactions with their hosts. Nevertheless, host switching is observed for viruses with a high degree of adaptive plasticity and a high mutation rate, such as certain coronaviruses. SARS-CoV-2 spilled over to humans possibly from bats and since this event has adapted to optimize its transmission between humans. However, traditionally, pathogenic viruses are studied in experimental animal models, in which specific virus-host interactions may not be modelled correctly, forcing viruses to adapt and limiting the use of these models. Studying SARS-CoV-2 pathogenesis and biology, as well as identifying potential treatments therefore benefits from the development of in vitro cell culture systems that closely mimic human physiology. We have established human organoid systems of the intestines, airways and alveoli to study the pathogenesis, cell biology and evolution of SARS-CoV-2. Combining these systems with CRISPR/Cas9 gene editing technology allows the identification of realistic antiviral drug targets. In addition, our findings unveil human organoids as powerful tools to phenotype SARS-CoV-2 variants-of-concern and identify correlates of fitness for SARS-CoV-2. Organoids are emerging as versatile tools to study SARS-CoV-2, laying a foundation for future pandemic responses.

SA40

Effects of SARS-CoV-2 infection on airway epithelial ion transport

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Introduction: SARS-CoV-2, the cause of COVID-19, belongs to the β -coronavirus subgroup that includes SARS-CoV-1 and MERS. SARS-CoV-2 utilizes the ACE2 receptor/TMPRSS2 protease complex expressed on the apical surfaces of respiratory epithelial cells as an entry mechanism. Removal of infectious agents from the airways is accomplished by mucociliary clearance (MCC), which requires a delicate balance between secretion and reabsorption of fluids. Ion channels such as the epithelial sodium channel (ENaC), the cystic fibrosis transmembrane conductance regulator (CFTR), and the calcium-activated chloride channel, TMEM16A, play key roles in regulating airway surface liquid homeostasis. SARS-CoV-2 encodes 4 major structural proteins: envelope (E), spike, membrane, and nucleocapsid proteins, which are required to produce a complete viral particle. CoV E proteins oligomerize to form viroporin channels that have been suggested to conduct cations and therefore may also affect airway hydration.

Objectives: To elucidate the impact of SARS-CoV-2 on ion transport in airway epithelia and to determine whether the SARS-CoV E protein can be used as a novel and effective target to treat COVID-19 as well as future SARS outbreaks.

Methods: We performed viral infections of primary airway epithelial cultures with CoV or CoV E. Because SARS-CoV-2 research requires BL3 facilities, the human CoV, NL63, which causes minor cold-like symptoms but shares the ACE2 entry mechanism with SARS-CoV-2, was used as a surrogate. We conducted measurements in Ussing chambers to determine ion channel function of CFTR, ENaC, and TMEM16A in the presence of CoV or SARS-CoV-2 E. In addition, we studied intracellular localization of CFTR, ENaC and SARS-CoV-2 E in primary airway cultures by immunofluorescence microscopy, and analyzed protein processing and expression by Western blotting. Furthermore, CoV E peptide studies in planar lipid bilayers by single-channel measurements were performed by fusion of membrane vesicles from HEK-293 expressing SARS-CoV-2 E or utilizing artificial lipid vesicles with reconstituted SARS-CoV-2 E peptides and conducting single-channel measurements.

Results: CoV infection substantially affected ion channel function in primary airway epithelia with a significant reduction in ENaC activity, while function of CFTR and TMEM16A, similar to responses observed after airway inflammation (1), were enhanced. Furthermore, we demonstrated that SARS-CoV-2 E conducts cations, such as Na^+ and K^+ and notably, that hexamethylene amiloride (HMA) inhibits CoV E channels and reduces the titer of CoV.

Conclusions: CFTR and ENaC were recently shown to be localized to secretory cells (2), where they regulate airway hydration required for MCC. Proper ENaC function requires proteolytic cleavage by the serine protease furin (3). We are currently exploring whether expression of viral spike protein, which contains the same furin cleavage sequence as ENaC, could interfere with ENaC processing. The

most likely host-infection sequence for SARS-CoV-2 begins with infection of ciliated cells in the nose, followed by viral replication and shedding. Subsequent spreading of viral particles from the nasal cavity by inhalation results in infection of bronchiolar ciliated cells and, ultimately, alveolar cells (4,5). Understanding the role of CoV-2 E may be key to developing therapies to inhibit viral propagation in nasal and bronchial epithelia, before SARS-CoV-2 spreads to alveoli.

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SA41

Assessment of SARS-CoV-2 transmission potential under experiment settings and in an acute healthcare setting

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Ferrets have been extensively characterised as models for SARS-CoV-2 transmission experiments, showing fairly robust infection and transmission. we assessed whether Spike mutations were selected for during experimental ferret infections in our laboratory, and, having detected a virus with a single Y453F mutation, we analysed the effect of this change on replication in ferrets, and in primary human airway epithelial cells. We confirm that these mutations likely adapt virus to mink/ferrets by enhancing entry into cells expressing mustelid ACE2. In addition, we assessed whether this single amino acid change altered neutralization of pseudovirus or live virus by antibodies in convalescent sera of individuals infected and recovered from COVID-19 during the first wave in the UK.

We performed a prospective cross-sectional observational study in a multi-site London hospital. Air and surface samples were collected from seven clinical areas, occupied by patients with COVID-19, and a public area of the hospital. Three or four 1.0 m³ air samples were collected in each area using an active air sampler. Surface samples were collected by swabbing items in the immediate vicinity of each air sample. SARS-CoV-2 was detected by RT-qPCR and viral culture; the limit of detection for culturing SARS-CoV-2 from surfaces was determined. Viral RNA was detected on 114/218 (52.3%) of surfaces and 14/31 (38.7%) air samples but no virus was cultured. The proportion of surface samples contaminated with viral RNA varied by item sampled and by clinical area. Viral RNA was detected on

surfaces and in air in public areas of the hospital but was more likely to be found in areas immediately occupied by COVID-19 patients than in other areas (67/105 (63.8%) vs. 29/64 (45.3%) (odds ratio 0.5, 95% confidence interval 0.2-0.9, $p=0.025$, Chi squared test)). The high PCR Ct value for all samples (>30) indicated that the virus would not be culturable. Our findings of extensive viral RNA contamination of surfaces and air across a range of acute healthcare settings in the absence of cultured virus underlines the potential risk from environmental contamination in managing COVID-19, and the need for effective use of PPE, physical distancing, and hand/surface hygiene.

SA42

Coordinated functions of STIM-Orai complexes fine tune calcium flux across nanojunctions of the ER/SR, plasma membrane and mitochondria

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In essentially all eukaryotic cells, activation of phospholipase C (PLC)-coupled receptors leads to inositol1,4,5-trisphosphate (IP_3)-dependent depletion of endoplasmic reticulum (ER) Ca^{2+} stores. ER Store depletion is sensed by Stromal-interaction molecule 1 (STIM1), which then activate Orai1 channels located in the plasma membrane (PM). STIM1/Orai1 coalesce within ER-PM junctions and generate Ca^{2+} nanodomains crucial for activation gene programs, including those driven by Calcineurin/nuclear factor for activated T-cells (NFAT) that regulate cell motility, growth, and metabolism. ER-mitochondria contact sites and functional PM-mitochondria interactions further shape receptor-evoked Ca^{2+} signaling to fine-tune cell function. In turn, mitochondrial Ca^{2+} uptake and extrusion, through the mitochondrial Ca^{2+} uniporter (MCU) and the $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX) respectively, are crucial for proper mitochondrial function and for coupling receptor stimulation to cellular bioenergetics. While the essential role of STIM1 and Orai1 in receptor evoked Ca^{2+} signaling is well understood, little is known about the physiological activation and the choreography of interactions between the five STIM1,2/Orai1,2,3 proteins natively expressed in all mammalian cells. Here we will discuss our recent findings revealing that coordinated functions of the five STIM1,2/Orai1,2,3, IP_3 receptors, MCU and NCLX at the ER-PM and ER-mitochondria contact sites translate the strength of agonist stimulation to precise levels of Ca^{2+} signaling and NFAT induction, ensuring the fidelity of complex mammalian Ca^{2+} signaling.

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SA43

ER-dependent scaling of site-specific calcium transients in astroglia

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Astroglia are an abundant and electrically non-excitable cell type in the brain, which can profoundly modify neuronal activity. For the latter, store-dependent calcium transients triggered by G-protein-coupled receptor are indispensable. Such calcium transients vary strongly in their spatial and temporal characteristics. They can be observed as fast, local elevations in small microdomains of the fine and fussy branches but also as large, slower waves propagating throughout a single branch, the whole astrocytic territory or even from cell to cell. While the functional relevance of these transients is well characterized, it is incompletely understood how the amplitude and waveform of these transients are controlled.

The IP₃ receptor type 2 is a key mediator of endoplasmic reticulum (ER)-dependent calcium elevations in astrocytes. The open probability of these receptors depends on the cytosolic calcium concentration. As the resting calcium concentration shows variations within and between astroglia, it could be responsible for site-specific scaling of calcium transients.

To explore this hypothesis in acute brain slices and in vivo, we used two-photon excitation microscopy and quantified calcium concentrations and their changes by analyzing the fluorescence lifetime or intensity of suitable indicators. Independent of the type of calcium transients (spontaneous, agonist- or behavior-induced) and recording technique, we observed a positive correlation between the maximum (peak) of the transient and a negative correlation between the scale (amplitude) and the local cytosolic calcium concentration. Mechanistically, the latter relationship is linked to store-dependent calcium entry into the cytosol. Importantly, deliberately increasing or decreasing the cytosolic resting calcium concentration altered calcium transient peaks and amplitudes accordingly, which indicates that the local resting calcium concentration dynamically controls the scale of local calcium transients.

In summary, our findings uncovered basic generic rules of calcium signal formation in astrocytes.

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SA44

Calcium signalling across the cell-wide web and its nuclear envelope invaginations: coordinating cellular process and gene expression by directing site-specific calcium flux

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A decade ago the panjunctional sarcoplasmic reticulum hypothesis was proposed (van Breemen *et al.*, 2013), that incorporated the idea that a network of tubules and quilts capable of auto-regulating their calcium content and determining junctional calcium concentration through loading and unloading at membrane-membrane nanojunctions. By this mechanism it was postulated that local calcium signals could be targeted to control a variety of cellular processes, from contraction and metabolism to transcriptional activities. In accordance with this, we recently identified a cell-wide network of distinct cytoplasmic nanocourses with the nucleus at its centre, demarcated by nanojunctions (<400 nm across) between sarcoplasmic reticulum (SR) and other organelles, that restrict calcium diffusion and thus provide for signal segregation in a manner facilitated by nanocourse-specific calcium pumps and release channels (Duan *et al.*, 2019). Ryanodine receptor subtype 1 (RyR1) supports relaxation of arterial myocytes by unloading calcium into peripheral nanocourses delimited by plasmalemma-SR junctions, fed by sarco/endoplasmic reticulum calcium ATPase 2b (SERCA2b). Conversely, stimulus-specified increases in calcium flux through RyR2/3 clusters selects for rapid propagation of calcium signals throughout deeper extraperinuclear nanocourses and thus myocyte contraction. Beyond these, nuclearenvelope invaginations and SERCA1 demarcate further diverse networks of cytoplasmic nanocourses that receive calcium signals through discrete RyR1 clusters that do not freely enter the nucleoplasm, yet impact gene expression through epigenetic marks segregated by their associated invaginations. Critically, this circuit is not hardwired and remodels for different outputs during cell proliferation.

Regardless of their functional subdivision, within cytoplasmic nanocourses all path lengths from calcium release site to targeted signalling complex are on the nanoscale. With picolitre volumes of cytoplasm lying within the boundaries of each nanocourse, relatively small net increases in local calcium flux (1-2 ions per picolitre) will be sufficient to raise the local concentration into the affinity ranges of most cytoplasmic calcium binding proteins (Fameli *et al.*, 2007; Fameli *et al.*, 2014). It is intriguing, therefore, that recent studies have established that unicellular organisms employ sequential computational logic to engage behaviour selection (Dexter *et al.*, 2019). Sequential logic can be actioned by progression through a series of circuit memory elements called flip-flops, which store a single bit (binary digit) of data, by switch and reset between 0 and 1. By analogy I propose that nanocourse-specific calcium binding proteins operate as local “switches”, their position

and reset directed, in part, by changes in local calcium flux. In this way coincident increases in calcium flux could be triggered in two distant parts of the cell-wide web at the same time (Duan *et al.*, 2019), enabling coordination of multiple cellular functions through either sequential, or parallel logic processing. This draws obvious parallels to mechanisms of conduction in carbon nanotubes, that transmit charge carriers through discrete conduction channels, enabling memory, logic and parallel processing. Thus, by analogy, our observations point to the incredible signalling potential that may be afforded by modulating “quantum calcium flux” on the nanoscale, in support of network activities within cells with the capacity to permit stimulus-dependent orchestration of the full panoply of diverse cellular processes.

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SA45

Molecular drivers of progressive renal injury in sickle cell disease

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Renal injury and progressive nephropathy is a major consequence of sickle cell disease (SCD) and is a cause of early mortality in this patient population. However, little is known about the molecular drivers that promote sickle cell nephropathy (SCN). Endothelin (ET) is a vasoconstrictor and pro-inflammatory peptide that is up-regulated in SCN. This presentation will provide preclinical data showing that specific blockade of the ET/ETA receptor pathway reduces the incidence of SCN. We will also provide data showing that endothelial-derived ET may specifically mediate SCN-induced immune cell activation, especially the Th17 pathway. Thus, we propose that the ET/ETA receptor pathway interacts with the activation of the Th17 pathway to promote SCN.

SA46

Clinical progression of proteinuric disease

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Urinalysis is an integral part of the assessment and management of individuals with kidney diseases. The finding of proteinuria in patients with kidney diseases is a poor prognostic sign for both for future renal function and cardiovascular disease. The role of proteinuria as an independent risk factor for renal function loss is now well recognized. The failing kidney is characterized histologically by tubulointerstitial inflammation, tubular cell apoptosis, tubular atrophy and fibrosis, and these changes correlate with the degree of proteinuria. Multiple clinical trials have indicated that antiproteinuric strategies are, in general, renoprotective. Such observations have led to the suggestion of a causal relationship between proteinuria and renal inflammation or scarring. Understanding the mechanisms underlying proteinuric nephropathy has attracted intense interest. It is now clear that the association between proteinuria and progression is causal and strong biological plausibility has been demonstrated. Targeting proteinuria is thus a rational target for clinical trial interventions.

SA47

A model of megalin trafficking in the proximal tubule

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The polarized epithelial cells that comprise the proximal tubule (PT) have a specialized apical endocytic pathway that allows for the high-capacity endocytosis necessary to recover essential nutrients and to maintain a protein-free urine. Impairments in this pathway result in tubular proteinuria, which can worsen to cause more severe kidney disease. Megalin and cubilin, multi-ligand receptors at the apical surface of the epithelial cells, bind proteins in the ultrafiltrate and internalize them via receptor-mediated endocytosis. Ligands are sorted from receptors in endocytic compartments, and the receptors are recycled back to the surface through structures called dense apical tubules (DATs). The molecular identities of the compartments involved in sorting and recycling in PT cells and the kinetics of megalin trafficking through them are unknown. Understanding megalin's endocytic trafficking itinerary and the key regulatory steps in this pathway is important for discerning the basis of proteinuric diseases and devising therapies. To address this, we identified endocytic compartment markers in a previously developed opossum kidney (OK) cell culture model that recapitulates morphologic and functional features of the PT *in vivo*. The fraction of total megalin colocalizing with each compartment marker was quantified by Mander's coefficient from deconvolved confocal images. Most megalin is localized primarily in Rab11-positive compartments at steady state while a much smaller fraction of megalin is localized in Rab4-positive structures. Surface biotinylation based assays revealed that only a small fraction of total megalin is present at the apical surface at steady state and that megalin is rapidly internalized from the surface. The half-life of surface megalin was also assessed. Our biochemical and quantitative colocalization data was used to construct and refine a kinetic model of megalin trafficking in the PT. These data suggest that recycling is the rate-limiting step in regulating the fraction of total megalin available at the surface. Together, our results provide valuable insight into the spatial organization of the endolysosomal

system and the itinerary and regulation of megalin traffic in the PT. Our kinetic model will be used to identify how genetic mutations and other conditions that cause tubular proteinuria disrupt traffic along the endocytic pathway.

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SA48

Megalín and cubilin function in proteinuric disease

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Introduction

An important role of the kidney proximal tubule is to reabsorb glomerularly filtered peptides and proteins. This occurs by receptor-mediated uptake by two large multiligand endocytic receptors, megalin and cubilin, which are highly enriched on the apical surface of proximal tubule epithelial cells. The process is highly efficient as reflected by the fact that in the healthy kidney, urine is practically devoid of protein. However, kidney disease with glomerular injury increases the load of filtered plasma proteins, in particular albumin, leading to proteinuria.

Objectives

Our objective is to understand the molecular basis of proximal tubule protein reabsorption and how it is regulated under normal and pathological conditions, with focus on the megalin and cubilin receptor system and its role in proteinuric disease.

Method

In our studies, we use a variety of molecular and cellular techniques as well transgenic animal models, to investigate the renal handling of filtered proteins by the megalin and cubilin receptors.

Results

Our research and that of co-workers has over the years established the essential role of the megalin and cubilin receptor system in modulating urinary protein excretion. Using a genetic model of glomerular kidney disease in combination with gene knockout of the receptors, we have further recently explored the implications of receptor dysfunction under nephrotic-range proteinuria. We

observed massive albumin uptake in proximal tubules under nephrotic proteinuria, which was efficiently blocked by megalin and cubilin receptor knockout. In addition, we found evidence of potential distinct roles of the cubilin and megalin receptor in the uptake of albumin at normal filtered levels and nephrotic levels of albumin.

Conclusion

Renal tubular reabsorption and rescue of filtered proteins, including albumin, is dependent on the megalin and cubilin receptors. Dysfunction of the receptors causes mild tubular proteinuria whereas glomerular kidney disease with increased filtration of plasma proteins overwhelms the receptors, causing nephrotic range proteinuria accompanied by massive protein uptake in the proximal tubules. These findings have implications for the pathophysiology and diagnosis of proteinuric renal diseases.

Ethics

All animal experiments were performed in accordance with the EU Animal Welfare Act for humane treatment of vertebrate animals and licenses issued by the Danish Animal Experiments Inspectorate.

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SA49

Novel insights into the role of intestinal NHE3 as a therapeutic target

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The sodium-hydrogen exchanger isoform 3 (NHE3) is abundantly expressed in the gastrointestinal tract and plays an essential role in sodium/fluid absorption and acid-base homeostasis. However, understanding the precise role of intestinal NHE3 has been challenging due to the lack of a suitable animal model. To circumvent this problem, we generated a tamoxifen-inducible intestinal epithelial cell-specific NHE3 knockout mouse model (NHE3IEC-KO). Before tamoxifen administration, the phenotype and blood parameters of NHE3IEC-KO were unremarkable compared with control mice. After tamoxifen administration, NHE3IEC-KO mice have undetectable levels of NHE3 in the intestine. NHE3IEC-KO mice develop watery, alkaline diarrhea in combination with a dilated small intestine, cecum and colon. They have higher fluid intake compared to controls due to persistent diarrhea. The mortality rate in NHE3IEC-KO is ~25% after 3 weeks. We found that NHE3IEC-KO mice exhibit metabolic acidosis, lower blood bicarbonate levels, hyponatremia and hyperkalemia associated with drastically elevated plasma aldosterone levels. These results demonstrate that intestinal NHE3 has a

significant contribution to acid-base, Na⁺ and volume homeostasis, and lack of intestinal NHE3 has consequences on intestinal structural integrity. Based on the physiological role of NHE3, pharmacological inhibition of intestinal NHE3 is an interesting treatment strategy. In fact, nonabsorbable NHE3 inhibitors have been developed, and preclinical as well as clinical trials indicate possible pharmacological use in fluid overload, hypertension, chronic kidney disease, hyperphosphatemia, and constipation.

Acknowledgements :- This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases [grant number 1R01DK110621 (to T.R.)]; American Heart Association Transformational Research Award [grant number 19TPA34850116 (to T.R.)]; the LeDucq Foundation (to R.A.F.); the Danish Medical Research Council; the Novo Nordisk Foundation; American Heart Association predoctoral fellowship [grant number 18PRE33990236 (to J.X.)]; and L.T. by a postdoctoral fellowship [grant number 19POST34400026].

SA50

Transporters feat. the lung: What do we learn from current understandings.

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This talk will provide an overview of recent knowledge on the (patho)physiological and pharmacological functions of organic cation transporters in the lung. The organic cation transporters (OCT) 1, 2 and 3 and novel organic cation transporters (OCTN) 1 and 2 of the solute carrier 22 (SLC22) family are involved in the various cellular transport mechanisms of endogenous compounds such as neurotransmitters and L-carnitine and further play an important role in the interaction with inhaled drugs such as beta2-agonists and anticholinergic drugs. They may be associated with the development of chronic lung diseases such as asthma and chronic obstructive pulmonary disease (COPD). In particular OCTN2 may be a potential target to enhance the pulmonary delivery of inhaled drugs for better therapeutical responsiveness in patients. The talk will further elucidate this and discuss future options of OCT/N molecular targets for therapeutic approaches.

SA51

Npt2a as a target for treating hyperphosphatemia with and without kidney disease

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The precise regulation of the body's phosphate (P_i) homeostasis is a critical task. Treatment of hyperphosphatemia, which becomes inevitable in later stages of chronic kidney disease (CKD), is limited to dietary P_i restriction and oral P_i binders. Two transport proteins mediate renal P_i reabsorption, the sodium-phosphate cotransporters Npt2a and Npt2c. The former mediates the majority of renal P_i reabsorption (70-80%), which is a hormonally regulated process and requires parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). Currently, no renal P_i cotransporter is a pharmacological target. Studies were performed *in vitro* and *in vivo* employing a novel Npt2a inhibitor (Npt2a-I, PF-06869206). In opossum kidney (OK) cells, a model of the proximal tubule, the Npt2a-I caused a dose-dependent decrease of Na^+ -dependent P_i uptake (IC_{50} : $\sim 1.4 \mu\text{mol/L}$). Michaelis-Menten kinetics in OK cells identified an ~ 2.4 -fold higher K_m for P_i in response to Npt2a inhibition with no significant change in apparent V_{max} . *In vivo*, the Npt2a-I induced a dose-dependent increase in urinary P_i excretion in wild-type mice (ED_{50} : $\sim 23 \text{ mg/kg}$), a finding completely absent in Npt2a^{-/-} mice. The observed decrease in plasma P_i in WT mice is also absent in Npt2a^{-/-} mice. Surprisingly the Npt2a-I-induced increase in urinary Na^+ excretion was unaffected in Npt2a^{-/-} mice, a response possibly mediated by an off-target acute inhibitory effect of the Npt2a inhibitor on open probability of the epithelial Na^+ channel (ENaC) in the cortical collecting duct. The effects on urinary P_i excretion and plasma P_i were also observed in a 5/6 nephrectomy (Nx) model but were somewhat attenuated. In addition, Sham and 5/6 Nx mice show a similar decrease in PTH in response to Npt2 inhibition. In summary, Npt2a inhibition is a possible treatment option in conditions where hyperphosphatemia is present, e.g., in severe CKD, acute tumor lysis syndrome, rhabdomyolysis, hemolysis, hyperthermia, profound catabolic stress, or acute leukemia.

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SA52

Pathophysiological significance of the prostaglandin transporter SLCO2A1 in lung inflammation

Takeo Nakanishi¹

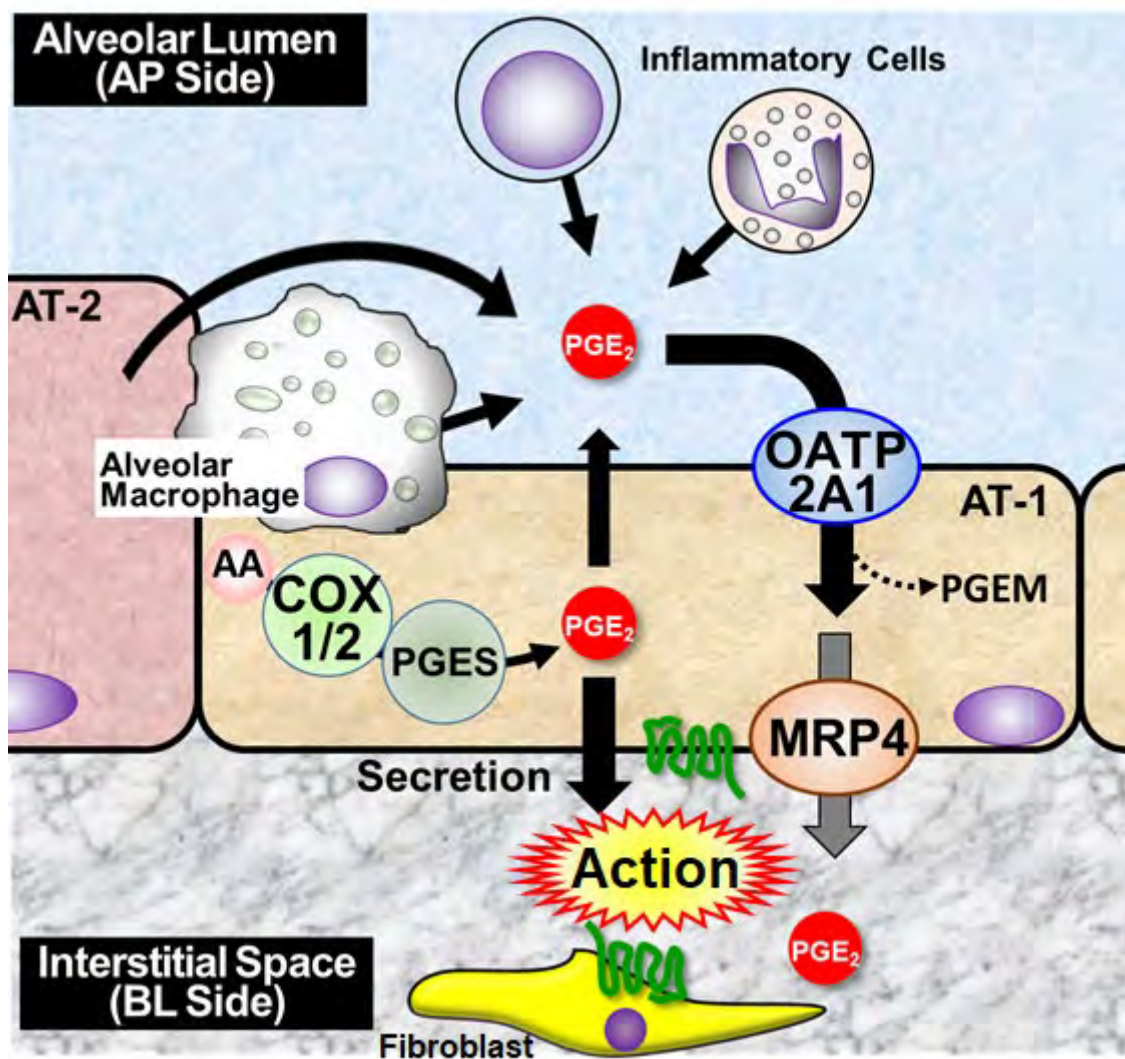
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Solute carrier organic anion transporter family member 2A1 (SLCO2A1) is a plasma membrane transporter consisting of 12 transmembrane domains and mediates cellular uptake of major prostaglandins (PGE₂, PGF_{2α}, and PGD₂) and, to a lesser extent, thromboxanes (e.g., TxB₂). Recent whole-exome analyses demonstrated that recessive inheritance of *SLCO2A1* mutations causes primary hypertrophic osteoarthropathy (PHO) and chronic enteropathy associated with *SLCO2A1* (CEAS), suggesting that impaired function of *SLCO2A1* is associated with a disorder of PG metabolism (1). In the lungs, there is significant evidence that the anti-fibrotic action of PGE₂ results in the increased sensitivity of fibroblasts to apoptosis. Although the importance of PG biosynthesis and signaling is well established, much less is known about the contribution of transporters to PGE₂ action in the respiratory regions. Our laboratory has been investigating the role of *SLCO2A1* in

actions of PGE₂ in the lungs since we observed more severe pulmonary fibrosis in bleomycin-treated *Slco2a1*-deficient mice (*Slco2a1*^{-/-}) (2). This study aimed to understand the pathophysiological significance of SLCO2A1 in pulmonary inflammation.

We conducted immunohistochemistry and transport studies using type 1 alveolar epithelial cell-like (AT1-L) cells transdifferentiated from type 2 alveolar epithelial cells prepared from rats and mice. Anti-Slco2a1 immunoreactivity was detected mainly in the alveolar epithelium. *Slco2a1* deficiency remarkably reduced cellular uptake of PGE₂ by AT1-L cells and increased PGE₂ in bronchoalveolar lavage (BAL) fluid of mice. Transcellular transport of PGE₂ across the monolayer of rat AT1-L cells from the alveolar lumen (AP side) to the interstitial space (BL side) was greater than that in the BL-to-AP direction (3). Permeation coefficient of PGE₂ was significantly reduced in the presence of SLCO2A1 inhibitors and, to a lesser extent, ceefourin-1 (an MRP4 inhibitor), suggesting that SLCO2A1 transports PGE₂ from alveolar lumen to stromal tissues in cooperation with MRP4 (Figure 1).

We further investigated the impact of SLCO2A1 on lung inflammation. BAL fluid cell analysis indicated more severe inflammation occurred in *Slco2a1*^{-/-} on day 5 after BLM intratracheal instillation, and *Slco2a1* deletion increased mRNA expression of pro-inflammatory cytokines (Tnf- α and Il-1 β) and chemokine (Ccl5) in BAL cells (4). Male *Slco2a1*^{-/-} exhibited significantly higher amounts of released Il-1 β and PGE₂ concentration in BAL fluid than female *Slco2a1*^{-/-}. Thus, *Slco2a1*-deficient male mice are likely more susceptible to airway inflammation. This observation is consistent with our recent report of NLRP3 inflammasome activation caused by increased peripheral PGE₂ level in dextran sulfate sodium-induced colitis mouse model (5). Finally, we found that cigarette smoke extracts (CSE) significantly reduced SLCO2A1-mediated PGE₂ uptake; therefore, SLCO2A1 inhibition by xenobiotics is considered a new rationale for lung toxicity. In conclusion, excess amount of PGE₂ due to impaired function of SLCO2A1 in the alveolar fluid may contribute to activation of inflammasomes in alveolar macrophages, resulting in aggravated lung inflammation. These results suggest that SLCO2A1 regulating PGE₂ biodistribution in the respiratory region is essential for lung homeostasis.



Reference 1 :- Nakanishi T et al, *Pharmacol Ther*, 223:107803, 2021

Reference 2 :- Nakanishi T et al, *PLOS ONE*, 10:e0123895, 2015

Reference 3 :- Nakanishi T et al, *J Pharmacol Exp Ther*, 368:317, 2019

Reference 4 :- Nakanishi T et al, *Toxicol Appl Pharmacol*, 405:115201, 2020

Reference 5 :- Nakata R et al, *Sci Rep*, 10:4883, 2020

Acknowledgements :- Grants-in-Aid for Scientific Research (KAKENHI 20K07018) from the Japan Society for the Promotion of Science, and a grant from the Smoking Research Foundation (Tokyo, Japan)

SA53

Probing photoreceptor physiology and pathophysiology in vivo using electrophysiology

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The electroretinogram (ERG) represents the summed electrical response of the retina to light stimuli. It can be recorded from human subjects non-invasively *in vivo* using electrodes placed around the eye. In response to flashes of light of a range of strengths, an initial negative-going wave is observed (the a-wave), followed by a positive-going wave (the b-wave). Stimulus protocols can be employed to estimate dark-adapted rod-driven and cone-driven response components. Application of mathematical models to the a-wave allow parameters relating to retinal current flows to be extracted from the human eye. This can be applied to probe retinal function in health and disease. Examples will be given in this talk. The ERG can also be used to track kinetics of retinal light and dark adaptation, including by monitoring the b-wave elicited by dim flashes delivered during and following exposure to different backgrounds (Cameron et al., 2006). We used a similar protocol to track dark adaptation in healthy volunteers and patients with selected diseases. Participants' pupils were pharmacologically dilated. ERGs were recorded using conductive fibre electrodes in response to the dim white flashes (c.0.02 scotopic cd m⁻² s) delivered following the extinction of a white background (30 photopic and c.85 scotopic cd m⁻²), after steady state exposure to this background. Flash series were repeated every 2 min for 20-60 min, and b-wave amplitudes (normalised to their final level) were plotted against time in the dark. Participants gave informed consent; the study had local ethics committee approval and complied with the tenets of the Declaration of Helsinki. The following participants underwent recordings: 7 healthy volunteers (aged 21-82 years); a patient (aged 50) with early-onset widespread retinal drusen; two patients (ages 42, 49), with molecularly confirmed Sorsby Fundus Dystrophy (SFD); a 70 year old with Vitamin A deficiency (VAD), both after treatment and at varying levels of deficiency. Responses were initially of low amplitude but then recovered gradually in the dark. In healthy participants (and the participant with early-onset drusen), recovery kinetics were similar: amplitudes reached the final level at c.20 min (and half the final level by c.10 min). The VAD patient showed normal recovery following treatment, but no recovery when markedly deficient, and a slowed recovery when mildly deficient. The SFD patients displayed recoveries similar to that found in the VAD patient when mildly deficient. Although abnormalities in SFD on clinical examination are largely in the macula, these findings confirm delayed recovery affecting the retina as a whole, consistent with "ocular vitamin A deficiency" in SFD. The protocol can be useful in investigating conditions in which photoreceptor dark adaptation might be impaired.

Reference 1 :- Cameron AM, Mahroo OA, Lamb TD (2006). Dark adaptation of human rod bipolar cells measured from the b-wave of the scotopic electroretinogram. *J Physiol.* **575**(Pt 2), 507-526.

Acknowledgements :- Fight for Sight UK and Birdshot Uveitis Society (24BU141); Fight for Sight UK (1409/10); TwinsUK receives support from the Wellcome Trust and Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London; Moorfields Eye Charity (R190037A).

SA54

Photoreceptor physiology: Cellular and molecular basis of rod and cone phototransduction

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Phototransduction in rod and cone photoreceptors utilises a G-protein cascade that is well understood at a cellular and molecular level. Rhodopsin (or its cone equivalent) is a typical G-protein-coupled receptor and, when activated by light, enzymatically activates the G-protein, transducin. Transducin in turn activates a cyclic-nucleotide phosphodiesterase (PDE6), increasing the hydrolysis of cGMP, and thereby causing closure of cyclic nucleotide-gated channels in the cell's plasma membrane. The activation phase of the light response in rods has been modelled accurately at a molecular level. An additional half-dozen proteins are involved in shut-off of the light response and light adaptation.

Remarkably, the homologous cascades in rods and cones use different isoforms of most of the main players. This duality can be traced back to the two rounds of whole-genome duplication (WGD) that occurred roughly 500 million years ago in a chordate ancestor of all jawed vertebrates, or in some cases to more ancient duplications (Lamb, 2020). For transducin α , for the PDE6 catalytic and regulatory units, and for the CNGC α and β subunits, this rod versus cone distinction arose at the first round (1R) of WGD. The HGNC names of the respective rod / cone isoforms of these genes are: *GNAT1* / *GNAT2*; *PDE6A+PDE6B* / *PDE6C*; *PDE6G* / *PDE6H*; *CNGA1* / *CNGA3* and *CNGB1* / *CNGB3*. For the visual pigments, three cone-type opsins with different spectral sensitivities had already evolved prior to 1R, along with pinopsin, which appears to have been the ancestral scotopic visual opsin; rhodopsin did not appear as a distinct isoform until 1R.

Likewise, most of the other proteins involved in response recovery and light adaptation exhibit distinct rod/cone isoforms, and they too arose through a combination of ancient (pre-1R) individual gene duplications and the first round of WGD.

The existence of these multiple isoforms underlies some marked differences in rod/cone manifestations in a variety of monogenic retinal disorders.

Recently, it has become apparent that the dimeric nature of the rod PDE6a/b plays an important role in enabling the rod to operate at very low intensities. Activation of the two catalytic subunits exhibits significant cooperativity, in that a single bound transducin elicits only a small fraction of the activity elicited when two transducins bind. This cooperativity provides immunity against spontaneous thermal activation of transducin, because appreciable PDE6 activity occurs only upon a coordinated burst of transducin activation. Such a noise-reduction mechanism appears crucial in enabling the rod to respond reliably to a single photon. However, this benefit comes at the cost of a short delay (of ~5 ms); this is probably immaterial to rods, but would be a severe disadvantage if it were to occur in cones which mediate responses at high temporal frequencies. Analysis of the dimeric PDE6 activation scheme provides a quantitative explanation for rod recovery from intense

flashes, and for the occurrence of an increase in the rod's 'dominant time constant' at high intensities.

Reference 1 :- Lamb TD (2020). Prog. Ret. Eye Res. 76, 100823.

SA55

Monogenic retinal disease: mechanisms and the landscape of novel therapies

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Inherited retinal diseases associated with single gene disorders affect about 1 in 4,000 people and although individually rare, as a group are the commonest cause of severe visual impairment in the working age population. The final common pathway to blindness is impaired function or death of photoreceptors. There is considerable phenotypic and genetic heterogeneity with variable visual prognosis. Some disorders present in infancy with severe visual impairment whilst in other conditions the first symptoms appear in adult life. In most cases the disease is confined to the eye, but the underlying genetic mutation(s) may result in syndromic disorders with additional involvement of other organs.

There have been a number of scientific advances that have had a major impact on the field in recent years. These include improved retinal imaging, including the ability to image individual photoreceptor cells in the human eye, next generation sequencing which has allowed a precise molecular diagnosis in the majority of patients and animal and iPSC models of disease which can be used to explore potential therapeutic approaches. Recently the first gene replacement therapy for an infantile onset retinal dystrophy has been approved by the FDA and is in current clinical practice. A number of other gene based clinical trials are underway.

This brief talk will discuss how precise molecular diagnosis and deep phenotyping has led to improved understanding of molecular pathogenesis and informed the development of clinical trials of novel therapies.

SA56

Restoring vision after photoreceptor degeneration using optogenetic techniques

Samantha De Silva^{1, 2, 3}

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Inherited retinal degenerations such as retinitis pigmentosa (RP) affect approximately 1 in 3000 people and are the leading cause of blindness in working age adults in England and Wales. In these, typically monogenic, conditions there is progressive degeneration of photoreceptors, however inner retinal neurons such as bipolar cells and ganglion cells remain largely structurally intact, even in end-stage disease.

Optogenetics is a method of neuromodulation that has wide applicability in neuroscience, and utilises light to activate neurons that have been engineered to ectopically express a light-sensitive protein. Given this naturally occurs in the eye when light triggers phototransduction in rods and cones, an intuitive application of optogenetic techniques would be to induce light sensitivity in remaining retinal cells and restore vision in end-stage retinal degenerations.

Developing an effective optogenetic approach requires consideration of multiple factors including the light-sensitive protein that is used, the method of gene delivery and the target cell for expression. A range of photosensitive proteins have been investigated including microbial opsins e.g. channelrhodopsin and halorhodopsin; mammalian opsins e.g. rhodopsin, cone opsin and melanopsin, and engineered ion channels. These vary in sensitivity, kinetics of their response and wavelength of light causing peak stimulation.

Gene therapy using adeno-associated viral vectors (AAV) has been extensively investigated for gene transfer to the retina, with the first gene therapy now licensed for the treatment of a rare retinal disorder. Modification of surface amino acid residues on AAV vectors, the promoter driving gene expression and administration via subretinal or intravitreal injection, all impact vector efficacy and which retinal cell type expresses the light sensitive protein. This in turn influences potential visual output: for example transducing bipolar cells, the most distal remaining neuron in visual circuitry in end-stage RP, may preserve processing of signals within the retina, but thus far these cells have proven most difficult to target.

In this talk, we will explore different optogenetic approaches with a focus on human melanopsin gene therapy (De Silva et al, 2017*), and the current clinical trials now underway to evaluate optogenetic gene therapy in reversing visual loss in end-stage retinitis pigmentosa.

Reference 1 :- *De Silva SR. et al, (2017), Proceedings of the National Academy of Sciences, 114, 11211 - 11216

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Placental endocrine insufficiency programs atypical behaviour in mothers and their offspringDavid Harrison¹, Hugo Creeth¹, Hannah Tyson¹, Raquel Boque Sastre¹, Anthony Isles¹, Rosalind John¹¹Cardiff University, Cardiff, United Kingdom

Fetal growth restriction (FGR) is a major threat to human health impacting neonatal survival and increasing the risk of behavioural and metabolic disorders later in life, a phenomenon described as fetal programming or developmental origins of disease. Higher than normal placental expression of the imprinted gene *PHLDA2* has been reported in a number of studies of FGR, fetal death and low birth weight. We modelled this specific alteration and demonstrated that two-fold increased expression of *Phlda2* is sufficient to reduce birth weight by 10% followed by rapid postnatal catch-up (1). We further showed that *Phlda2* functions to negatively regulate the endocrine compartment of the placenta (2) and, through this function, influences the behaviour of wild type mothers towards their offspring (3). Being born small and being exposed to suboptimal maternal care are both risk factors for behavioural disorders in human populations. We therefore undertook a behavioural and molecular characterisation of *Phlda2* transgenic and non-transgenic littermates alongside concurrently bred fully wild type cohort. Offspring exhibited increased anxiety-like behaviours, deficits in cognition and atypical social behaviours alongside changes in the transcriptional signatures of key brain regions with males relatively more impacted than female (4). Our work establishes, for the first time, the experimental paradigm that placental endocrine insufficiency programs atypical behaviour in both mothers and their offspring. Importantly, we have evidence that this mechanism may contribute to the co-occurrence of low birth weight and perinatal mood disorders in human populations.

Reference 1 :- Isolating the role of elevated *Phlda2* in asymmetric late fetal growth restriction. Tunster, SJ, Van De Pette, M, and **John, RM**. *Dis Model Mech*. 2014 Oct;7(10):1185-91.

Reference 2 :- The imprinted *Phlda2* gene modulates a major endocrine compartment of the placenta to regulate placental demands for maternal resources. Tunster SJ, Creeth HD, **John RM**. *Dev Biol*. 2016 Jan 1;409(1):251-60.

Reference 3 :- Maternal care boosted by paternal imprinting in mammals. Creeth, HDJ, McNamara, GI, Tunster, SJ, Boque-Sastre, R, Allen, B, Sumption, L, Eddy, JB, Isles, AR and **John, RM**. *PLOS Biology*. 2018 Jul 31;16(7):e2006599.

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SA58

Development of the gut microbiome in early life

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Following birth, neonates are rapidly colonized by microbes, which play important roles in health and disease. The gut contains the largest density of microorganisms, termed the gut microbiome, which plays fundamental roles in protection from pathogens, immune system training, and the breakdown of dietary compounds. Our research has shown that over the first year of life the infant gut microbiome is highly dynamic, providing a window of opportunity in which to seed a potentially beneficial microbiome to reduce the risk of early- and later-life disease risk. For instance, in a recent study of term infants, we showed that birth mode and breastfeeding are the most important variables for shaping the early life microbiome, which are directly correlated to an increased risk of obesity, allergy, asthma, and other disorders later in life. This, early life host-microbiome crosstalk and immune development is hypothesised to have important roles in long-term health.

Our research has made important advances in the role of the preterm infant microbiome in health and disease. Unlike infants born at term, extremely preterm infants (<32 weeks gestation) have immature intestinal architecture and an underdeveloped immune system. They are also less likely to be vaginally delivered and breastfed, and they receive limited exposure to microbes during the first months of life, leading to a reduction in potentially beneficial bacteria. Because the preterm gut can become leaky, translocation of microbes into the bloodstream and/or intestinal cell death represent major problems in this vulnerable population. However, evidence from my group and others has shown that certain types of bacteria, such as *Bifidobacterium*, may increase gut and immune maturation. The latest work from my group further shows specific components of human milk are linked to colonisation by *Bifidobacterium* which together contribute toward the health or disease of an infant.

While such associations provide insights, it is not possible to determine cause or effect. To advance this work, we have recently developed a novel model derived from primary human intestinal organoids that accurately recapitulates physiologically relevant oxygen conditions. This allows the co-culture of intestinal organoids with enteric anaerobic bacteria (i.e., oxygen sensitive bacteria that reside in the gut lumen). A better understanding of the interaction between bacteria and infant gut epithelial cells holds incredibly exciting possibilities to better predict, diagnose, and manipulate the microbiome of preterm infants at risk of disease.

OC01

Perivascular excitation tunnelling as a novel mechanism of cardiac reperfusion arrhythmias

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Introduction: Reperfusion after myocardial ischaemia can lead to fatal arrhythmias, in part due to heterogeneities in electrophysiology (EP) across affected tissue. Understanding the spatiotemporal dynamics of this pathophysiological cardiac electrical behaviour may improve outcomes.

Objective: Elucidate EP mechanisms underlying ischaemia-reperfusion arrhythmias.

Methods and Results: In experiments involving panoramic optical mapping of transmembrane voltage of Langendorff-perfused rabbit hearts, a region of the left ventricle was independently perfused by cannulation of the anterior branch of the left circumflex coronary artery with physiological solution, which was switched to and from no-flow ischaemia, simulated ischaemia solution, or a solution that mimicked a specific aspect of ischaemia (hyperkalaemia, hypoxia or acidosis). When local no-flow, simulated ischaemia, hyperkalaemic or hypoxic solution (n = 6, 22, 8, 5, respectively) was switched to physiological perfusion, we observed preferential recovery of electrical excitability of myocardium along the main branch of the perfused coronary vessel ('perivascular excitation tunnelling', PVET). In a subset of hearts, PVET resulted in re-entrant arrhythmias. In contrast, local acidosis experiments showed no loss of excitability and subsequently no PVET (n = 5). Assessment of tissue perfusion showed that myocardium closest to major arteries was first perfused and likely underlies the surprisingly fast recovery of excitability in perivascular myocardium. Data were used to inform a computational model of ischaemia-reperfusion to further explore mechanisms of arrhythmia inducibility. The computational model reproduced PVET and illustrated the quantitative plausibility of PVET as a cause for re-entry. Simulations predicted that step-wise reperfusion strategies could reduce EP heterogeneity and thus vulnerability to arrhythmias, however experimentally step-wise or gradual reperfusion (n = 5 and n = 2) increased the heterogeneous period, resulting in increased arrhythmogenesis.

Conclusions: We observed a novel PVET-based re-entry mechanism upon coronary reperfusion, suggesting that detrimental reperfusion-induced gradients arise in the myocardium along coronary vessels. Initial step-wise or gradual recovery of normal perfusion tests were not less arrhythmogenic than instant recovery of physiological flow. Further research is required to identify 'smart reperfusion' approaches favourable in the clinical setting.

Acknowledgements :- SFB1425

OC02

***In silico* investigation of interactions between antiviral agents repurposed for COVID-19 and the hERG potassium channel.**

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Although vaccines are of major importance in the battle against the Sars-CoV-2 virus responsible for COVID-19, issues including vaccine hesitancy, availability, virus evolution and incomplete vaccine effectiveness mean that effective treatments are also required. There is currently no Sars-CoV-2 specific antiviral treatment, but a number of existing antiviral agents are under investigation for repurposing as Sars-CoV-2 treatments. Amongst these are remdesivir and favipiravir (Wadaa-Allah et al, 2021) and the ribonucleoside analogue molnupiravir (also known as EIDD-1931; Sheahan et al, 2020) which is currently in human trials (NCT04405739 and NCT04405570). Concerns have been raised about potential risks of arrhythmia, including drug-induced QT interval prolongation, in COVID-19 patients (e.g. Carpenter et al, 2020). As the cardiac hERG potassium channel is a major culprit in drug-induced arrhythmia, the purpose of this investigation was to determine in silico the ability of remdesivir, favipiravir and molnupiravir to interact with known drug binding determinants in the hERG channel pore region. Established computer models of the pore domain of hERG (Helliwell et al, 2018; Dickson et al, 2020) were employed and antiviral molecules were docked using GOLD v2020.1 and GOLD version 5.6. The side chains of S6 domain aromatic residues (Y652 and F656) were unconstrained during docking simulations. Rotamer sampling was maximally set to 300,000 generations. Remdesivir could be accommodated in the open state but not closed state channel models. In open channel models, remdesivir could interact with F656, Y652 on the S6 domain and L622 and S624 residues near the channel selectivity filter. Additionally, part of the remdesivir molecule could advance towards lateral side pockets and interact with the F577 S5 residue. Favipiravir is a much smaller molecule and could be accommodated in both open and closed channel models and interact with known binding residues. However, its small size meant that it made comparatively few simultaneous binding contacts in the open state and weak binding contacts in the closed state. Molnupiravir is intermediate in size between favipiravir and remdesivir. When docked to the open hERG structure it was able to make favourable interactions with the S6 aromatic residue Y652 and with residues under the selectivity filter (e.g. S624), but was unable simultaneously to access lateral side pockets and the F577 residue. Molnupiravir could be accommodated in the central cavity of the closed hERG model, however the fit was relatively poor with a potential to interact with residues under the selectivity filter and contact both the S6 aromatic residues: Y652 and F656. The results of this in silico study indicate that all 3 antiviral agents studied can interact with hERG channels; the small size of favipiravir makes it likely to have reduced hERG liability. These in silico findings provide a rational basis for further in vitro and in vivo investigations in order to compare the ability of these agents to produce inhibition of hERG channel current and delay ventricular repolarization.

Acknowledgment

The authors acknowledge research funding from the British Heart Foundation (PG/17/77/33125, PG/17/89/33414 and PG/20/10252).

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OC03

Gain of function mutations in the human ether-a-go-go-related gene (hERG) act to increase cell surface expression of the channel complex

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Long QT syndrome (LQTS) and short QT syndrome (SQTs) are arrhythmogenic disorders, characterised by altered cardiac action potential (AP) repolarization and subsequent risk of arrhythmia and sudden death. The *human Ether-à-go-go-Related Gene (hERG/KCNH2)* encodes the pore forming subunit of channels carrying the 'rapid' delayed rectifier current I_{Kr} , a key constituent of AP repolarization. Loss of function (LOF) mutations in *hERG* act to delay repolarization and cause LQTS type 2 (LQT2) (Schwartz et al, 2009). Conversely, gain of function (GOF) *hERG* mutations act to accelerate repolarization and underlie SQTs Type 1 (SQT1) (Hancox et al, 2018). LOF *hERG* mutations can reduce I_{Kr} current via different mechanisms: abnormal transcription/translation, defective protein trafficking, defective channel gating and/or altered ion permeation (Delisle et al, 2004). For LQT2 the most common mechanism is defective trafficking (Anderson et al, 2014). SQT1-causing GOF *hERG* mutations have been shown to modify the kinetic properties of I_{Kr} (Hancox et al, 2018), but their effects on trafficking are not well established.

To investigate whether GOF *hERG* mutations can alter hERG channel trafficking we utilised *LI-COR*[®] based 'On-Cell' and 'In-Cell' Western assays to quantitatively measure the effects of two GOF mutations (N588K and T618I) and two LOF mutations (G601S and A561V) on the cell surface 'On-Cell' and total 'In-Cell' expression level of the hERG1a channel in transiently transfected Human Embryonic Kidney 293 cells. The trafficking status of mutant hERG channels was assessed under both homozygous and heterozygous expression to mimic patient phenotype. For full methodological details of this assay please refer to (Al-Moubarak et al, 2020). Data are from five independent repeats (n=5) and are presented as mean $\pm$ S.E.M. Statistical analysis was performed using One Way ANOVA and a Dunnett's multiple comparison test. Our data show that the GOF SQT1 causing mutants N588K and T618I significantly increased cell surface expression of the hERG channel when compared to the wild-type (WT) level (homozygous: 1.05 ± 0.12 arbitrary fluorescent units ($\times 10^{-7}$) and heterozygous: 1.09 ± 0.13) under both homozygous (N588K: 156.5% of WT level, 1.64 ± 0.16 , $p < 0.05$; T618I: 193.3% of WT level; 2.03 ± 0.17 , $p < 0.001$) and heterozygous (N588K+WT: 139.9% of WT level,

1.53±0.11, p<0.05; T618I+WT: 152.4% of WT level; 1.66±0.10, p<0.05) expression. In contrast, the loss of function mutants G601S and A561V significantly reduced the cell surface expression level of the hERG channel under both expression states (homozygous: G601S: 32.5% of WT level, 0.34±0.04, p<0.05; A561V: 36.3% of WT level, 0.38±0.05, p<0.05) (heterozygous: G601S: 59.1% of WT level, 0.65±0.04, p<0.05; A561V: 51.6% of WT level, 0.56±0.08, p<0.05). For all mutants the total cellular hERG channel expression level was not significantly (p>0.05) different from the WT channel, indicating that these mutations do not act to perturb translation.

The alterations to the trafficking status of the hERG channel when expressed in the heterozygous state highlights that these mutants exert dominant phenotypes. In addition, our study provides evidence that augmented trafficking may be an important contributor to the disease mechanism for specific SQT1 causing GOF *hERG* mutations.

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Acknowledgements :-

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OC04

Insights into human healthy lower limb perfusion asymmetry during rest

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Introduction

Perfusion differences related to posture/position and arterial blood pressure (BP) in the upper and lower limbs are long known. Some pathologies, such as critical limb ischemia, diabetes, and peripheral vascular disease, accentuate these differences. Variations between left and right lower limb perfusion during rest were reported in healthy subjects in the upright position. Although poorly understood, recent studies have suggested that anatomical and morphometric asymmetries of

femoral arteries in paired legs could explain these haemodynamical differences. Our recent observations suggest that these might result from various factors other than BP.

Aims/objectives

To analyze the association of sex, age, and body mass index (BMI) with perfusion asymmetries of the lower limbs.

Methods

Perfusion was measured with LDF (laser Doppler flowmetry) at the 3rd metatarsophalangeal dorsal region in both feet. 139 healthy participants of both sexes were recruited. Age, BP (systolic – SYS; diastolic – DIA), height, and weight were taken. Measurements were obtained in supine and upright position. Procedures respected all the principles of good clinical practice for human studies research.

Participant data was grouped into Young (< 30 years) and Older Adult (> 30 years old), and BMI categories (Normal weight < 25.0; overweight/obese $\geq$ 25.0). As 99.3% (138/139) of participants presented different LDF values for each limb, LDF ratios between the “dominant foot” (i.e., higher LDF value) and “contralateral” (i.e., lower LDF value) were calculated. The mean LDF value of each individual was also determined, and a new categorical variable was designed, dividing the individuals into mean LDF quartiles (Q1 to Q4). Statistical analysis was performed with SPSS (v.22.0). Parametric or non-parametric tests were performed to assess differences between variables. A 95% level of confidence was adopted.

Results and Discussion:

Both men and women were of similar age (Mann-Whitney; $p=0.846$). 67.6% of all participants exhibited a BMI lower than 25 kg/m². SYS was higher in men (t-test; $p=0.048$), but no differences between sexes were found in DIA nor in BP between age groups. Participants with higher BMI had higher DIA ($p=0.003$). The ratio between limbs showed wide intra-individual differences (0 to 678% Median: 28.36; IQR 11.19 – 59.87). Both sexes had similar ratios, while older adults and normal BMI individuals had lower ratios ($p>0.05$). Individuals in the supine position had significantly higher ratios ($p=0.002$).

Considering mean LDF for perfusion assessment, significant differences were observed regarding sex (higher in males, $p=0.013$), age (higher in adults, $p<0.0001$), and BMI (higher in overweight, $p<0.0001$). Logistic regression models showed that BMI had a higher impact than sex or age on perfusion (Q4/Q1 – aOR 4.37 [1.13 – 16.95; $p=0.032$]). Moreover, regression models adjusted for BP, sex, age and BMI, confirmed that the body position was associated with higher ratios between limbs (High/Low – aOR 2.76 [1.27 – 5.99, $p=0.010$]).

Conclusions

Our data suggests that body position and BMI are the most important determinants for these resting lower limb perfusion differences. Future studies should focus on the relationship between dominance, perfusion and lifestyle factors with known effects e.g. tobacco use and exercise.

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OC05

Dimethylarginine dimethylaminohydrolase levels in blood outgrowth endothelial cells

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Introduction.

Dimethylarginine dimethylaminohydrolase (DDAH) is a major endogenous regulatory enzyme controlling asymmetric dimethylarginine (ADMA) levels. ADMA, which inhibits nitric oxide synthase (NOS), is a mediator elevated in numerous disease states and several diseases associated with vascular dysfunction have been associated with aberrant levels of DDAH (Arrigoni et al., 2003; Palm et al., 2007). Investigating the endothelial cell (EC) function of individuals and assessing the nitric oxide (NO) pathway including the DDAH pathway, has been limited to invasive techniques and animal studies. Recent developments in culture techniques to extract progenitive endothelial cells from whole blood, known as blood outgrowth endothelial cells (BOEC) or endothelial colony-forming cells (ECFC), means that endothelial cells can be isolated in a non-invasive manner from a relatively small amount of donor's blood and represent an individual's epigenetic makeup (Paschalaki et al., 2013). We therefore sought to measure DDAH and NOS mRNA in these cells to see if this was a viable model for assessing the NO pathway.

Methods.

The study was approved by the Kingston University Faculty Research Ethics Committee (Reference 1617/024). Blood outgrowth endothelial cells (BOECs) were isolated and cultured from male and female adult healthy donors (n=5). BOECs were isolated as previously described until the formation of a characteristic cobble-shaped morphology (Ormiston et al., 2015). BOECs were confirmed to have classical EC surface marker expression (CD31, CD144, low CD34) using flow cytometry. Extracted BOECs and HUVECs (Sigma-Aldrich) were cultured under standard conditions and plated for 2-3 days, until confluent and treated under control or inflammatory conditions (TNF- α , 10ng/ml) for 24 hours. RNA was extracted using TRIzol™ Reagent (Invitrogen) and cDNA synthesis performed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). qRT-PCR was executed using primers for *NOS2*, *NOS3*, *DDAH1* and *DDAH2* mRNA, with *ACTB* and *GAPDH* used as reference genes. Analysis used the $2^{-\Delta\Delta Ct}$ method for quantification.

Results.

NOS3 expression was identical between HUVEC and BOEC and decreased following inflammatory stimulation to an equivalent level in both cell types (BOEC $p=0.0275$ and HUVEC $p=0.0367$, $n=5$). *NOS2* was not detected under quiescent conditions or following inflammatory stimuli.

[DDAH2 mRNA levels were greater than DDAH1 in both BOEC and HUVEC \(DDAH2>DDAH1 BOEC 15.2x \$p=0.0001\$, HUVEC 4.7x \$p=0.0248\$ \).](#) Under quiescent and inflammatory conditions, *DDAH1* gene expression did not differ between BOEC and HUVEC. However, there was 2.9x more *DDAH2* gene expression in BOEC than HUVEC at rest ($p=0.0489$, $n=5$) and following incubation with TNF- α , *DDAH2* mRNA increased in HUVEC so that the two cell types expressed equivalent amounts.

Conclusions.

This is the first time that DDAH expression has been examined in BOEC. BOEC express more *DDAH2* than HUVEC under resting conditions with both cell types expressing more *DDAH2* mRNA than *DDAH1*. There was no difference in the expression of *NOS3* in either EC type. *DDAH2* is known to contribute to endothelial cell NO bioavailability, and as an endothelial cell model, these cells may provide better insight into an individual's endothelial health than HUVEC.

Keywords.

BOEC; endothelial colony-forming cells; ECFC; DDAH; Nitric oxide; NO.

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Acknowledgements :- This work was funded by Animal Free Research UK.

OC06

Circadian clocks in diabetic retinal endothelial cells

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Background: Diseases such as diabetes have been reported to disrupt circadian rhythms and circadian disruption emerges as an important factor in the prognosis of disease outcomes and treatment success. Our objective was to investigate whether diabetes affects circadian gene expression in endothelial cells and the mechanisms involved in this.

Methods: Induced Pluripotent Stem Cell-Derived Endothelial Cells (iPS-ECs) from healthy and diabetic patients were sequenced and differential analysis was performed. Genes related to circadian rhythms were identified. Primary human retinal endothelial cells (HRECs) were cultured in vivo in hyperglycaemic and hypoxic conditions to validate the results. Cells were synchronised with 50% serum shock and repeated samples collected every 2 hours over a 36 hour period. Circadian gene expression was measured using RT-PCR.

Results: ip-ECs from diabetic patients had a 5.7 fold reduction in *Dec2* mRNA expression and a 4.0 fold increase in *Bmal-2*. Synchronised HRECs under hypoxic conditions showed a more robust circadian oscillation but lower amplitude of *Bmal-1* and *Cry1* mRNA expression, indicating an effect of hypoxia on circadian rhythmicity. Four weeks of hyperglycaemic conditions resulted in a slight increase of *Bmal-1* and a reduction in *Cry1* and *Cry2* mRNA expression. Expression of *Dec2* was most affected by hyperglycaemia. Hypoxia had a significant effect in reducing the expression of the majority of circadian genes.

Conclusions: Diabetic conditions resulted in a specific reduction of *Dec2* expression in both patient derived iPS-ECs and HRECS in hyperglycaemic conditions. Hypoxia alone had a more pronounced effect on circadian gene expression and rhythmicity compared to hyperglycaemia alone.

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OC07

Flipped classroom and small-group teaching in physiology: complementary or redundant?

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Physiology teaching at the University of Cambridge has historically involved whole-cohort lectures, supplemented by weekly, small-group teaching sessions called ‘supervisions’. Supervisions typically include 2-4 students led by an academic specialist, who asks questions and sets problems to solve. ‘Flipped-classroom’ teaching (FCT) similarly involves prior preparation of course material, followed by students working on tasks set by the instructor within the class itself. Active learning of this nature, requiring students to think through problems, ought to be particularly beneficial in physiology, a subject in which the underlying concepts can be challenging, and the implications of dysfunction complex and profound. Indeed, FCT has previously been used in physiology teaching at other universities, leading to improved exam performance, but with mixed results in terms of student satisfaction (e.g. Street et al., 2015; Entezari and Javdan, 2016; Rae and O'Malley, 2017). Because Cambridge students regularly receive opportunities for active learning in a small-group context, we wanted to establish whether they would perceive FCT as redundant.

We introduced FCT to three undergraduate lecture series in digestive physiology. A three-lecture pilot course given to 187 first-year biologists in 2018 was followed by six-lecture courses to 386 first-year medical and veterinary students, and to 39 second-year biologists, the following academic year. Students were given material to prepare in advance. In the live presentations, they were given group tasks including multiple-choice questions, calculations, compare and contrast, and working out the clinical implications of certain conditions. Student feelings about the new style of teaching were assessed through questionnaires. In order to avoid leading the participants in their answers, they were asked more general questions about learning and understanding, backed up with the opportunity for open comments. The overlap between flipped-classroom teaching and supervisions was explored in detail through interviews.

Feedback on the 'flipped' courses was very positive, the mean scores for all three cohorts showing that students generally believed that they had learned more, understood more and felt better-prepared for the exams, in comparison with traditional lecturing. The main complaint related to a perceived increase in preparation time, as Rae & O'Malley (2017) had also found. Strikingly, only 13 of the 265 completed questionnaires even mentioned supervisions, many of these referring to the continued need for small-group support. While students recognised the parallels between the two teaching methods when prompted in the interviews, they explained that the intensive nature of small-group teaching felt very different to working anonymously within a large lecture theatre.

Despite the similarities in the active learning exercises that might be set in small-group and FCT sessions, we conclude that they represent very different experiences for the students involved. There is no evidence to suggest that our students found any redundancy between these types of teaching. As a result, we adapted flipped-classroom physiology teaching to an online format for this academic year, and it seems likely to be rolled out more widely in this university in the future.

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OC08

Reaching Consensus on the Core Concepts of Physiology using the Delphi Method and Development of an Assessment Framework

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There are a set of core concepts ('big ideas') that are central to the discipline of Human Physiology and thus important for students to understand and demonstrate their capacity to apply the knowledge. However, a preliminary study indicated poor mapping of an existing set of core concepts (1) in subject learning outcomes across physiology curricula in undergraduate degree programs across Australian universities.

The first aim of this project was to reach Australia-wide agreement on the core concepts of physiology using the Delphi method – an iterative process that explores agreement and disagreement amongst participants to achieve representative consensus. In the first phase, physiology educators from 25 of 40 Australian universities agreed to be part of a Task Force, which agreed on seven core concepts of physiology. In the second phase, national consensus was reached on the seven core concepts following a survey to physiology educators across Australian universities of which 125 responded. The agreed core concepts are currently being 'deconstructed' into subsidiary sub-concepts.

The second aim of the project is to incorporate the agreed core concepts into an Assessment Framework which will improve assessment of learning outcomes. This is a pressing concern - in a pilot study, expert analysis of high stakes assessments from various Australian universities has indicated a pre-occupation with testing mainly discipline knowledge. To move beyond assessment of memorised material (itself important) to analytical, practical and creative aspects of science, we have developed an assessment framework based on the Organisation for Economic Cooperation and Development's Programme for International Student Assessment (PISA) 2015 Science framework (2). The framework maps assessment to the student's ability to explain phenomena, interpret data, and evaluate, design and conduct scientific enquiry. We have incorporated the agreed core concepts into this framework, which also maps against threshold learning outcomes and subject learning objectives. The complete framework can then be used to design assessment that not only measures knowledge gain, but also conceptual knowledge, higher-order thinking and the ability to practice science. To date, we have trialled assessments against the Assessment Framework with consistent results that it is achieving its goals - highlighting gaps and providing critical feedback to those involved in the design of assessments.

In conclusion, we have reached national agreement on the core concepts of physiology, which when embedded will provide some standardisation across undergraduate physiology curricula across Australia. In turn the Assessment Framework will provide essential information on current assessment practices and drive a more sophisticated understanding of student achievement, while at the same time, building assessment capability and improving teaching and learning practices in physiology.

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Acknowledgements :- This work was partially funded by the David Jordan Teaching Excellence Award from The Physiological Society UK (2019) to Dr Kathy Tangalakis.

OC09

Enhancing interactivity within multimodal physiology classes: Student perceptions of Kahoot! quizzing between online and face-to-face sessions.

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Recently, many tertiary institutions have been rethinking the way courses are presented to students. This has resulted in subjects, previously taught as face-to-face, moving to a blended or multimodal delivery. This has placed various challenges on tertiary educators who have needed to rapidly seek learning activities that can remain effective, regardless of whether students are attending in-person or online (1, 2). One such method, interactive quizzing, is a tool that could facilitate this requirement. This study aimed to compare learner perceptions of the interactive quizzing platform Kahoot! when used in either a face-to-face or online setting within a health sciences and medical course. A total of 174 first-year health sciences and medical students from an Australian university enrolled in this study. Two study groups were formed based on whether the participants were enrolled in a face-to-face (n = 72) or online (n = 102) provision of their subject. Participants attended a one-hour physiology lecture, either in a face-to-face class or online during live sessions, then completed a 10-item Kahoot! interactive quiz based on the session content. Following the provision of the quiz, participants completed a four-question Likert scale survey related to their experiences and provided written responses to three open-ended questions regarding their perceptions of using the interactive quizzing platform. Overall, participants in both the face-to-face and online learning groups highly rated their learning experience using interactive quizzing. There were no significant differences (Student's two-tailed t-test) between experiences from using Kahoot! during a face-to-face or online session. In particular, responses from the face-to-face and online participants on the Likert scale (1 = strongly disagree, 5 = strongly agree) for the statement "I enjoyed using Kahoot!" were 4.71 ± 0.54 and 4.81 ± 0.68 , respectively ($p = 0.3$). Three overall themes emerged from qualitative analysis of student perceptions that were comparable between the two groups. These themes were (1) interactive quizzing is enjoyable, (2) interactive quizzing is engaging, and (3) interactive quizzing helps my learning. Participants utilising interactive quizzing in an online setting reported increased engagement whilst learning due to the fun, eye-catching environment and interactive nature of the platform, despite being isolated or not in the same room as their cohort. This study identifies Kahoot! as a teaching tool that is equally effective regardless of whether the students attended either face-to-face or online. With many tertiary institutions currently split between online, face-to-face or mixed-mode curricula, and an increasing reliance upon virtual and online resources (3-5), it is increasingly important to highlight technology that can be rapidly and easily utilised to suit all students equally within multimodal classes.

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OC10

Students' Experiences and Perceptions of Physiology Online Learning amid the COVID-19 Pandemic in Nigerian Universities

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Introduction:

The emergence of the novel COVID-19 outbreak had significant effects on human endeavors globally. During the COVID-19 pandemic, several institutions and medical schools globally transited to online learning, as evident in few Nigerian Universities (1).

Objective:

This study explores the experiences and perceptions of medical students regarding Physiology e-learning amid the COVID-19 lockdown.

Methods:

An online descriptive, cross-sectional study was conducted between July and September 2020. Current undergraduate medical students aged 17 years or older across five private-owned Universities who transited to virtual learning constituted the study population. Using Google forms, a web-based questionnaire was administered to participants via WhatsApp messenger. The questionnaire was developed using validated questions from previously published studies.

Results:

Overall, 200 participants responded (response rate = 65%). Of the 190 valid responses, 48 (25.3%) were males, and 157 (82.6%) were in the age bracket of 17-20 years. Of the 190 participants who engaged in learning Physiology online during the lockdown, 78 (41.1%) were pursuing the Bachelor of Medicine and Bachelor of Surgery (MBBS) program, 69 (36.3%) Bachelor of Pharmacy (B.Pharm), while 16 (8.4%) were studying Bachelor of Science in Physiology (Majors; B.Sc.). More than half (n = 122, 64.2%) of the study participants agree to actively participate in online classes, 68 (35.8%) had a suitable home environment for online learning, 32 (16.8%) agree that the quality of online teaching equals classroom teaching, while 36 (19%) students are satisfied with learning Physiology online. Furthermore, 135 (71.1%) participants prefer mornings as their learning periods compared to afternoons, 149 (78.4%) supplement the online lectures with further private study, while only 46 (24.2%) students agree that online lectures only will sufficiently equip them with required Physiology knowledge.

Conclusion:

This study is imperative due to the novelty of the online learning mode in Nigeria. Thus, there is a need to encourage the consideration of adopting and integrating blended (online) learning in Physiology education in Nigeria.

Keywords: Physiology education; COVID-19; Pandemic; Nigeria; Online learning

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OC11

The Capstone Experience: Better preparing physiology graduates for the diversity of careers they go onto.

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Historically physiology students have undertaken a laboratory-based, fieldwork or literature project as their final year undergraduate research or Honours project, a requirement of the QAA Biosciences Benchmark statement. However, very few students are going onto careers in research. Our aim therefore, was to develop alternative educational experiences to research projects, which better prepared our students for the diversity of careers they go onto.

We have adopted and implemented capstone experiences, a high impact education practice¹, with its focus on student personal and professional development rather than gaining research experience.

Over the last 15 years we have progressively developed, in a collaborative partnership with students and external partners, a sector-leading portfolio of traditional research projects offered alongside science or industry-focused capstones (e.g. stakeholder opinion, commercial reports, and those with a civic or societal focus (e.g. science in schools, educational development), in the same module or course². Students select the project that best addresses their individual developmental needs and/or future career intentions. By offering a broad inclusive portfolio of opportunities, each developing different skills and attributes, there is something for every student, an opportunity to excel irrespective of background. To enable them to fully showcase both their project outputs, and their knowledge, skills and understanding to us as educators, potential employers and most importantly themselves, we give them free choice of their primary assessment tool e.g. an academic paper, commercial report or reflective e-portfolio.

Students have wholeheartedly grasped this opportunity, excelling academically. Their module marks are significantly higher than students undertaking traditional research projects (2020: mean $\pm$ SD = 71.4 $\pm$ 4.4% vs 68.4 $\pm$ 5.8%, $p < 0.05$, capstone vs research). In 2020-21, 27% selected capstones as their first choice of project, a massive cultural shift given laboratory projects have traditionally been viewed as the “gold-standard”. Similarly, 44% of educators contributing to the module mentor one or more capstones.

Our work has had significant impact beyond our Institution, leading to revision of both the Royal Society of Biology³ and the Institute of Biomedical Sciences’ project accreditation criteria. To support colleagues globally during the 2020-21 Covid pandemic, we delivered online workshops and created guides for both students and educators^{4,5}. These have had 11,000 views from over 50 Countries in 9 months.

This case study has showcased the potential of capstones to provide an inspirational, transformative and translational educational experience for students. However, it is only the start of a journey. Capstones are traditionally conservative in nature, a taught course, extended essay or senior seminar series. In order for educators globally to fully realise the transformative and translational potential of capstones, and develop global graduates equipped with the skills and attributes to become leaders in whatever field they go onto, we collectively need to engineering in additional purposes e.g. interdisciplinarity, global grand challenges, trans-national educational experiences.

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Reference 2 :- Lewis DI (2020) *Physiology News* 119: 10-11.

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Reference 4 :- Lewis DI (2020) <https://bit.ly/ChoosingBioCapstone>

Reference 5 :- Lewis DI (2020) <https://bit.ly/BiosciCapstones>

COLLABORATIVE APPROACHES TO DELIVER INTEGRATED MEDICAL TEACHING: A case study

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Integrated medical courses, in which scientific knowledge is taught alongside clinical training, have proved successful as a model for training doctors. Ideally, integrated learning is embedded throughout the curriculum; however, in practice multiple issues such as resourcing, timetabling etc., can act as barriers. In this study we describe an innovative integrated teaching block combining self-directed online learning with tutor lead, hands on practice. The module was conceived partly to mitigate for teaching disruption caused by the COVID pandemic and optimise learning opportunities. The module aimed to develop clinical reasoning skills and highlight the reliance that these skills have on an understanding of the underlying medical sciences. Interactive online cases containing videos of simulated clinical scenarios that integrated content across Clinical Skills, Anatomy and Physiology were created. Key themes taken from teaching of the respiratory, cardiovascular, and abdominal systems were included. The session was delivered to year 1 of the MBBS programme at Barts and the Royal London, QMUL (cohort size of approximately 400 students). Students were provided with the opportunity to critically evaluate the online scenarios and then apply their knowledge in small group, tutor lead practical sessions. Module evaluation was collected across the whole cohort. The feedback questionnaire asked students to rate statements on a five-point scale from completely agree to completely disagree. Results revealed that overall, 89% of student found the session extremely useful, with the remainder scoring it as useful (Figure 1).

A total of 93% of students agreed that integrated teaching block helped to link an understanding of anatomy, physiology and clinical skills when interpreting clinical scenarios and 82% agreed that the cases helped to put the learning into context. Students were asked to comment on what was particularly good about the Integrated Teaching Block. The quantitative data was supported by responses from individual students.

“the clinical skills, anatomy and physiology sessions were all linked by scenarios which helped gain an overall understanding of the modules.”

“I loved how seamless the integrated teaching week was where anatomy, physio and clinical skills are intertwined appropriately w the scenarios given”.

“I gained so much more confidence by being able to learn clinical skills in person, as I feel like I can now link anatomy and physiology to an actual skill and being able to examine another person.”

When asked what could be improved most students felt that more sessions could have been provided. Building on the success of this module and lessons learned, we are applying a similar approach in year 2 of the medical course. The pandemic has provided significant challenges to delivering practical teaching, especially to large cohorts. However, by working across disciplines we show that it is possible to provide valuable blended learning opportunities for medical students.

Overall how useful was the Integrated Teaching Block?

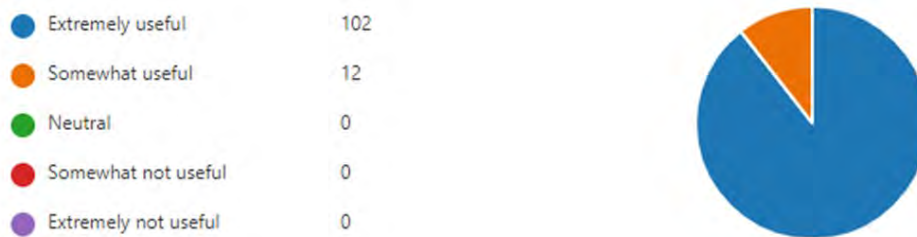


Figure 1: Student evaluation of the usefulness of the year 1 integrated teaching block (n=114 students).

Acknowledgements :- We would like to thank the rest of the teaching team who contributed to this work, Dr Hakim and Ms Jones, and the technical staff.

OC13

The relationship between insulin sensitivity and menstrual cycle phase is modified by BMI, fitness and physical activity: Results from NHANES 1999-2006.

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The menstrual cycle is an important biological rhythm in women of reproductive age, characterised by rhythmic variation in hormone levels. Cyclical fluctuations in hormone profiles across the menstrual cycle are associated with alterations in metabolic control [1,2] with insulin sensitivity reduced during the luteal phase [2]. However, to date, research has yielded inconsistent results.

In this study we firstly aimed to characterise the variation in insulin sensitivity and associated metabolites across the menstrual cycle. Secondly, we aimed to investigate the role of BMI, physical activity and cardiovascular fitness on variation in insulin sensitivity and associated metabolites across the menstrual cycle.

Data from 1906 pre-menopausal women in NHANES cycles 1999-2006 were analysed. Participants in NHANES completed an at-home interview and physical examination. Menstrual cycle day was retrospectively assigned using questionnaire responses to “number of days since last period started”.

All data for each participant are relevant to this single timepoint within their menstrual cycle. Rhythmic variation of plasma glucose, triglyceride, insulin, homeostatic model of insulin resistance (HOMA-IR) and adipose tissue insulin resistance index (ADIPO-IR) across the menstrual cycle were analysed using cosinor rhythmometry. Cosinor fits a cosine curve to data and calculates the MESOR (rhythm adjusted mean), amplitude (half the predictable variation within a cycle) and acrophase (time of highest value within a cycle). Participants were assigned low or high categories of BMI, physical activity and cardiorespiratory fitness and category membership included in cosinor models as covariates.

Rhythmicity was demonstrated by a significant cosine fit for glucose (MESOR: $85.1 \pm$ amplitude: 1.2 mmol/L; $p = 0.014$), but not triglyceride (87.7 ± 2.8 mg/dL; $p = 0.369$), insulin (9.8 ± 0.4 mmol/L; $p = 0.470$), HOMA-IR (2.1 ± 0.1 mmol/L; $p = 0.461$) or ADIPO-IR, (9.7 ± 0.6 mmol/L; $p = 0.335$). When covariates were included, rhythmicity was observed when adjusting for: 1. BMI: glucose ($p < 0.001$), triglyceride ($p < 0.001$), insulin ($p < 0.001$), HOMA-IR ($p < 0.001$) and ADIPO-IR ($p < 0.001$); 2. Physical activity: glucose ($p < 0.001$), triglyceride ($p = 0.006$) and ADIPO-IR ($p = 0.038$); 3. Cardiorespiratory fitness: triglyceride ($p = 0.041$), insulin ($p = 0.002$), HOMA-IR ($p = 0.004$) and ADIPO-IR ($p = 0.004$). Triglyceride amplitude, but not acrophase, was lower in low physical activity category compared to high (3.1 vs 7.2 mg/dL, $p = 0.018$; 12 vs 27 d, $p = 0.675$). No significant differences in amplitude nor acrophase were observed for glucose, insulin, HOMA-IR and ADIPO-IR between respective high and low covariate categories ($p > 0.05$).

In conclusion, our study confirms previous reports showing insulin sensitivity undergoes small, yet statistically significant, rhythmic cycling across the menstrual cycle. This is the first study to demonstrate a modifying effect of BMI, physical activity and cardiorespiratory fitness on variation in insulin sensitivity and associated metabolites across the menstrual cycle. These findings demonstrate that menstrual cycle phase is an important consideration when assessing insulin sensitivity in clinical practice or research, especially in populations with high BMI or low cardiorespiratory fitness. Furthermore, this provides direction for investigation into the therapeutic benefit of targeting BMI and physical activity to mitigate disturbances in insulin sensitivity across the menstrual cycle.

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OC14

Modulation of IAPP aggregation and calcium-signaling are key mechanisms underlying Urolithin B protection against amyloidogenesis in diabetes

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Introduction: Diabetes is one of the major causes of premature illness and mortality worldwide. Disease onset and progression has been associated to intracellular aggregation of Islet Amyloid PolyPeptide (IAPP), or amylin, which impairs proper function of pancreatic beta-cells and insulin secretion. As disease progresses, amyloid deposition in the pancreas appears in 90% of individuals, constituting a histopathological hallmark of the disease. The potential inhibition of IAPP aggregation, allowing the improvement of beta-cell functionality, remains a therapeutic target yet unexplored. Diet is an essential source of bioactive compounds. Encouraged by the reported activity of dietary (poly)phenols (PP) against diabetes [1], we hypothesized that low molecular weight metabolites resulting from PP metabolism in the human body may modulate IAPP aggregation.

Objectives: Benefiting from an in-house library of predicted bioavailable PP metabolites, the objectives of this study were (a) the identification of metabolites potentially preventing IAPP aggregation and (b) the characterization of their activity towards the improvement of beta-cell function.

Methods: *In silico* testing of putative interactions of PP metabolites and IAPP (NMR structure 2L86 from Protein Data Base) was performed using Auto Dock Vina software. The best hit was assayed in cell-free systems using synthetic IAPP by means of Thioflavin-T assays and Transmission Electronic Microscopy. Metabolite-mediated protection was investigated by means of genetic (mutation analysis), biochemical (ELISA, enzymatic activity assays), cellular (fluorescence microscopy) and molecular biology (flow-cytometry, immunoblotting, RNA sequencing) approaches [2], using eukaryotic reporter systems expressing human IAPP [2,3] and INS-1 832/13 pancreatic beta-cells challenged with IAPP aggregates. Proper statistical analyses were performed using GraphPad and results of at least three biological replicates were included.

Results: Among approximately 200 PP metabolites tested *in silico*, Urolithin B (UroB) emerged as the best performing molecule interacting with IAPP. In cell-free assays, UroB interfered with the kinetics of IAPP fibril formation and modulated the size and morphology of IAPP fibrils. The cytoprotective effects of UroB were first assessed in yeast models recapitulating human IAPP aggregation and cytotoxicity [2]. UroB prevented IAPP-induced cell death, reduced the size of IAPP aggregates and decreased IAPP insoluble fractions. Mechanistic studies revealed the involvement of autophagy, cell antioxidant responses and calcium-signaling in UroB-mediated protection. Also, UroB protection in beta-cells challenged with hyperglycemia and hyperlipidemia was associated with the attenuation of oxidative stress. Furthermore, transcriptomic analysis indicated calcium-signaling as the top molecular pathway enriched in IAPP-exposed cells treated with UroB compared to the untreated control. Consistent with these data, and the key role of calcium-signaling in insulin secretion, UroB stimulated insulin secretion in IAPP-exposed cells under hyperglycemia.

Conclusions: Our data reveals UroB bioactivity for the alleviation of IAPP pathological processes by several mechanisms. UroB interacts with IAPP, and activates autophagy and antioxidant defenses to protect cells against the proteotoxic effects of IAPP accumulation. Noteworthy, UroB modulates calcium-signaling as an important intervenient in insulin secretion. The promising

results, which still need to be validated in more complex systems, open a new venue for the exploitation of dietary urolithins as inhibitors of IAPP aggregation with potential implication for diabetes.

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Reference 2 :- [2] Raimundo et al. (2020). *European Journal of Nutrition* 59, 1329-43.

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Acknowledgements :-

Fundação para a Ciência e a Tecnologia (FCT) through projects PTDC/BIA-MOL31104/2017 to RM, PD/BD/135504/2018 to AFR, and UIDB/04567/2020 and UIDP/04567/2020 to CBIOS.

OC15

Brain-Carotid-body link in dysmetabolic states: where and how this interaction occurs?

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Introduction: Metabolic diseases, like type 2 diabetes (T2D) and obesity are highly incident diseases worldwide caused in part by hypercaloric diets [1]. Recently, we demonstrated that the activity of the carotid body (CB) is increased in prediabetes and T2D animals [2], and in prediabetic patients [3] and that the resection of the carotid sinus nerve (CSN), the nerve that links CB to the brain, prevented and reversed the metabolic alterations induced by hypercaloric diets in rats [2,4]. The nucleus of the tractus solitarius (NTS) is an integrative center of CSN signals distributing information to nucleus within other regions of the brain like the hypothalamus [5]. We propose to discriminate the type of integration and distribution of CSN metabolic signals to understand the brain regions involved in the CB-dependent metabolic control and complete the circuit between CB-brain-peripheral tissues in the scenario of metabolic disorders.

Material & Methods: Cryopreserved brains of male Wistar sham vs CSN-transected control and HF rats (19 weeks diet treatment) were cut in coronal sections (40um) following the coordinates from bregma to englobe NTS region from caudal (-14mm), to medial (-12,5mm) and rostral (-11mm) regions and 2 important regions of the hypothalamus the paraventricular nucleus (PVN) and the arcuate (ARC). Free-floating immunofluorescence for delta-FosB, an early gene used to measure brain activity was performed. Slices were visualized in a confocal microscope and images were used to count the fluorescence intensity correspondent to delta-FosB staining. Experiments followed the European Union Directive for Protection of Vertebrates Used for Experimental and Other Scientific Ends (2010/63/EU) and were approved by the NOVA Medical School Ethics Committee and the Portuguese Authority for Animal Health. Data was analyzed and differences were calculated using

One-Way ANOVA with Turkey's multiple comparison test and considered significantly different with p-values < 0.05.

Results: Delta-FosB relative fluorescence was not altered in control animals undergoing CSN denervation in all NTS regions. Also, HF diet did not promote any alteration in delta-FosB relative fluorescence in all NTS regions. However, CSN denervation in HF animals promoted a significant decrease in delta-FosB relative fluorescence in the rostral (ctl=100%±14.4% vs. HF+Den=59.5%±16.3%; p=0.037) and medial regions (ctl=100%±2.7% vs HF+Den=86%±3.3%; p=0.0046) of the NTS, without alterations in the caudal region. HF diet did not change delta-FosB fluorescence in the sub-region of PVN and ARC at -1.8mm from bregma, but CSN denervation in control and HF animals promoted an increase in delta-FosB fluorescence in this sub-region of PVN (ctl=100%±10.1%, ctl+Den=234.8%±80.2% (p=0.021); HF=105.4%±34.3% vs HF+DEN=171.9%±4.3, p=0.032) without alterations in the ARC on this coordinate. At -2.16mm the HF diet promoted an increase in delta-FosB staining in the PVN that was reverted by CSN denervation (ctl=100%±0.0% vs HF=150.2%±5.1%, p=0.005; HF vs HF+DEN=85.3%±9.6%, p=0.003) again without alterations the ARC.

Conclusions: We can conclude that the signals from CB integrated in the NTS affect the hypothalamus, particularly the PVN. Further experiments must be done to explore neuronal populations within these regions of the hypothalamus that are modulated by CB in states of dysmetabolism.

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Reference 2 :- Ribeiro et al. Diabetes. 2013; 62(8):2905-16

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Acknowledgements :-

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OC16

Rescue of diabetes-induced HIF1α suppression recovers cardiac function and metabolism in the type 2 diabetes

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Introduction and Aims:

Cardiovascular disease is the leading cause of mortality in people with type 2 diabetes (T2D), with more rapid progression into heart failure post-myocardial infarction (MI). Hypoxia-inducible factor (HIF) is a master transcription factor regulating over 1000 genes critical for adapting the heart to survive when oxygen is restricted (hypoxia), as occurs following an MI [1]. We questioned whether diabetes impaired HIF signalling, contributing to decreased recovery post-MI, and whether pharmacologically targeting HIF was a novel treatment strategy for the diabetic heart.

Methods:

T2D was induced in the rat using high-fat diet (6 weeks) in combination with a single low-dose of streptozotocin (25mg/kg) (n=6) [2]. Human induced pluripotent stem cells were differentiated and matured into contracting cardiomyocytes (n=4), according to our published protocol [3]. Insulin resistance was induced in the human and rodent (HL1) cardiomyocytes by culturing in high palmitate (0.3mM), high glucose (12mM) and high insulin (50nM).

Results:

Insulin resistant human and rodent cardiomyocytes, when challenged with hypoxia (2% oxygen for 16 hr), had blunted activation of HIF1 α protein, and decreased transcription of downstream HIF targets, including genes involved in anaerobic glycolysis, angiogenesis and autophagy. T2D hearts had decreased HIF1 α activation within the myocardium in response to ischaemia. Blunted HIF1 α activation suppressed the reprogramming of metabolism in hypoxia, maintaining a more mitochondrial oxidative metabolism rather than shifting towards anaerobic oxygen-efficient glucose metabolism. When challenged with whole body hypoxia (11% oxygen for 3 weeks in a hypoxia chamber), impaired HIF signalling in T2D rats resulted in blunted cardiac hypoxic adaptation and decreased cardiac function.

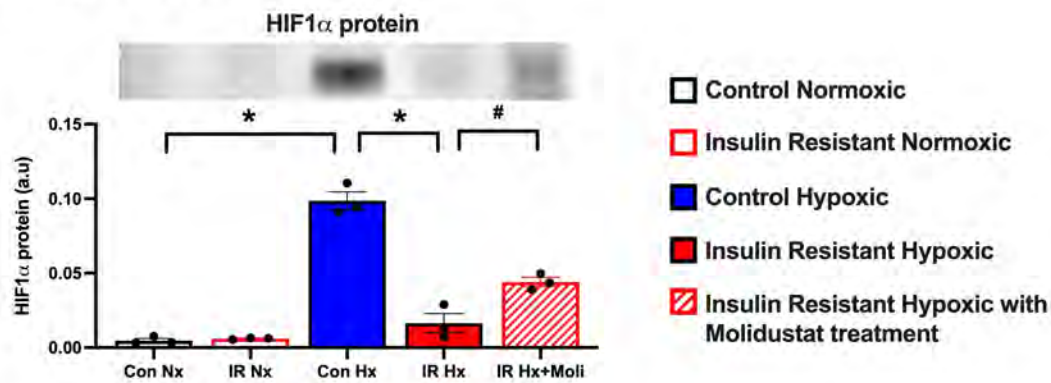
Mechanistically, decreased HIF1 α in T2D was due to the increased fatty acid (FA) concentrations within the circulation (hyperlipidaemia), and not related to the hyperglycaemia or hyperinsulinemia. Long chain FA, palmitate and oleate, were able to suppress HIF1 α activation in response to hypoxia in a concentration-dependent manner, and could be reversed by blocking fatty acid entry into the cardiomyocyte. FA effect on HIF1 α was mediated indirectly via changes in Krebs cycle intermediates. We demonstrate that increased succinate is required for optimal cardiac HIF1 α activation in hypoxia, and that FA indirectly suppress succinate concentrations, causing suppressed HIF1 α in T2D.

Finally, HIF1 α activators are undergoing phase III clinical trials for the treatment of renal anaemia associated with chronic kidney disease. We postulated that Molidustat, a HIF1 α activator developed by Bayer, could be repurposed for the T2D heart, to restore HIF1 α signalling and function post-MI. Molidustat (50 μ M for 16hr) activated HIF1 α and downstream HIF signalling in insulin resistant human cardiomyocytes, overcoming the inhibitory effect of FA (Figure). In T2D rats, Molidustat (5mg/kg for 5 days) reprogrammed cardiac metabolism, increasing glucose metabolism and decreasing fatty acid oxidation and lipotoxicity. This culminated in increased cardiac function and increased recovery post-MI in Molidustat-treated T2D rats.

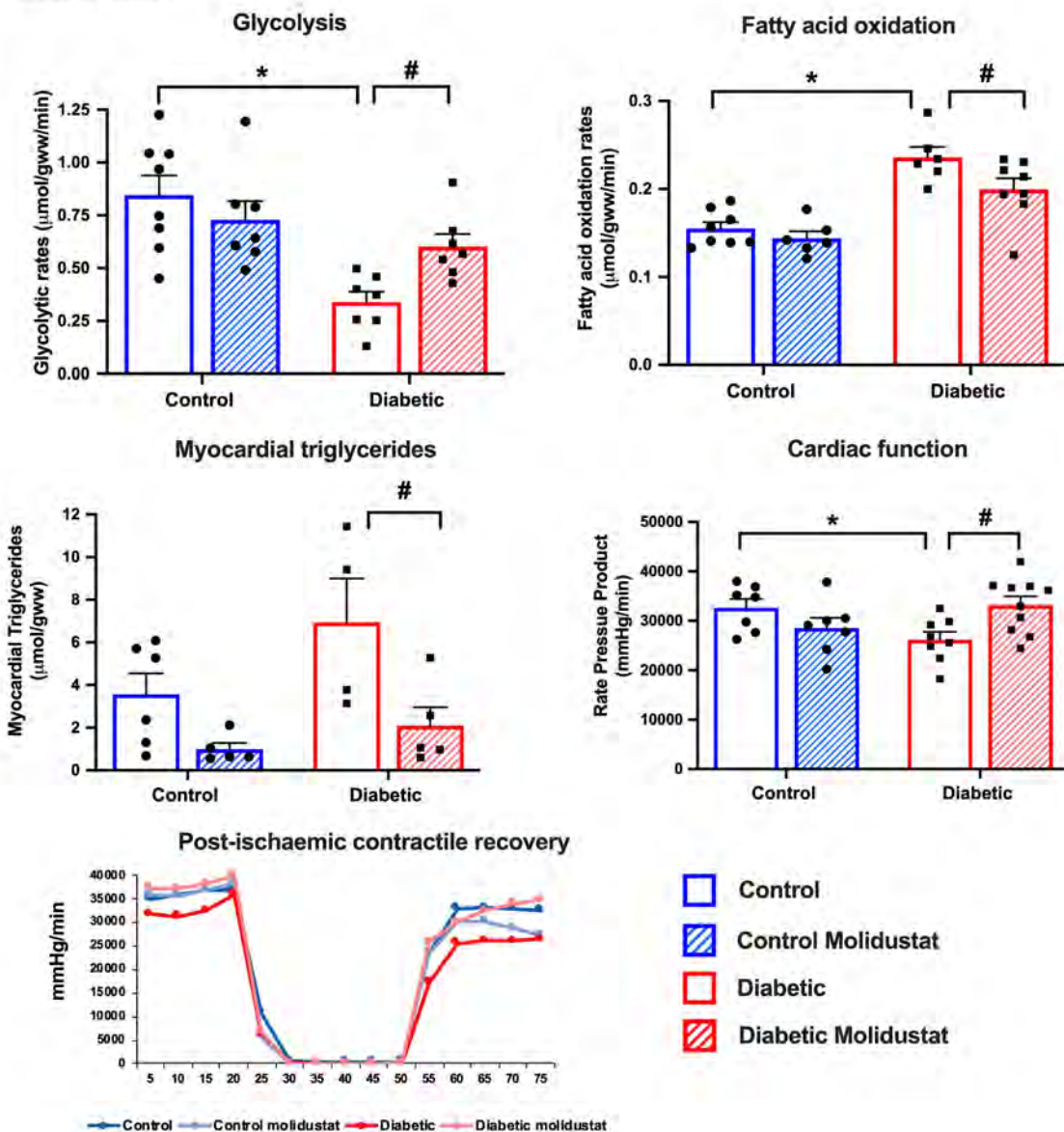
Conclusions:

Elevated FA in T2D suppress cardiac HIF1 α activation in response to ischaemia, which culminates in blunted beneficial adaptation to hypoxia. Pharmacological activation of HIF1 α with Molidustat provides a route to improve cardiac metabolism and function in T2D, and can be rapidly repurposed for clinical translatability.

Human Cardiomyocytes



Rodent Hearts



Reference 1 :- Heather, L.C. and K. Clarke, *Metabolism, hypoxia and the diabetic heart*. J Mol Cell Cardiol, 2011. **50**(4): p. 598-605.

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OC17

MrgD is essential for a classic pattern of gene expression in brown adipose tissue

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Background: Renin-Angiotensin System (RAS) is essential for blood pressure control and electrolytic homeostasis. Besides its classical mechanisms, RAS also represents a link between obesity and its consequences. Angiotensin II/AT1 overactivity reinforces obesity implications, and Angiotensin-(1-7)/Mas activity improves metabolic parameters. Alamandine is a newly described peptide part of the RAS. Alamandine shares functional and structural similarities with Angiotensin-(1-7) and its effects are mediated by a different receptor, MrgD (Lautner et al., 2013). Therefore, we believe that alamandine, closely participates in the RAS-metabolism interaction by mediating different signaling pathways.

Aim: Our goal was to evaluate the consequences of the genetic deletion of alamandine receptor, MrgD, on the metabolism of C57BL6/J mice and the mechanisms underlying the possible alterations.

Methods and Results: All experimental protocols were approved by UFMG ethics committee. Obesity was induced by high glucose diet (HG) for 8 weeks; control groups were feed with standard diet (ST). Our initial results indicate MrgD as an important influence for brown adipose tissue (BAT). We showed that MrgD/KO mice have diminished BAT regardless of age compared with wild type mice (WT); (i) 5-day-old mice, P value= 0.0246, N=5; (ii) 8-week-old mice, P value<0.0001, N=27 and (iii) 16-week-old mice, P value=0.0007, N=18. Moreover, WT obese mice, decreased not only MrgD expression in BAT (P value<0.0001, N=7) as also decreased alamandine circulating levels (P value=0.0017, N=8). Therefore, to identify potential target of MrgD signaling in BAT, RNA sequencing with RNA isolated from BAT of obese and lean MrgD/KO mice was performed (n=4). HG diet led to a transcriptional regulation in BAT of 1148 genes between WT-HG vs WT-ST groups. In contrast, intriguingly, only 45 genes were regulated between MrgD/KO-HG vs MrgD/KO-ST. Therefore,

MrgD/KO-HG group failed in regulating more than 1100 genes in response to the HG diet. MrgD/KO-ST showed a deeply down regulation of transcripts pattern in BAT compared with WT-ST; of 476 regulated genes 445 were down regulated. The comparison of BAT transcriptomes between MrgD/KO vs WT mice was mainly marked by the regulation of genes encoding AMPK, extracellular matrix (ECM) components and ion channels.

Conclusion: MrgD receptor absence led to a significant change in gene expression pattern in BAT. It deviated the tissue towards a profound transcripts' depletion and to a response absence to the caloric challenge. In the view of regulated genes and enrichment analysis, we conclude that alamandine/MrgD activation contributes to RAS-metabolism interaction. MrgD expression is important to BAT protection via maintenance of cell structure, ribosomal structure, mitochondrial structure and proper signaling via ATP, calcium, and AMPK.

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OC18

Inhibition of the mechanistic target of rapamycin complex 1 (mTORC1) pathway partially restores hyperglycaemia-associated mitochondrial dysfunction in pancreatic islets

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Aims: Hyperglycaemia leads to impaired mitochondrial respiration in pancreatic islets. The nutrient-sensing mechanistic target of rapamycin complex 1 (mTORC1) pathway is hyperactivated in islets from patients and animals with Type 2 diabetes. We aimed to investigate if inhibition of mTORC1 could restore mitochondrial efficiency in diabetic islets.

Methods: We utilised the β V59M mouse model whereby hyperglycaemia/diabetes (>20mmol/l) is initiated via a tamoxifen-inducible K_{ATP} channel activating mutation in pancreatic beta cells. mTORC1 signalling was determined by the ratio of phosphorylated (p-S6) and total ribosomal protein S6 (tot-S6) using standard western blotting methods. Following isolation, islets were incubated with/without

10 μ mol/l S6 kinase inhibitor PF-4708671 (S6Ki) for 48 hours. Oxygen Consumption Rate (OCR) was monitored using the extracellular flux analyser (Seahorse Bioscience, Inc.).

Results: mTORC1 signalling is increased in diabetic islets (p-S6/total-S6 ratio: control = 0.36 ± 0.02 vs diabetic = 0.70 ± 0.082 AU, $p < 0.05$; $n=6$) but incubation with S6Ki reduced this to levels observed in control islets. Diabetic islets displayed an attenuated glucose-stimulated OCR, in comparison to control islets. However, this was significantly improved following incubation with S6Ki (diabetic = 34.45 ± 10.72 vs diabetic+S6Ki $203.94 \pm 39.24\%$ increase in OCR above baseline, $p < 0.001$; $n=7-8$). The ATP-synthase inhibitor, Oligomycin, produces significantly less inhibition of OCR in diabetic islets, compared to control, but this response was restored via mTORC1 inhibition (diabetic = 55.01 ± 9.44 vs diabetic+S6Ki = $240.32 \pm 9.71\%$ decrease in OCR, $p < 0.001$; $n=7-8$).

Conclusion: Our results suggest that hyperactivation of mTORC1 signalling is partially responsible for mitochondrial dysfunction in diabetic islets and may be involved in regulating ATP-synthase activity.

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OC19

Modulation of action potential firing by TMEM16A and TMEM16B proteins in mouse vomeronasal sensory neurons.

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The vomeronasal organ (VNO) is a chemosensory organ present in most mammals. The main function of the VNO is to detect pheromones regulating the physiology and social behavior of the organism. Chemosensory neurons in the VNO, named vomeronasal sensory neurons (VSNs), express vomeronasal receptors in the specialized knob/microvilli region where signal transduction takes place. Signal transduction cascade is poorly understood in VSNs and some steps still remain obscure. The two calcium-activated chloride channels, TMEM16A and TMEM16B, are expressed in the knob/microvilli region of VSNs [1,2,3], however, their physiological role in VSNs is still controversial. We have previously reported that TMEM16A is an essential component to mediate the calcium-activated chloride currents in VSNs [4]. Moreover, a recent report showed that deletion of both TMEM16A and TMEM16B proteins lead to a reduced capability of spontaneous and evoked firing in VSNs, but did not modify male-male aggression, a VNO-mediated behavior [5]. Taking advantage of *Tmem16a* conditional knockout (cKO) and *Tmem16b* KO mice, we aimed to evaluate the individual contribution of TMEM16A and TMEM16B proteins to the physiology of VSNs. By immunohistochemistry, we confirmed the expression of TMEM16B protein in *Tmem16a* cKO mice and the expression of TMEM16A protein in *Tmem16b* KO mice. Then, we evaluated calcium-activated

chloride currents in VSNs using the whole-cell patch-clamp technique. We confirmed that TMEM16A is the principal contributor to calcium-activated chloride currents in VSNs, while TMEM16B contribution to those currents seems to be minor (WT-16AcKO: $p < 0.01$; Dunn–Hollander–Wolfe after Krustal-Wallis). To check the role of calcium-activated chloride currents in VSNs, we first evaluated voltage-gated currents and passive membrane properties in WT, *Tmem16a* cKO and *Tmem16b* KO VSNs ($n=75$). We did not find differences between the three groups ($p=0.21$; Krustal-Wallis), indicating that TMEM16A and TMEM16B proteins do not contribute to passive membrane properties or to voltage-gated currents in VSNs. Finally, we investigated the spontaneous ($n=136$ cells) and evoked activity ($n=54$ cells) in VSNs from acute slices of VNO, using the loose-patch recording technique. We found that deletion of TMEM16A protein affects spontaneous activity pattern modulating the inter-spike interval (ISI) ($p < 0.001$; Kolmogorov-Smirnov), while deletion of TMEM16B protein did not affect spontaneous activity. Furthermore, deletion of either *Tmem16a* or *Tmem16b* did not affect the frequency of spontaneous activity ($p=0.62$; Krustal-Wallis). Then, we evaluated the spiking properties of VSNs in response to urine stimulation. We found that VSNs from both KO mice conserved the capability of response to urine stimulation when compared with WT. We did not find an alteration in frequency of response or response duration ($p=0.71$; Krustal-Wallis), while we found differences in the ISI distribution ($p < 0.001$; Kolmogorov-Smirnov), meaning that either deletion of TMEM16A or TMEM16B protein alters the spiking pattern of VSNs during the response to urine. We concluded that, while TMEM16A is the main contributor to calcium-activated chloride currents in VSNs, both TMEM16A and TMEM16B proteins modulate action potential firing during spontaneous and evoked activity. Also, our results indicate that TMEM16A and TMEM16B proteins can work as heterodimers in the knob/microvilli region of VSNs.

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OC20

Hypoxia induced carbonic anhydrase mediated dorsal horn sensory neuron activation and induction of neuropathic pain

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Neuropathic pain such as that seen in diabetes mellitus, results in part from central sensitisation in the spinal cord dorsal horn. However, the mechanisms responsible for sensory neuron activation remain unclear. There is evidence that disturbances in the integrity of the vascular network can be a causative factor in the development of neuropathic pain through alterations in the microenvironment of the dorsal horn sensory neuron. Here we show that induction of hypoxia at the level of the dorsal horn leads to the onset of pain hypersensitivity. All animal experimentation was performed in line with the ARRIVE guidelines, with experimental procedures reviewed by the local Animal Welfare and Ethics Review Boards and performed in accordance with the UK Home office animals (Scientific procedures) Act 1986 and EU Directive 2010/63/EU. Using adult (30g) C57.bl6 male mice, nociceptive behavioural assays (von Frey hair – mechanical hypersensitivity and Hargreaves test – heat hypersensitivity) were performed with baseline nociceptive withdrawal thresholds determined prior to drug administration. Mice were treated with either intrathecal injection (under recovery gaseous anaesthetic isoflurane ~2% O₂) of vehicle control (PBS; n=12) or 1mM Dimethylxalylglycine (DMOG; n=12) and in an additional group intraperitoneal acetazolamide (ACZ; n=12). Nociceptive behavioural analysis (data provided as mean±SEM) was performed post intrathecal injection for upto 24 hrs, which demonstrated pronounced mechanical (Veh = 1.53g±0.04 vs DMOG=1.22g±0.05; ***p<0.001 One Way ANOVA) and heat hypersensitivity (Veh = 10.6s±1.1 vs DMOG=6.3s±2.2; ***p<0.001 One Way ANOVA) following DMOG administration. Lumbar spinal cord tissue was collected at 24hrs post DMOG administration demonstrating induced increased hypoxia signalling in the dorsal horn, depicted by increased expression of hypoxia markers hypoxia inducible factor 1 α and carbonic anhydrase 7 as well as markers of neuronal excitation (increased fos expression) depicted by western blot (n=3 per group), immunohistochemistry (n=3 per group) and RNAseq (n=5 per group) analysis. This was corroborated by electrophysiological recordings of lumbar spinal cord tissue slices demonstrating increased dorsal horn sensory neuron activity following treatment to induce hypoxia signalling. Furthermore, inhibition of carbonic anhydrase activity through intraperitoneal injection of Acetazolamide in combination with DMOG treatment inhibited hypoxia induced mechanical (DMOG + ACZ = 1.59g±0.026; ***p<0.001 One Way ANOVA) and heat hypersensitivity (DMOG + ACZ =11.86s±2.84; ***p<0.001 One Way ANOVA). This investigation demonstrates that induction of a hypoxic microenvironment in the dorsal horn, is an integral process by which sensory neurons are activated to initiate neuropathic pain states.

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Selenomethionine mis-incorporation results in redox-dependent Nav1.4 channel gain-of-functionRama Hussein¹, Marwa Ahmed¹, Stefan H. Heinemann¹¹*Center of Molecular Biomedicine, Department of Biophysics, Friedrich Schiller University Jena and Jena University Hospital, Jena, Germany*

Selenomethionine (SeMet) mis-incorporation is a process where SeMet replaces methionine (Met) residues in proteins due to the high structural similarity between the two amino acids. The rate of mis-incorporation is dependent on the availability of SeMet to the protein synthesis machinery. SeMet content can reach to 90% of total selenium content in some plants consumed by humans. Moreover, SeMet is a supplement of selenium, an essential micronutrient with cancer chemopreventive properties. SeMet gets readily absorbed via the methionine intestinal transporters and contributes to the methionine pool in the liver. Therefore, long-term intake of high SeMet doses results in the accumulation of the amino acid in muscle, heart and liver tissue proteins (Burk et al. 2015). Despite the high rates of incorporation of SeMet in proteins and its stronger susceptibility to oxidation compared to methionine, little is known about the effect of SeMet mis-incorporation on electrical excitability and ion channels. Fast inactivation of voltage-gated sodium channels (Nav) is essential for proper action potential generation, and even minute impairment of inactivation can result in adverse phenotypes. Surprisingly, Nav inactivation depends on the Ile-Phe-Met motif in the DIII-IV linker because oxidation of that Met results in marked loss of inactivation (Kassmann et al. 2008). Here we examined the impact of SeMet mis-incorporation on the function of skeletal muscle Nav1.4 channels.

HEK293T cells were transfected with DNA coding for rat Nav1.4. Subsequently, cells were cultured in media supplemented with varying mole-fractions of Met vs SeMet (total concentration 200 μ M). After about 24 h, currents were measured in the whole-cell patch-clamp configuration.

In 100% SeMet medium, where almost all Met residues in a protein are expected to be replaced with SeMet, Nav1.4 channels (which harbors 66 Met residues) exhibited normal activation and inactivation properties with a slight right-shift in voltage dependence (Fig. 1). While 300-s exposure to 100 μ M of the Met-specific oxidant chloramine T (ChT) had almost no impact on rNav1.4 channels under control conditions, rNav1.4_{SeMet} channels responded with an almost complete loss of inactivation and about 60% reduction of the peak current amplitude at -20 mV (Fig. 2). Partial substitution of SeMet in culture media resulted in a graded response. The impact of oxidation on rNav1.4_{SeMet} was reversible with 1.5 mM dithiothreitol (DTT), while loss of inactivation on control channels induced by higher ChT concentration persisted even after DTT application. This observation is an indirect proof for SeMet being incorporated in Nav1.4 because oxidation of SeMet to methionine selenoxide is spontaneously reversible (Carroll et al. 2017). Great part of the redox sensitivity of Nav1.4_{SeMet} channel inactivation can be attributed to the IFM motif because mutation M1305L (resulting in IFL) diminished the fraction of non-inactivating current from 0.77 ± 0.09 (wild

type) to 0.12 ± 0.02 ($n = 4-5$). The molecular origin for the current amplitude reduction remains to be determined.

SeMet incorporation in Nav1.4 channel proteins coinciding with oxidative insults may affect electrical excitability of neurons and myocytes to result in hyperexcitability pathologies, such as cardiac arrhythmias and neuropathies, similarly to congenital Nav channel gain-of-function mutations.

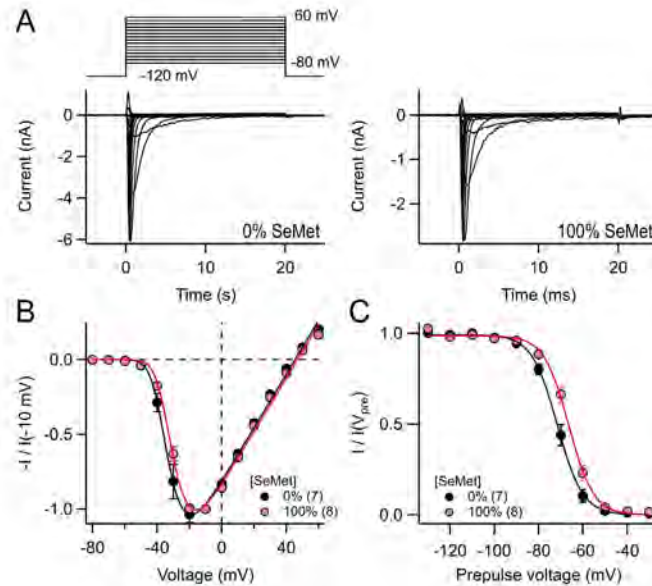


Figure 1. Voltage dependence of rNav1.4 channel activation and inactivation. (A) Whole-cell current traces of rNav1.4 channels in response to the pulse protocol shown on top. Cells were either cultured in Met-containing (*left*) or SeMet-containing medium (*right*). (B) Current-voltage relationship normalized to the current at -10 mV with superimposed fits estimate half-maximal activation voltage: -42.0 ± 0.6 mV for 0% SeMet and -38.9 ± 0.8 mV for 100% SeMet. (C) Voltage dependence of inactivation with superimposed fits to a single-component Boltzmann function (half-maximal inactivation voltage: -71.6 ± 0.2 mV for 0% SeMet and -66.4 ± 0.4 mV 100% SeMet. Data are mean $\pm$ S.E.M, n values in parentheses.

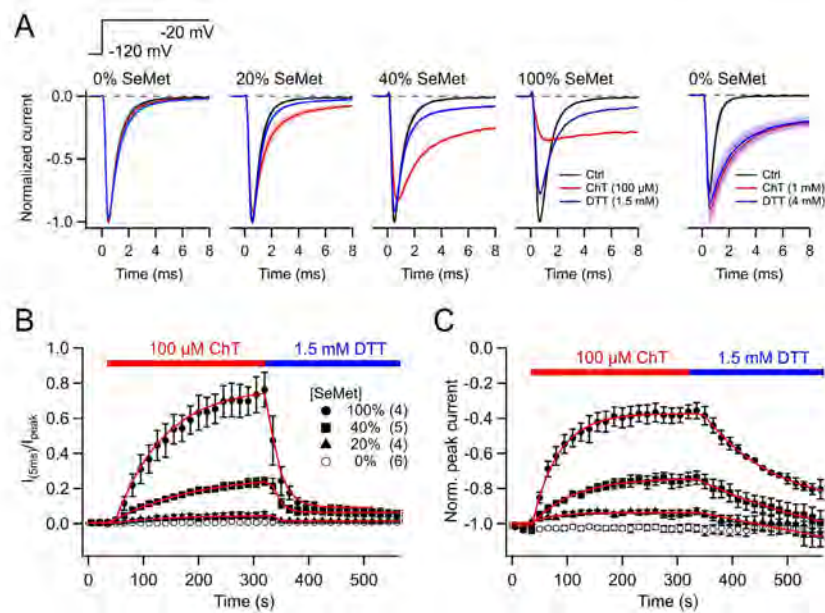


Figure 2. Influence of SeMet mole fraction on oxidation sensitivity of Na_v1.4. (A) Superposition of mean current traces of rNa_v1.4 channels in response to pulses to -20 mV. Cells were cultured with the indicated mole fraction of SeMet vs Met in the culture medium. The current traces are normalized to the mean maximum current under control conditions. Red traces were recorded 300 sec after application of 100 μM ChT, blue traces another 270 sec after application of 1.5 mM DTT. In the rightmost panel, 1 mM ChT and 4 mM DTT were applied. (B) Fraction of non-inactivating current as a function of time with indicated application of ChT and DTT and different culture conditions. (C) Normalized maximal current amplitude at -20 mV as a function of time from the same experiments as in (B). Data are mean ± S.E.M, n values in parentheses.

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OC22

Determining the Mechanisms of Long Term Pain in Childhood Cancer Survivorship

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Chemotherapy induced peripheral neuropathy (CIPN) commonly occurs following platinum-based chemotherapy (e.g. cisplatin). A typical adverse effect is a delayed but lasting chronic pain, that

remains into adulthood. Such a complication is prevalent in about 50% of childhood cancer survivors and it decreases quality of life. Presently, there are no preventative treatments and no condition-tailored analgesia. Being dose-dependent, a major concern is that this may negatively impact on chemotherapy treatment, preventing the administration of chemotherapy at the optimal effective doses and premature discontinuation of therapy. Exposing dorsal root ganglion (DRG) sensory neurons to platinum-based chemotherapy such as cisplatin, sensory neurodegeneration is exhibited. In previous studies, 24hr cisplatin treatment of 50B11s (an immortalised nociceptive sensory neuronal cell line) resulted in inhibited neurite outgrowth and increased cleaved caspase 3 (CC3) expression [Vencappa et al., 2015]. In rodent models, however, following exposure to cisplatin early in life and subsequent washout, aberrant nociceptor intraepidermal sensory nerve fibre (IENF) growth is induced, a hallmark of chronic pain and putatively mediated by Tropomyosin receptor kinase A (TrkA) [Hathway et al., 2018]. In this study, the hallmarks of CIPN and TrkA dependent signalling were investigated. SH-SY5Y cells and DRGs underwent 24hr treatment with varying concentrations of cisplatin (0-10µg/ml). Cisplatin-induced neurodegeneration occurred in a dose-dependent manner (**** $P < 0.0001$, * $P < 0.05$ One Way ANOVA) alongside increased phosphorylation of DNA damage markers, p53 and Histone H2A.X. TrkA expression remained unchanged following treatment with cisplatin. TrkA mediated aberrant sensory nerve fibre growth following CIPN is believed to be nerve growth factor (NGF) driven. SH-SY5Y cells treated with NGF (1nM) showed increased phosphorylated TrkA expressions. All experimental procedures involving animals were performed in accordance with the UK Home office animals (Scientific procedures) Act 1986 and reviewed by Nottingham Trent University Animal Welfare and Ethics Review Boards. Male Wistar rats (~250g) intraplantar injected (under recover gaseous isoflurane anaesthetic ~2% O₂) with NGF (1µM) showed increased PGP9.5+ve IENF growth in the plantar skin (* $P < 0.05$ Mann Whitney Test $n = 3$ per group) and increased mechanical sensitivity (* $P < 0.05$ Two Way ANOVA; $n = 6$ per group) up to 5hrs post-treatment versus the vehicle control group. In addition, Wistar rats injected with NGF (1µM) and accompanied with either TrkA inhibitor, GW441756 (intraperitoneal 2mg/kg) or NGF neutralising antibody (intraperitoneal 1mg/kg) presented no mechanical behaviour sensitization (* $P < 0.05$ Two Way ANOVA) when compared to NGF+vehicle ($n = 3$ all groups). It appears that the NGF-TrkA pathway can sensitize nociceptor sensory neurons, thus potentially causing cisplatin-induced survivorship pain.

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OC23

The role of the HCN channel receptor in neuropathic pain: a feasibility study of ivabradine in patients with chronic peripheral neuropathic pain

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Introduction

Hyperpolarization-activated cyclic nucleotide gated (HCN) ion channels mediate repetitive action potential firing in the heart and nervous system [1]. The HCN2 isoform is expressed in nociceptors and preclinical studies suggest a critical role in neuropathic pain [2,3]. Ivabradine is a non-selective HCN blocker that is available for prescription but licenced only for cardiac indications. Mouse data suggest that ivabradine is equianalgesic with gabapentin for neuropathic pain [3].

Aims

We sought to translate these findings to patients with chronic peripheral neuropathic pain.

Method

The study was conducted in accordance with the spirit and the letter of the Declaration of Helsinki, the conditions and principles of International Conference on Harmonisation's Good Clinical Practice, the protocol and applicable local regulatory requirements and laws. The study was registered prospectively (ISRCTN68734605), and ethical approval obtained from the London-Bromley Research Ethics Committee (16/LO/1901). Written informed consent was obtained from every participant before any study-related activity was performed. We adopted an open label design; administering incrementally increasing doses of ivabradine, titrating according to heart rate up to 7.5 mg twice daily. All participants gave daily pain ratings on an 11-point numerical rating scale (NRS).

Results

Seven participants completed the study. There was no significant treatment effect on the primary endpoint, change-from-baseline in mean NRS score (reduction = 0.878, 95% CI = -2.07 to 0.31, p=0.1). Exploratory analysis using linear mixed models revealed a highly significant but modest relationship between ivabradine dose and daily NRS pain score ($c2(1)=74.6$, $p<0.001$), with a reduction of 0.12 ± 0.01 (SEM) NRS points per mg. The two participants with painful diabetic neuropathy responded particularly well.

Conclusions

These preliminary data suggest there may be an analgesic effect of ivabradine at higher doses, possibly in patients with diabetic neuropathic pain. Importantly, there were no adverse effects. This suggests that ivabradine, a peripherally-restricted drug (devoid of central nervous system side effects) is well tolerated in patients with chronic neuropathic pain. Ivabradine is now off-patent and its analgesic potential merits further investigation in clinical trials.

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Acknowledgements :-

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OC24

Selenomethionine mis-incorporation in TTX-sensitive voltage-gated sodium channels of mouse dorsal root ganglia

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Selenomethionine (SeMet) is widely marketed as anti-aging food supplement. Due to its structural similarity to methionine (Met), SeMet is loaded on Met tRNA and is efficiently incorporated into proteins during translation. Unlike Met, SeMet is readily oxidized; furthermore, reduction of methionine selenoxide occurs non-enzymatically, e.g. in the presence of reducing agents such as dithiothreitol (DTT).

Met oxidation in voltage-gated sodium channels (Nav channels) has a gain-of-function effect as it leads to impairment of fast inactivation (1). Even small gain-of-function changes in neuronal Nav channels can cause neuronal hyperexcitability and contributes to the pathology of, e.g., epilepsy and erythralgia (2). When skeletal muscle Nav1.4 channels are heterologously expressed in a SeMet-rich medium, they incorporate SeMet which makes them sensitive to oxidation. Here we address the question if and to which degree SeMet is incorporated into neuronal Nav channels in native neurons and how this might alter their function.

We chose mouse dorsal root ganglia (DRG) nociceptor neurons because of their relevance for pain signaling, their likely exposure to oxidative stress, and their rich repertoire of tetrodotoxin-sensitive (TTX-s) (mostly Nav1.3, Nav1.6, Nav1.7) and TTX-resistant Nav channels (Nav1.8, Nav1.9). Because the inactivation of TTX-r channels is markedly slower than that of TTX-s channels, we employed a double-

knockout mouse model (Scn10a/11a^{-/-}), lacking functional Nav1.8 and Nav1.9, to gain access to TTX-s Nav currents in isolation.

Wild type (C57BL/6J) and Scn10a/11a^{-/-} mice were sacrificed according to protocols approved by the local animal welfare committee. DRGs were extracted, neurons were separated by enzymatic digestion and seeded on poly-L-lysine-coated glass cover slips in high-glucose Gibco Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 4% penicillin/streptomycin. Met concentration in this medium was about 200 μ M. To introduce SeMet to the cultures, Met-free medium with a SeMet concentration of 150 μ M was prepared. 18 to 24 h after seeding the cells, the culture medium was partially replaced with the SeMet-containing medium to achieve a SeMet fraction of 0, 5, 10, 40, or 100% of the combined Met and SeMet molarity. Whole-cell patch-clamp measurements were performed 16-24 h later.

Even with 100% SeMet, cells from both strains were viable at the time for measurements and showed functional expression of Nav channels. TTX (1 μ M) blocked Nav currents of Scn10a/11a^{-/-}. In DRGs from Scn10a/11a^{-/-} mice under 40% SeMet conditions, the Met oxidant chloramine T (ChT, 100 μ M) markedly impaired fast inactivation and diminished the maximal current amplitude, both effects reversible under 2 mM DTT (Fig. 1). Even SeMet fractions of only 5% resulted rendered TTX-s channels sensitive to oxidation to yield measurable sustained currents (Fig. 2).

The reversibility of ChT-mediated changes by DTT is a clear indication of SeMet incorporation into the channel proteins demonstrating that the protein turnover in DRG neurons occurs on the order of several hours. The observed oxidation-dependent gain of function in even low SeMet concentrations suggests a possible involvement for SeMet mis-incorporation in SeMet toxicity and motivates investigations into how high-SeMet diet might affect neuronal excitability.

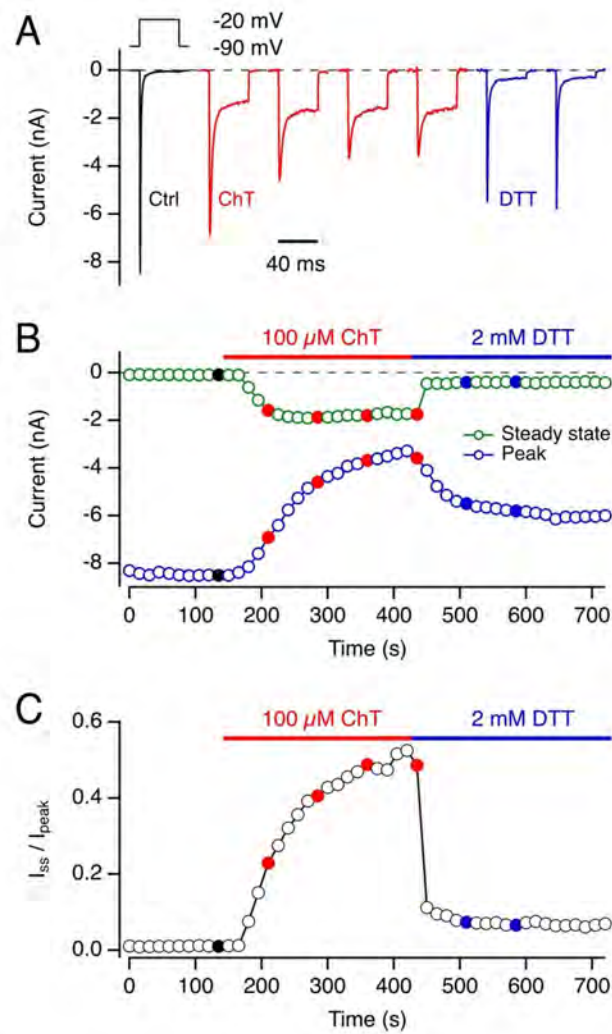


Figure 1 TTX-s Na_V currents in murine DRG cells cultured in SeMet-rich medium. (A) Sample Na_V traces, at 75 s intervals, recorded from a cell cultured in 40% SeMet medium in response to step depolarization to -20 mV, from -90 mV holding potential, before and after application of ChT (100 μM) and subsequent application of DTT (2 mM). (B) Peak current and steady state current (between 10 and 20 ms) recorded from the same cell as a function of time. (C) Ratio of steady state and peak current ratio for all recorded traces for the same cell.

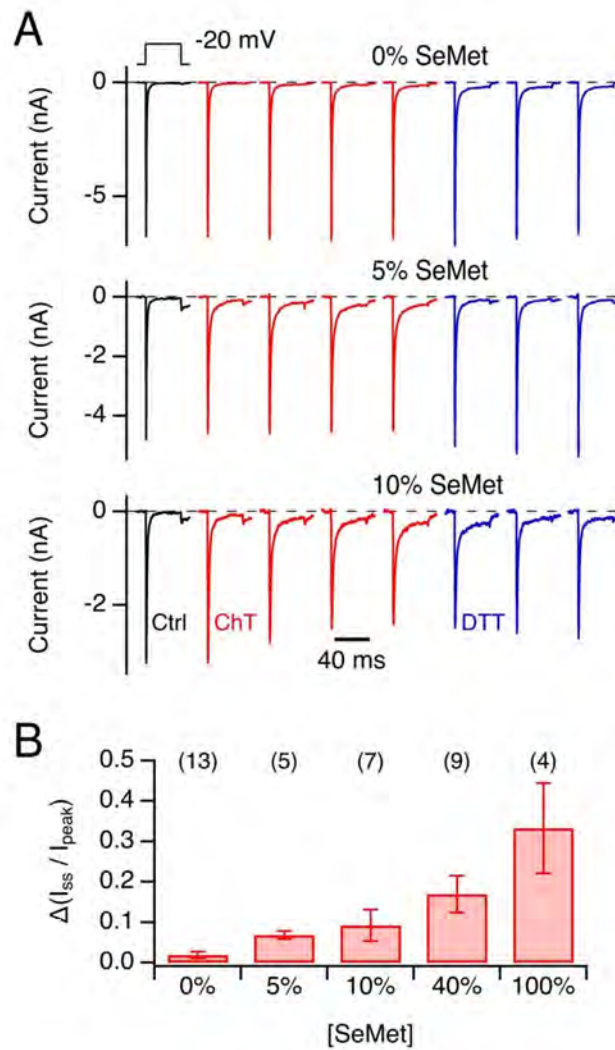


Figure 2 ChT sensitivity of mouse DRG TTX-s Na_v currents correlates with the SeMet concentration relative to Met. (A) Sample Na_v traces, at 75 s intervals, from cells cultured in 0, 5, 10% SeMet medium in response to step depolarization to -20 mV, from -90 mV holding potential, before and after application of ChT (100 μ M) and subsequent application of DTT (2 mM). (B) The mean change in the ratio between the steady state current and the peak current before and 3 min after ChT application. Data are mean $\pm$ sem, n in parentheses.

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Acknowledgements :- Support by the Research Training Groups RTG 2155 (MA) and RTG 1715 (RH) financed by the German Research Foundation (DFG). We thank Prof. Dr. Ingo Kurth for generating the Scn10a/11a-/- mice.

OC25

Respiratory coupling of muscle sympathetic nerve activity in healthy postmenopausal women and young adults.

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Muscle sympathetic nerve activity (MSNA) is modulated by respiration such that lung stretch receptor activity during inspiration inhibits sympathetic activity. Respiratory sympathetic coupling is preserved in healthy older versus younger men (1), but whether a similar preservation occurs in healthy postmenopausal versus premenopausal women is not known. Thirteen postmenopausal women (PMW; 55 [9] years), 12 young women (YW; 25 [5] years) and 12 young men (YM; 26 [7] years) gave written informed consent to participate (demographic data available for 9/13 PMW, 11/12 YW, 12/12 YM). YW took part in the early follicular phase of the menstrual cycle or low-hormone phase of oral contraception. PMW had been amenorrhoeic for at least one year without hormone replacement therapy. MSNA (peroneal microneurography), respiration (respiratory belt), heart rate (ECG) and continuous blood pressure (brachial intra-arterial catheter) were recorded over five minutes of quiet rest. MSNA was sampled during 20 % intervals of inspiration and expiration and quantified as two respiratory phases: mid to late expiration (60-100 % of expiration and 0-60 % of inspiration) and inspiration to post-inspiration (60-100 % of inspiration and 0-60 % of expiration) (1). Two-way mixed ANOVA tested for an interaction between group and respiratory phase, whilst one-way ANOVA or Kruskal-Wallis test tested for group differences in demographic, haemodynamic and percentage change data. Data are presented as mean $\pm$ SD or median [interquartile range]. Body mass index (24.2 ± 2.5 , 23.7 ± 1.0 , 24.7 ± 2.2 kg/m², $p=0.317$), mean arterial pressure (97 [14], 98 [12], 88 [8] mmHg, $p=0.065$), heart rate (64 ± 7 , 62 ± 10 , 58 ± 9 beats/min, $p=0.247$) and breathing rate (13 [5], 14 [2], 13 [6] breaths/min, $p=0.818$) were similar in PMW, YW and YM. Overall MSNA burst incidence was higher in PMW (79 [17] bursts/100 HB) versus YW (53 [13] bursts/100 HB, $p<0.0005$) and YM (51 [11] bursts/100 HB, $p<0.0005$). There were no significant interactions between group and respiratory phase for heart rate, sum of burst area/s, or mean burst area/s, however all showed significant main effects of group and respiratory phase. There were significant interactions between group and respiratory phase for burst incidence and burst frequency ($p=0.004$ and $p=0.029$). Simple main effects analysis revealed that PMW showed a higher burst incidence versus YW and YM during both respiratory phases (mid to late expiration 83 ± 12 versus 62 ± 17 and 65 ± 11 bursts/100 HB, $p=0.002$ and $p=0.009$; inspiration to post-inspiration 72 ± 12 versus 39 ± 11 and 41 ± 8 bursts/100 HB, $p<0.0005$ and $p<0.0005$), with similar results seen for burst frequency. However, PMW exhibited a smaller percentage reduction in MSNA during inspiration to post-inspiration versus mid to late expiration than YW and YM (-10.3 [9.7] versus -37.9 [16.8] and -38.2 [11.6] % change in burst

incidence, $p=0.003$ and $p=0.001$; -10.9 ± 10.6 versus -31.6 ± 14.8 and -33.2 ± 14.5 % change in burst frequency, $p=0.001$ and $p=0.001$). These data indicate that, whilst respiratory sympathetic coupling is preserved in PMW, inspiratory inhibition of MSNA is smaller in PMW versus YW and YM.

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OC26

Vertebral artery hypoplasia and brainstem blood flow in patients with hypertension

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Hypertension is highly prevalent worldwide and is a risk factor for several diseases (1), but its aetiology is poorly understood (2). Cushing's hypothesis proposes that hypertension is a compensatory mechanism that aims to correct cerebral blood flow (CBF) to regions of the brain responsible for cardiovascular autonomic control. These include the brainstem, within which the medulla plays an important role in autonomic regulation of the cardiovascular system.

Our group previously showed an increased prevalence of vertebral artery hypoplasia (VAH) and a reduction in CBF in hypertensives compared to normotensives (3). A further analysis of this data was performed to investigate the impact of VAH on brainstem CBF, and whether this varies between normotensives, treated hypertensives and untreated hypertensives.

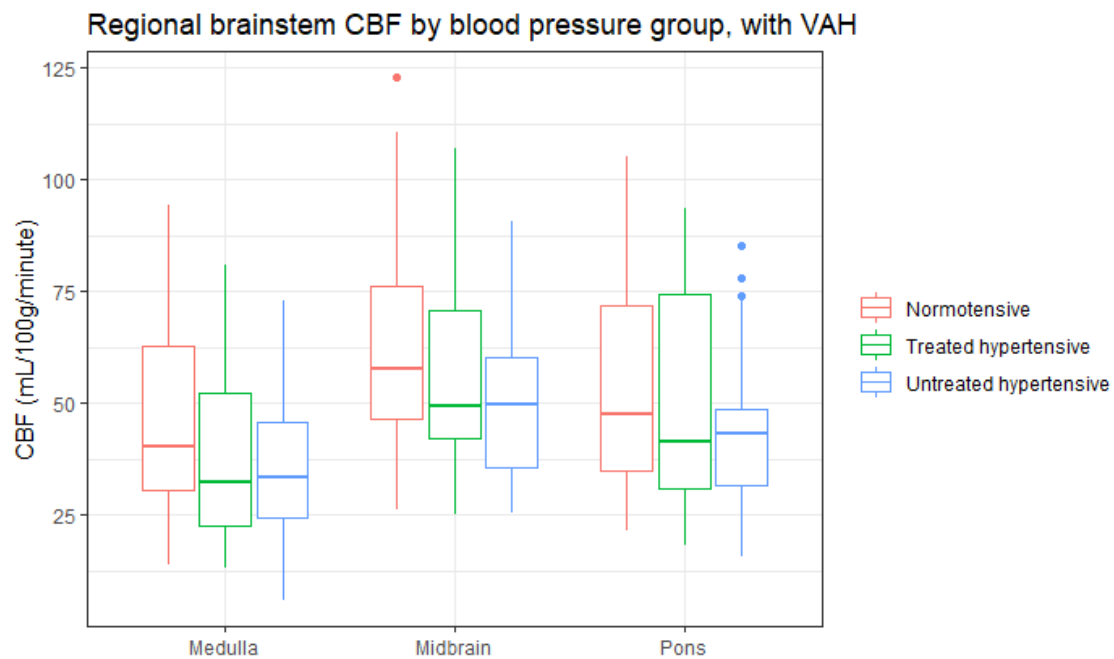
Whole brain CBF maps acquired using arterial spin labelling magnetic resonance imaging (ASL MRI) were available for 80 participants (25 normotensives, 26 untreated hypertensives and 35 untreated hypertensives). Anatomical segmentation of a T1-weighted structural MRI scan was performed for each participant. The segmented regions of interest were applied to the CBF data to generate regional CBF values.

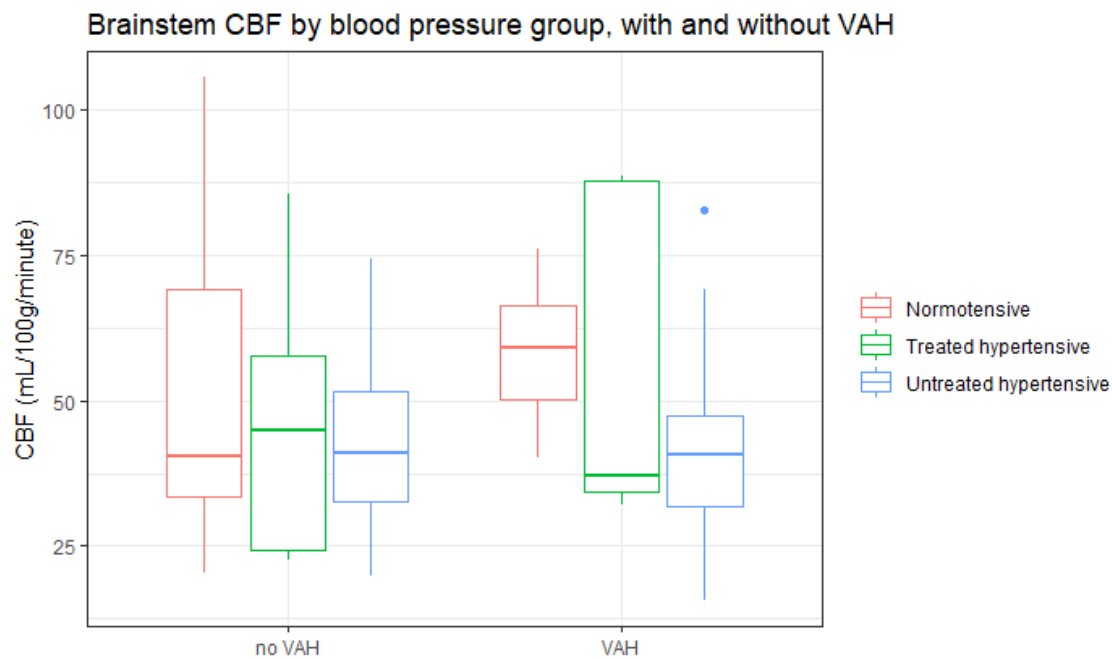
A two-way ANCOVA was conducted to compare the effect of VAH (present or absent) and blood pressure (normotensive, treated hypertensive or untreated hypertensive) on brainstem CBF. The interaction between blood pressure and VAH was also modelled. Age, BMI and sex were included as covariates. To account for the positive skew of CBF data, values are reported as median [interquartile range]. There was no significant effect of VAH ($F(1) = 0.07$, $p = 0.79$, partial $\eta^2 = 0.001$), blood pressure ($F(2) = 2.74$, $p = 0.07$, partial $\eta^2 = 0.09$) or the interaction between VAH and blood pressure ($F(2) = 0.15$, $p = 0.87$, partial $\eta^2 = 0.01$) on CBF. However, there is a trend towards higher brainstem CBF in normotensives with VAH (normotensive CBF = 59 [16], untreated hypertensive CBF = 41 [16] and treated hypertensive CBF = 37 [54] mL/100g/minute) compared to those without VAH

(normotensive CBF = 47 [35], untreated hypertensive CBF = 41 [19] and treated hypertensive CBF = 45 [34] mL/100g/minute).

Subsequently a three-way ANCOVA was conducted to investigate the interaction between regional brainstem CBF (medulla, pons or midbrain), VAH and blood pressure. A similar trend was observed towards higher CBF in normotensives with VAH in all three brainstem regions including the medulla (medulla CBF with VAH 44 [23] (normotensive), 33 [23] (untreated), 25 [8] (treated) mL/100g/minute). There was no significant interaction between VAH, blood pressure and regional brainstem CBF ($F(4) = 0.32$, $p = 0.86$, partial $\eta^2 = 0.006$).

A trend towards a higher brainstem CBF in normotensives with VAH is unexpected and contrary to Cushing's hypothesis. On the other hand, within the VAH group, a trend towards a lower regional brainstem CBF in hypertensives with VAH raises the possibility that VAH creates a fixed resistance to brainstem CBF in hypertensives. The cross-sectional nature of the data precludes any inferences about causation. An adequately powered longitudinal study is required to further explore the causative nature of these relationships.





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OC27

The response of atrial pacemaker activity to α -adrenergic stimulation is inhibited by the type 1 adenylyl cyclase inhibitor ST034307

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Atrial arrhythmias, including atrial fibrillation (AF), are a major mortality risk and a leading cause of stroke. Current pharmacological treatments for AF are poorly tolerated and may increase the risk of fatal ventricular arrhythmias. The IP₃ signalling pathway has been proposed as an atrial specific target for AF therapy, and our work has demonstrated a link between atrial IP₃ signalling and activation of calcium sensitive adenylyl cyclases AC1 and AC8 [1]. Here we demonstrate that a selective inhibitor

of AC1, ST034307 [2], inhibits changes in chronotropy, but not inotropy, induced by activation of the IP₃ pathway by the α -adrenoceptor agonist phenylephrine (PE).

Experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986. Data are presented as mean $\pm$ SEM. Whole left, and right mouse atria were mounted in organ baths containing 10ml physiological salt solution (PSS), maintained at 37°C with 95% O₂ and 5% CO₂ and connected to a mechanical force transducer. Spontaneous beating rate of right atria, and tension generated by left atria electrically paced at 5Hz, was recorded for the duration of experiments. Dose response curves were generated for PE (0.1-30 μ M) in the presence of 1 μ M metoprolol to inhibit beta-adrenergic signalling. PE increased beating rate of right atria with an increase of $12.1 \pm 1.61\%$ observed at 30 μ M ($n=12$). In the presence of 1 μ M ST034307, this increase was reduced (an increase of $7.43 \pm 1.44\%$ at 30 μ M; $n=10$, $P < 0.05$, two-way repeated measures ANOVA). PE increased the tension generated by left atrial contraction by $13.3 \pm 1.9\%$ ($n=5$). This increase in tension was not altered in the presence of 1 μ M ST034307 ($11.4 \pm 3.68\%$, $n=3$, $P > 0.05$).

Further experiments were performed using isolated guinea pig atrial cells loaded with Fluo-5F-AM to record changes in calcium transient amplitude (CaT) generated by 10 μ M PE in the presence and absence of 1 μ M ST034307. Atrial myocytes were isolated from guinea pig hearts using enzymatic digestion and retrograde Langendorff perfusion ($n=7$). Cells were loaded with Fluo-5F-AM, perfused with PSS at $35 \pm 2^\circ\text{C}$ and field stimulated at 1Hz. CaTs were recorded and imaged using an inverted spinning disk confocal microscope connected to an EMCCD camera (iXON 897). 10 μ M PE induced a 35% increase in CaT from 3.56 ± 0.84 to 4.84 ± 1.04 ($n=6$, $P < 0.05$, paired t-test). In the presence of 1 μ M ST034307, CaT increased by 39% from 1.94 ± 0.34 to 2.69 ± 0.36 ($n=15$, $P < 0.05$, paired t-test), indicating that ST034307 does not inhibit the increase in CaT in response to PE.

These results demonstrate AC1 is involved in the response of atrial pacemaker activity to α -adrenoreceptor stimulation, likely as a result of regulation of pacemaker currents within the sino-atrial node, and downstream of IP₃ receptor activation. Conversely, AC1 activation does not appear to be involved in the inotropic response of atrial cells at either the whole-tissue or cellular level. These data support further investigation of cardiac AC1 as a potential pharmacological target for the treatment of atrial arrhythmias.

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OC28

An organelle proteomics method to study endolysosomal proteins in a goat model of atrial fibrillation

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Introduction: Atrial Fibrillation (AF) is one of the most commonly occurring arrhythmias and is associated with an increased risk of stroke, heart failure and significantly higher morbidity and mortality rates. The pathophysiology of AF is not well understood. Lysosomes are recognized as the major location for degradation of both intracellular and extracellular macromolecules and contain more than 50 identified acid hydrolases. The ability of lysosomes to act as Ca²⁺ stores and participate in calcium signaling processes is relatively understudied. Studies have investigated the role of lysosomal calcium channels in fundamental cellular processes and their involvement in disease mechanisms¹. Understanding the role played by lysosomes in the mechanisms underlying AF is vital for the development of pharmacological therapies to treat AF.

Methods: This study was carried out in accordance with the principles of the Basel Declaration and regulations of European directive 2010/63/EU. The local ethical board for animal experimentation of the Maastricht University approved the protocol. Goats were maintained in AF for 6 months. Following open chest sacrifice experiments (n=3 persistent AF and n=3 sham controls), left atrial tissue biopsies were swiftly snap frozen in liquid nitrogen. Samples were prepared for organelle proteomics using a method recently developed in our lab². We performed label-free quantitative proteomics on tissue lysate (TL), endolysosome (EL) and mitochondria (Mito) fractions.

Results: Raw mass spectrometry data was obtained from label-free quantitative (LFQ) proteomics. The protein intensities were quantified and analysed using Perseus 1.6.15.0, and the differential experiment groups of AF vs Sham control displayed a 42.8% variability under principal component analysis. Significantly upregulated proteins were identified by volcano plot.

The major molecular networks were studied using Gene Ontology, KeGG, Panther and Cytoscape pathways. Confirmation of regulated lysosomal proteins discovered in proteomics were followed up with molecular techniques including Western blotting for Ras related protein 11 (RAB11), N-ethylmaleimide-sensitive factor (NSF), Glycogenin-1(GYG1) and lysosomal assays such as beta galactosidase and beta hexosaminidase. The EL fraction showed enrichment for EL specific proteins

such as lysosomal alpha glucosidase (GAA), Clathrin light chain (CLTB), T-complex protein 1 subunit beta (CCT2), vacuolar protein-sorting-associated protein 25 (VPS25) and many more.

Discussion: We observe in KEGG pathways analysis - mitochondrial TCA, OXPHOS, glycolysis and the AMPK pathway protein upregulation, highlighting that there could be an increase in ATP energy demand in AF. The upregulation of proteins in endocytosis and protein processing in endoplasmic reticulum suggest increased vesicular trafficking potentially to support the increased metabolic energetics and recycling of the cellular waste. However, the downregulation of autophago-lysosome fusion proteins such as dynein and dynamin indicate a possible disruption in the cellular degradation of macromolecules in the atrial cell during chronic AF. Further confirmation of P62, LC3 II/I ratio and whether they increase, indicating a blockage of autophago-lysosome and autolysosome fusion, is required. Our omics data provides evidence to support the role of the lysosome as an important intracellular organelle which may play a role in AF pathology.

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OC29

A novel SCN3b mutation in a Brugada Syndrome patient

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Brugada Syndrome (BrS) is characterised by right precordial ST-segment and T-wave changes and an increased risk of potentially fatal ventricular arrhythmias. Several mutations in the SCN3b gene encoding the cardiac voltage-gated sodium channel (Nav1.5) β 3 subunit have been identified in patients with BrS [1,2]. Here, we investigate the role of a novel SCN3b mutation in producing BrS phenotypes.

A 54-year old man presented with palpitations and dizziness. The diagnostic type 1 Brugada pattern was present and subsequently seen intermittently on a 24-hour ECG. Next-generation sequencing analysis of a 12 gene panel revealed a heterozygous 3-base deletion (ACG) from SCN3b position 412 to 414. The variant yields an in-frame deletion of a single threonine at position 138 (T138), localised within the Greek key motif- β sheet of the extracellular immunoglobulin-like domain of the $\beta 3$ -subunit. HEK293 cells stably expressing Nav1.5 were transiently transfected with eGFP labelled full-length $\beta 3$ ($\beta 3$ -WT), T138 deleted- $\beta 3$ ($\beta 3$ - Δ T138) or empty (eGFP) vectors as appropriate. Whole cell patch clamp protocols imposed initial, 100 ms duration, activating steps to test voltages varied in 5 mV increments between -140 and + 45 mV from a -120 mV holding potential to measure I_{Na} current (I_{Na}) activation. A further 50 ms duration step to a fixed -40 mV voltage assessed the resulting I_{Na} inactivation. Data (means $\pm$ SEM) were compared with One-Way ANOVA followed by Tukey post-hoc tests.

$\beta 3$ - Δ T138 expressing cells showed significantly decreased peak Na current densities I_{Na} compared to $\beta 3$ -WT (-213.0 $\pm$ 62.0 (n=8) vs -499.7 $\pm$ 111.0 pA/pF (n=7); P=0.043), but similar voltages of half maximal steady state activation $V_{1/2}$. WT- $\beta 3$ showed depolarized shifts in $V_{1/2}$ of steady-state inactivation relative to that of the eGFP cells (-72.67 $\pm$ 1.38 (n=9) vs -80.77 $\pm$ 1.85 mV (n=11); P=0.042), but this was not observed with the Δ T138- $\beta 3$ mutant (eGFP vs $\beta 3$ - Δ T138: -80.77 $\pm$ 1.85 mV (n=11) vs -79.95 $\pm$ 1.96 mV, (n=13), P=0.990). To analyse the functional impact of the mutant in the heterozygous state, I_{Na} recordings were made in cells co-expressing $\beta 3$ -WT and $\beta 3$ - Δ T138 ($\beta 3$ -WT/ Δ T138) at a 1:1 ratio. Peak I_{Na} was rescued by $\beta 3$ -WT/ Δ T138 ($\beta 3$ -WT vs $\beta 3$ WT/ Δ T138: -499.7 $\pm$ 111.0 pA/pF (n=7) vs -462.6 $\pm$ 41.7 pA/pF, (n=6), P=0.938). The $V_{1/2}$ of steady-state activation was unaffected with the $\beta 3$ -WT/ Δ T138 heterozygous mixture. The $V_{1/2}$ of steady-state inactivation of $\beta 3$ -WT/ Δ T138 showed increased variation but was not significantly different from $\beta 3$ - Δ T138 (-80.77 $\pm$ 1.85 mV (n=11) vs -78.40 $\pm$ 2.33 mV, (n=11), respectively, P=0.986). Co-immunoprecipitation and western blotting confirmed that the Δ T138 mutation did not disrupt the Nav1.5- $\beta 3$ interaction. In addition, biotinylation experiments revealed that the Δ T138 mutation did not prevent trafficking to and expression of the $\beta 3$ or Nav1.5 subunits on the cell surface membrane.

Our results demonstrate that the T138 deletion in the $\beta 3$ subunit reduced peak I_{Na} density and shifted channel inactivation to less depolarised potentials, hence reducing channel availability, contributing to a BrS phenotype. Nevertheless, the heterozygous expression of this mutant protein seems to ameliorate the effect on peak I_{Na} density, while maintaining more hyperpolarised channel inactivation possibly explaining the relatively mild phenotype exhibited by the patient.

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OC30

Evaluation of the murine experimental model of senescence SAMR1/SAMP8 for microRNA studies in Acute Myocardial Infarction

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Introduction: The Senescence-Accelerated Mouse Prone (SAMP8) and its control strain SAM Resistant (SAMR1) have been established as an experimental model to study age-associated vascular dysfunction (1), but scarcely used in epigenetic studies. The small non-coding RNA molecules microRNAs (miRNAs) can repress gene expression post-transcriptionally, and are being widely studied as potential biomarkers and therapeutic drugs in cardiovascular diseases.

Aims: We examine the suitability of SAMR1/SAMP8 as a physiological model to study age-related miRNA expression after inducing an acute myocardial infarction (AMI), based on the miRNA expression profile in AMI previously described in literature (2,3). We also determine the optimal time after the AMI to measure circulating miRNA levels.

Methods: Six-month old SAMR1 and SAMP8 (n = 6 per group) underwent AMI by ligation of the left anterior descending coronary artery and sham surgery. All interventions were performed under full anesthesia induced by inhalation of 5% isoflurane, and then maintained in 2% isoflurane. Animals were euthanized at different times (1h, 4h and 24h) and heart and blood were collected. AMI was confirmed by immediate discoloration of the left ventricle upon ligation, by ST segment elevation in the electrocardiogram (ECG), and by Triphenyltetrazolium chloride (TTC) staining of the non-ischemic areas of heart sections. Total RNA was obtained from serum (3) using miRNeasy Serum/Plasma Advanced Kit (Qiagen). Circulating miRNA considered as AMI biomarkers, miR-1, miR-133, miR-208 and miR-499 (2) were measured by qRT-PCR. Results are shown as mean $\pm$ SEM of the relative expression ($2^{-\Delta\Delta C_t}$), using U6 snRNA as endogenous control. *p*-values were calculated using ANOVA and statistical significance was considered when *p*<0.05. The investigation complies with ethical standards and was approved by the Ethics Committee for Animal Welfare of University of Valencia (2016/VSC/PEA/00135).

Results: Circulating values of miR-1, miR-133, miR-208 and miR-499 were significantly increased 4 hours after AMI when compared with sham group in both SAMR1 and SAMP8. While miR-1 totally rose at 4 hours after AMI (6.46 ± 1.32 , $p < 0.001$), miR-133, miR-208 and miR-499 levels exhibited a time-dependent upregulation, being maximal at 24 hours post-AMI ($p < 0.001$ vs. sham group). When samples from SAMR1 and SAMP8 were compared separately, the expression of all the analyzed miRNAs were lower in SAMP8 1h after AMI ($p < 0.05$). 4h after AMI, the ageing-related miRNAs miR-1 and miR-133 were higher in SAMP8 ($p < 0.05$), and 24h after AMI only miR-499 expression was significantly higher in SAMP8 ($p < 0.05$).

Conclusions: The serum levels of the four miRNAs mimicked the results obtained in humans and other studies performed in animals. With the combined use of the two strains, SAMR1 and SAMP8, the effect of ageing on miRNA expression after an AMI was preferably confirmed at 4 hours after AMI. Therefore, this study proposes the SAMR1/SAMP8 mice as a suitable model to study the effects of AMI and ageing in circulating miRNA expression.

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OC31

Functional analysis of two epithelial sodium channel isoforms in rodents

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Introduction: The epithelial sodium channel (ENaC) plays a key role in sodium homeostasis in tetrapod vertebrates. Four ENaC subunits (α , β , γ , δ) form heterotrimeric $\alpha\beta\gamma$ - or $\delta\beta\gamma$ -ENaCs. While the physiology of $\alpha\beta\gamma$ -ENaCs is well understood, the function of $\delta\beta\gamma$ -ENaC is unknown. The *SCNN1D* gene coding for δ -ENaC is a pseudogene in mice/rats, limiting research on δ -ENaC physiology. We investigated whether δ -ENaC is generally absent across rodents or limited to specific suborders.

Methods: The presence of potentially functional *SCNN1D* genes was assessed for all currently sequenced rodent genomes. Two-electrode voltage-clamp and single-channel patch-clamp electrophysiology were used to record transmembrane currents of human and guinea pig $\alpha\beta\gamma$ - and $\delta\beta\gamma$ -ENaCs expressed in *Xenopus* oocytes. Sodium self-inhibition (SSI) and activation by extracellular protease (chymotrypsin, 2 mg/ml), two mechanisms controlling ENaC activity, were characterised as previously described (Wichmann et al. 2019). Data are reported as means $\pm$ standard error, 'n' indicates the number of experiments.

Results: While *SCNN1D* was lost in five rodent lineages, including Muridae (mice/rats), functional *SCNN1D* is present in species within 21 of 35 currently recognised rodent families. Fusion of *SCNN1D* exons 11 and 12 to a 'super-exon' was observed in the Hystricognathi, a suborder containing the Caviidae family (guinea pigs). The 'super-exon' causes intron DNA sequences to be translated into a structurally flexible part in the δ -ENaC extracellular domain. Whole-cell and single-channel electrophysiology revealed that guinea pig $\delta\beta\gamma$ -ENaCs generate robust amiloride-sensitive currents. Amiloride-sensitive currents generated by guinea pig $\delta\beta\gamma$ -ENaC ($-6.03 \pm 0.79 \mu\text{A}$, $n = 20$) were significantly larger than those of $\alpha\beta\gamma$ -ENaC ($-2.1 \pm 0.21 \mu\text{A}$, $n = 19$, $p < 0.0001$, Student's unpaired t-test with Welch's correction), comparable to human ENaCs ($\delta\beta\gamma$ -ENaC: $-10.23 \pm 3.25 \mu\text{A}$, $n = 10$; $\alpha\beta\gamma$ -ENaC: $-6.52 \pm 3.19 \mu\text{A}$, $n = 10$, $p = 0.0191$, Student's unpaired t-test). The single channel conductance of guinea pig $\alpha\beta\gamma$ - and $\delta\beta\gamma$ -ENaC were $4.43 \pm 0.19 \text{ pS}$ ($n = 10$) and $4.21 \pm 0.35 \text{ pS}$ ($n = 7$), respectively. In both species, the magnitude of SSI was greater in $\alpha\beta\gamma$ -ENaCs compared to $\delta\beta\gamma$ -ENaCs (guinea pig $\alpha\beta\gamma$ -ENaC SSI: $46.52 \pm 2.71 \%$, $n = 18$, guinea pig $\delta\beta\gamma$ -ENaC SSI: $16.09 \pm 2.49 \%$, $n = 17$, $p < 0.0001$, Student's unpaired t-test; human $\alpha\beta\gamma$ -ENaC SSI: $57.68 \pm 2.21 \%$, $n = 10$, human $\delta\beta\gamma$ -ENaC SSI: $17.72 \pm 0.99 \%$, $n = 10$, $p < 0.0001$, Mann-Whitney U-test). Extracellular chymotrypsin stimulated guinea pig $\alpha\beta\gamma$ -ENaC currents by 1.52 ± 0.08 fold ($n = 13$), but not $\delta\beta\gamma$ -ENaC currents (0.78 ± 0.03 fold, $n = 15$, $p < 0.0001$, Mann-Whitney U-test). Similarly, human $\alpha\beta\gamma$ -ENaC currents increased 1.78 ± 0.11 fold ($n = 9$), but not $\delta\beta\gamma$ -ENaC currents (1.13 ± 0.06 fold, $n = 9$, $p = 0.0002$, Student's unpaired t-test with Welch's correction).

Conclusion: δ -ENaC is not generally absent from rodents but was independently lost in five lineages. Guinea pigs have two functional $\alpha\beta\gamma$ - and $\delta\beta\gamma$ -ENaC isoforms with biophysical and regulatory features similar to human orthologues. Guinea pigs represent a commercially available rodent model for studying mammalian δ -ENaC.

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OC32

Neuropeptide W (NPW) treatment ameliorates acetic acid-induced colonic injury in rats by upregulating colonic blood flow and depressing cyclooxygenase-2 expression

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Introduction: Neuropeptide W (NPW) regulates food intake and energy homeostasis, and was recently shown to have anti-oxidant and anti-inflammatory effects^{1,2}. The present study was aimed to investigate the possible beneficial effects of NPW treatment on colonic injury in rats induced with colitis.

Methods: Following the ethical approval by Marmara University Animal Care and Use Committee (33.2020.mar), Sprague Dawley female rats were randomised to control (n=8) or colitis groups (n=30) treated with saline or NPW (0.5, 1 or 5 µg.kg⁻¹.day⁻¹). Under light ether anaesthesia, colitis was induced by intracolonic administration of 1 ml of 5% (v/v) acetic acid diluted in saline through a polyethylene tube, which was positioned in the colon 8 cm past the anus, while intracolonic saline was instilled in the control group. Saline or NPW was injected subcutaneously immediately after the administration of acetic acid, and the treatments were continued in the following 4 days. At the 24th h following the last treatment, colonic blood flow was monitored under ketamine and chlorpromazine (100 and 10 mg.kg⁻¹, intraperitoneally) anaesthesia using a laser Doppler. Then, animals were decapitated and the distal 8 cm of the colon were removed for macroscopic scoring, determination of tissue wet-to-dry weight ratio and biochemical analyses. Colonic levels of interleukin (IL)-6 and tumour necrosis factor (TNF)-α were detected by commercial enzyme-linked immunosorbent assay (ELISA) kits, and malondialdehyde (MDA; showing lipid peroxidation), glutathione (GSH; antioxidant) and myeloperoxidase activity (MPO; indicator of neutrophil infiltration) levels in the colon were measured spectrophotometrically, while cyclooxygenase (COX) activity was determined by a fluorometric assay. COX-1 and COX-2 protein expressions were detected by western blotting. The statistical analyses of the data were carried out by one-way ANOVA using the GraphPad Prism 9.0 software.

Results: No significant differences in food intake, body weight, stool weight or colonic levels of IL-6, TNF-α and COX-1 expression were present among the experimental groups. Macroscopic score, wet/dry weight ratio, MPO activity, MDA level and COX-2 expression levels were increased in the colonic tissues of saline-treated colitis rats as compared to control group (p<0.05–0.001), while blood flow, GSH level and COX activity were significantly lower than those of the control group (p<0.05–

0.001). Colonic oedema was not reduced in NPW-treated groups, but macroscopic scores in NPW-treated (0.5 and 5 $\mu\text{g}\cdot\text{kg}^{-1}$) groups were not different than that of the control group. Colitis-induced elevation in MDA level was abolished with all 3 doses of NPW ($p<0.01$ - 0.001), but MPO activity was reduced only at its 5 $\mu\text{g}\cdot\text{kg}^{-1}$ dose of NPW ($p<0.05$). NPW, at the 5 $\mu\text{g}\cdot\text{kg}^{-1}$ dose, also elevated colonic blood flow ($p<0.05$) and replenished the depleted glutathione level ($p<0.05$). In NPW-treated groups (0.5 and 5 $\mu\text{g}\cdot\text{kg}^{-1}$), colitis-induced elevation in COX-2 protein expression was abolished ($p<0.001$), but the depressed COX activity was not altered by NPW treatment.

Conclusions: NPW ameliorated acetic acid-induced oxidative colonic injury in rats through the upregulation of colonic blood flow and the inhibition of COX-2 protein expression.

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Reference 2 :- Koyuncuoglu T et al. (2019). Acta Physiol 227, 87-87.

OC33

Regulation of the cystic fibrosis transmembrane conductance regulator (CFTR) by the nuclear bile acid receptor, farnesoid X receptor.

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Introduction and Aims: CFTR, a transmembrane Cl^- channel important in regulating intestinal fluid and electrolyte secretion, is implicated in the pathogenesis of a number of intestinal disorders. At physiological concentrations, bile acids, acting via the nuclear receptor, farnesoid X receptor (FXR), inhibit colonic epithelial CFTR expression. Dietary phytochemicals have been reported to have the capacity to modulate FXR signalling. Here, we set out to investigate mechanisms underlying FXR regulation of epithelial CFTR expression, and the potential for therapeutically targeting the receptor with dietary phytochemicals.

Methods: Polarised monolayers of T₈₄ colonic epithelial cells were treated with the FXR agonist, GW4064 (5 μM), in the absence or presence of a plant phytochemical, designated here as KFS1 (5 μM). CFTR and FXR expression were measured by qRT-PCR and immunoblotting. Expression of NF- κB , FOXA1, HNF1A, and CDX2, transcription factors that regulate CFTR expression, were measured by qRT-PCR. Nuclear translocation of NF- κB was measured by immunoblotting. Electrophysiological studies of T₈₄ cells were conducted in Ussing chambers. Studies on human colonic enteroids were

carried out with ethical approval from Johns Hopkins University School of Medicine Institutional Review Board, while studies of murine colonic epithelial enteroids were conducted with approval from the Institutional Review Board of the University of California San Diego.

Results: Treatment of T₈₄ monolayers with GW4064 significantly downregulated CFTR mRNA to 0.51 ± 0.06 fold after 12 hrs ($n=12$; $p<0.001$) and protein levels to 0.28 ± 0.06 fold after 48 hrs, compared to controls ($n=8$; $p<0.001$). Electrophysiological studies in Ussing chambers showed that GW4064 treatment for 48 hrs inhibited Cl⁻ secretory responses to the Ca²⁺-dependent agonist carbachol (CCh;100mM) and the cAMP-dependent agonist, forskolin (FSK;10mM) by 79.9 ± 7.5 % and 74.2 ± 8.9 %, respectively. Transcriptomic analysis of human colonic enteroids revealed FXR to be robustly expressed in secretory (crypt-like) cells and that its activation also induced CFTR downregulation. FXR activation did not alter expression or phosphorylation of the p65 subunit of NF- κ B, or inhibit its translocation to the nucleus. GW4064 downregulated FOXA1 mRNA expression by $33.2 \pm 5.2\%$ after 3 hrs ($n=4$; $p<0.05$), but had no effect on HNF1A or CDX2 expression. Treatment with the phytochemical, KFS1 (5 mM;24hrs), upregulated FXR mRNA and protein expression in T₈₄ cells and enhanced GW4064-induced downregulation of CFTR mRNA by 0.28 ± 0.05 fold ($n=8$; $p<0.01$) and protein by 0.25 ± 0.11 fold ($n=4$) after 24 hours. Similarly, KFS1 significantly upregulated FXR mRNA expression 2.3 ± 0.2 fold ($n=4$; $p<0.01$) compared to controls in murine colonic epithelial enteroids and enhanced GW4064-induced downregulation of CFTR mRNA 0.5 ± 0.1 fold ($n=4$; $p<0.05$) compared to GW4064 alone. Finally, KFS1 enhanced FXR inhibition of agonist-induced Cl⁻ secretory responses across T₈₄ cells mounted in Ussing chambers.

Conclusion: FXR regulates colonic epithelial CFTR expression and function by a mechanism which appears independent of NF- κ B, but which may involve FOXA1. By virtue of their ability to upregulate FXR expression, and thereby enhance its antisecretory actions, plant extracts containing KFS1 have excellent potential to be developed as targeted nutraceuticals for the treatment and prevention of intestinal disease.

Acknowledgements :- This work was supported by a Science Foundation Ireland Principal Investigator award to Dr Stephen Keely.

OC34

Impact of the F508del Mutation on Pig Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), a Cl⁻ Channel with Enhanced ATP-Dependent Channel Gating

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Dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR), an epithelial anion channel, causes the genetic disorder cystic fibrosis (CF) (1). F508del, the most common CF mutation,

disrupts CFTR processing and intracellular trafficking, reduces CFTR stability at the plasma membrane and alters channel gating (2). To understand CF pathogenesis and test new therapeutics, CF pigs with the F508del mutation were generated (3). Here, we investigate the single-channel behaviour of pig CFTR and the impact on it of the F508del mutation. Using the patch-clamp technique, we studied CFTR Cl⁻ channels in excised inside-out membrane patches from CHO cells transiently transfected with CFTR constructs voltage-clamped at -50 mV using a large Cl⁻ concentration gradient ([Cl⁻]_i, 147 mM; [Cl⁻]_o, 10 mM); temperature was 37 °C (4). Like human CFTR, pig CFTR formed low conductance Cl⁻-selective channels regulated by protein kinase A-dependent phosphorylation and intracellular ATP. However, distinct differences were observed in the frequency and duration of channel openings. At 1 mM ATP, channel openings of pig CFTR were 4-fold longer than those of human CFTR, whereas the long closures separating channel openings of pig CFTR were similar in duration to those of human CFTR. Consequently, the open probability (P_o) of pig CFTR (0.68 ± 0.12 , $n = 13$; means $\pm$ SD) was 2-fold higher than that of human CFTR (0.39 ± 0.07 , $n = 15$) (Student's unpaired t-test, $P < 0.0001$). To explore these gating differences, we examined the ATP dependence of P_o between 0.03 mM and 3 mM ATP. By fitting mean P_o data with Michaelis-Menten functions, we found that ATP regulated pig CFTR with increased apparent affinity and efficacy (human: $K_D = 180 \mu\text{M}$, $P_{o(\text{max})} = 0.61$, $r^2 = 0.96$, $n = 4 - 16$; pig: $K_D = 25 \mu\text{M}$, $P_{o(\text{max})} = 0.76$, $r^2 = 0.78$, $n = 4 - 7$). Consistent with previous studies (4, 5), the severity of the F508del mutation varied across species. Human F508del-CFTR exhibited a severe gating defect characterised by infrequent channel openings and marked thermostability demonstrated by P_o diminishing from ~ 0.15 to 0 within 8 minutes at 37 °C (4). By contrast, the F508del mutation had reduced impact on pig CFTR. First, the F508del mutation decreased the P_o of pig CFTR by only 0.5-fold (0.38 ± 0.18 , $n = 9$) (Student's unpaired t-test, $P < 0.001$ vs. pig wild-type CFTR), but that of human CFTR by 5-fold (0.08 ± 0.03 , $n = 7$) (Student's unpaired t-test, $P < 0.0001$ vs. human wild-type CFTR). Second, pig F508del-CFTR showed greater thermostability at 37 °C, with P_o only gradually declining from ~ 0.26 to ~ 0.10 over a 20-minute period ($n = 5$). We conclude that *i*) pig CFTR forms a regulated Cl⁻ channel with enhanced ATP-dependent channel gating, and *ii*) the F508del mutation has distinct consequences in human and pig CFTR. Thus, these data provide insights into species-specific differences that illuminate analyses of CFTR structure and function with the potential to inform the development of new CF therapeutics.

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Potential targets for underactive bladder treatments: Receptor-mediated contractions of the urinary bladder urothelium

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Introduction: Underactive bladder is a complex clinical condition with a limited amount of research focused on identifying the mechanisms underlying its presentation. Most commonly, patients present with urgency, weak stream, nocturia, and urinary frequency. Underactive bladder is a multifactorial condition that can result from a variety of pathological processes, including idiopathic, neurogenic, myogenic or functional. However, there is limited research into the mechanisms underlying this disorder. **Aim:** This study aimed to identify potential receptors which could mediate contractions of the urinary bladder urothelium (1-3). **Methods:** Porcine urothelial strips were mounted in gassed Krebs-bicarbonate solution at 37°C and the tissue baseline tension (grams) was recorded before and after the addition of agonists as per prior research (4, 5). Ethical approval was not required as tissues were sourced from the local abattoir after slaughter for the routine commercial provision of food. Data obtained was analysed using paired Students *t*-tests. **Results:** The urothelium exhibited strong contractions upon receptor stimulation, with particularly rapid responses to many of the G protein-coupled receptor agonists. Contractions were induced by (mean $\pm$ SEM): carbachol 4.06 ± 0.43 g (1 μ M, $P < 0.001$, $n = 11$); histamine 1.41 ± 0.29 g (100 μ M, $P < 0.01$, $n = 7$); 5-HT 4.38 ± 0.53 g (100 μ M, $P < 0.001$, $n = 8$); α, β -methylene ATP 1.88 ± 0.27 (10 μ M, $P < 0.001$, $n = 8$); and neurokinin-A 2.3 ± 0.25 g (300nM, $P < 0.001$, $n = 8$). **Conclusions:** The strongest contractions were induced by stimulation of the muscarinic and 5-HT receptors, with plans for follow-up experiments to identify the influence of calcium and second-messenger mechanisms involved. A greater understanding into the various mediators of contraction may help identify future therapeutic targets to be employed in the pharmaceutical management of underactive bladder. In addition, further categorising the contractile responses of these receptors will provide further insights into the methods of contraction within the urinary bladder wall.

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OC36

Physiology students can use a quality improvement approach to support a safe and efficient medication pathway in geriatric rehabilitation

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Quality improvement (QI) science enhances patient care and safety by using a structured experimental approach to learning and tests of change. We piloted the involvement of physiology honours students in such activities (in partnership with clinical staff) as part of their final year research project. We aimed to establish a comprehensive process map of medication pathways and identify areas for improvement in documentation standards. We also aimed to enhance the QI culture in a geriatric rehabilitation facility. We report how physiology students can apply QI principles to enhance medication usage and contribute to improving patient safety as part of a multidisciplinary team.

Background

In a geriatric rehabilitation ward located in a small district hospital, this study focused on improving medication safety for the patient population. Multi-morbidities and polypharmacy are complex but common issues in geriatric patients, therefore continuity of care is necessary inter-departmentally and during care transitions. This can be achieved through ensuring standardised documentation in a medical file to simplify medicines reconciliation (Med Rec). Medicines reconciliation is the process of accurately recording a person's medicines and should be completed as soon as possible upon admission to a hospital environment.

Specific project aims: (a) To accurately establish the medication pathway of Ward X. (b) To improve documentation for sources and accountability in prescriber identification in the Med Rec Forms to 95% by 31st July 2020. (c) To improve ward staff experience with the medical files to 95% by 31st July 2020.

Methods

Established QI methodology was utilised. This included process mapping, Pareto charts, questionnaires for quantitative and qualitative data, and 3 sequential Plan-Do-Study-Act cycles for

the final intervention. Presentations were held weekly during multidisciplinary team and supervisory meetings to update ward staff on progress.

Results

A detailed process map of the medication pathway of the ward was established.

The final intervention was a Table of Contents in patient notes, creating a standardised organisation system for the medical files to simplify completion of the Med Rec Form. Completion of the "Medication history taken by" section of the Med Rec Form increased from 46% to 80% for "Name", "Role", "Date"; scores for the medical file for nurses (n=3), doctors (n=2), and allied health professionals (AHPs) (n=4) increased significantly for efficiency, ease of use, and overall experience ($p=0.03$ for each parameter). The target of reaching 95% completion for parameters was not achieved but positive results occurred despite the ward undergoing drastic changes and reconfiguration during this project due to the onset of the global pandemic at the end of March 2020.

Conclusion

This intervention provided a clear, standardised organisational system which positively affected medicines reconciliation completion and user experience with the medical files. It also demonstrates that physiology undergraduate students can use a quality improvement approach to support a safe and efficient medication pathway in geriatric rehabilitation. Due to the success of this pilot, we aim to expand the involvement of medical science honours students to help our local healthcare facilities increase their quality improvement work.

Acknowledgements :- We thank the staff of Woodend Hospital, Aberdeen for taking the time to work with the student researcher and for their constructive feedback during the project.

OC37

Effect of High Intensity Interval Exercise on BDNF Isoforms and cognitive function in patients with type 2 diabetes

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Introduction: The continuous rise of type 2 diabetes (T2D) and its associated chronic complications such as peripheral neuropathy and cognitive dysfunction remains a global problem. Physical exercise has been shown to improve cognitive performance in many populations including T2D patient population. The mechanism via which exercise is believed to improve cognitive function is an increase in mBDNF levels. High-intensity interval exercise (HIIT) causes an increase in mBDNF levels which is associated with increased cognitive performance. ProBDNF, an isoform of BDNF, which has

opposing effects to that of mBDNF decreases with HIIT. There is a paucity of data on the roles of pro-BDNF in diabetes-induced cognitive dysfunction compared to the numerous reports on the function of mature BDNF. **Aims and objectives:** The aim of the study was to determine the effect of HIIT on cognitive performance in patients with T2D. **Methods:** 32 nondiabetic and 54 diabetic subjects were screened and enrolled into this study. Ethical clearance was obtained from the Kano state ministry of health and each subject signed informed consent prior to inclusion. The subjects were subjected to 6 HIIT sessions spread over 2 weeks. MoCA test was administered before starting (day 1) and after completion (day 6). 5mL of blood was also collected from each subject on the same days in plain and EDTA tubes. The blood in the plain tubes was used to collect serum for the determination of proBDNF and BDNF levels employing the ELISA technique. Plain tube blood was used for the determination of FBG (using Accucheck glucometer) and HbA1c test (using point of care). Data were analysed using the Wilcoxon rank test and dependent sample t-test. Results presented as Median (Min-Max). **Result:** proBDNF isoform was found to be significantly lower after HIIT (5.04 ± 0.20 vs 4.94 ± 0.14) with significant rise in BDNF levels were observed after (8.17 ± 0.30) exercise among the diabetics compared to baseline levels (5.32 ± 0.29). The total MoCA score increased in both groups. The scores in all the domains of the MoCA test except visuospatial domain were also found to be higher compared to the baseline values ($p < 0.05$). **Conclusion:** HIIE improved cognitive function in patients with T2D, apparently through increase in conversion of proBDNF isoform to mBDNF.

OC38

A proof-of-concept experimental study to quantify lobar gas exchange

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Introduction

Predicted post-operative lung function helps stratify lung cancer patients' mortality risk and potential suitability for resection¹. Carbon monoxide transfer factor and forced expiratory volume in 1 s tests are commonly used to provide global indicators of lung function. Gas exchange differs between lung regions in health and disease², and this heterogeneity may be exaggerated by lung cancer, limiting the usefulness of whole-lung function tests. Quantifying lobar contribution to overall pulmonary gas exchange could provide more accurate predictions of post-operative lung function.

Our study aimed to measure lobar oxygen uptake in a proof-of-concept experimental study.

Methods

Ethical approval (AREC ref.C98/16) was obtained and conformed with the NIH and ARRIVE guidelines³. Two pigs (29 kg) were studied in dorsal recumbency under general anaesthesia with intravenous infusion of ketamine, fentanyl and midazolam, and mechanical ventilation via tracheostomy. Mechanical ventilation was delivered in pressure-control mode with tidal volume of 10 mL/kg, respiratory rate 12 breaths per minute, inspiratory:expiratory ratio of 1:2, and inspiratory rise time of 0 s. A saline lavage surfactant-depletion lung-injury model was induced in one pig to study heterogeneous lungs⁴.

Fine-bore, fibre-optic PO₂ sensors (response time <150 ms) were inserted into a bronchoscope until the sensor tip was visible inside the main bronchus entering a lobe; the bronchoscope remained within the large airways⁵. Data for ~12 breaths were collected in each lobe and averaged to produce a single breath per lobe. PO₂ tidal variation was calculated as the peak-to-trough difference in the averaged breath. Tidal variation in lobar PO₂ (kPa) was then converted to tidal variation in oxygen concentration [Δ PO₂ (%)], dividing by 101.3 kPa.

Whole-lung volume CT scans were recorded during end-inspiratory and end-expiratory breath-holding manoeuvres under the same ventilation conditions as during the lobar PO₂ measurements. Lobar volumes were calculated via segmentation at 3 mm intervals; figure 1A, B and C illustrate the segmentation process. Gas volumes were calculated as

Lobar Gas volume (mL) = Lobar volume (cm³) x -[Mean voxel density (HU)]/1000

and lobar oxygen uptake calculated by multiplying tidal gas volume by Δ PO₂ (%).

Results

Figure 1D illustrates lobar PO₂ tidal variation, greater in the saline lavage lung injury model than in the control pig.

Figure 1 inset table presents lobar PO₂, end-inspiratory, end-expiratory and tidal volumes, and the associated lobar oxygen uptake. PO₂ tidal variation ranged from 4.7 to 20.3 kPa in different lobes. Lobar tidal volume ranged from 12.9 to 75.1 mL, with lobar oxygen uptake ranging from 0.6 to 11.9 mL/breath. Figure 1E shows oxygen uptake in both animals, visualising the difference between lobes, and overall pulmonary oxygen uptake between animals.

Conclusions

We demonstrated the feasibility of a novel technique to calculate lobar oxygen uptake in mechanically ventilated control and saline-lavage lung-injury pig models. The distribution of lobar contribution to gas exchange can vary significantly, even in patients with similar overall lung function, but tests currently used cannot determine gas exchange at the lobar level. Following refinement, the proposed technique could provide valuable insight to support the decision on patients' suitability for lobar resection.

Figure caption

Figure 1. Computed tomography scans and associated 3D reconstruction of the porcine lung, lobar partial pressure of oxygen (lobar PO_2) measured during a respiratory cycle, and lobar and whole lung oxygen uptake.

Panels A and B show the same axial slice from the same pig; panel A is the original image, and panel B shows the lobes labelled by colour (Left Caudal= green, Left Cranial= yellow, Right Accessory= red, Right Caudal= orange, Right Cranial= pink, Right Middle= blue). This colour coding is used in panels C, D and E also.

Panel C is a three-dimensional reconstruction of the control pig lungs at end-inspiration, viewed in the anterior-posterior direction. The model is labelled as follows: I= inferior, S= superior, R= right, L= left.

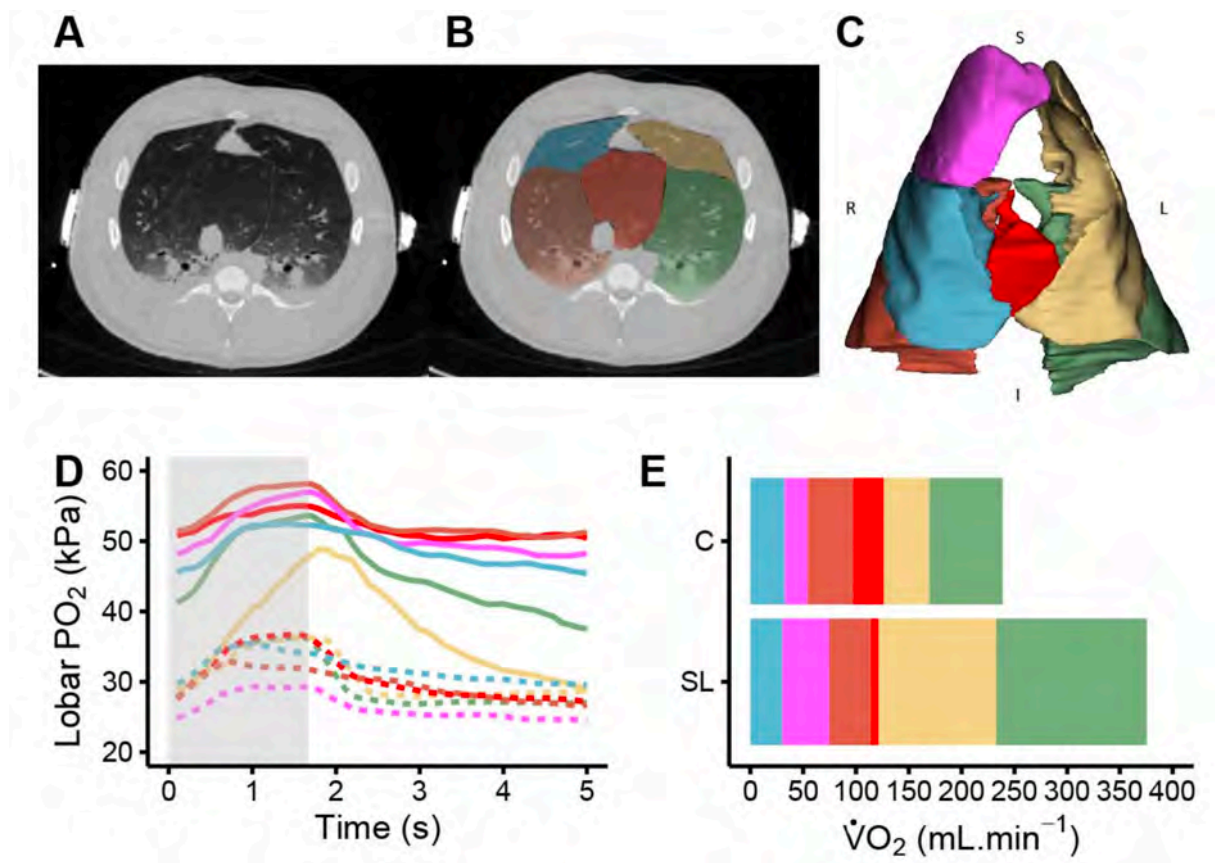
Panel D shows the lobar partial pressure of oxygen (PO_2) recorded with fibre optic technology at each 0.1 s interval during a respiratory cycle for each lobe (data averaged over at least 3 cycles); dashed and solid lines show values respectively for the control and saline lavage lung injury pig. The shaded (left) and clear (right) regions in panel D indicate inspiration and expiration respectively. Recordings were made during pressure-controlled mechanical ventilation, with tidal volume 10 mL/kg, I: E 1:2 and respiratory rate 12 breaths per minute.

Panel E shows lobar and whole lung oxygen uptake (mL/min).

Inset table: lobar densities and volumes were calculated from CT images recorded during end-inspiratory and end-expiratory breath holding manoeuvres. Lung segmentation started at the bifurcation of the trachea. Rounding to the nearest % performed. PO_2 = Partial pressure of oxygen, HU= Hounsfield units, C= Control pig, SL= Saline lavage lung injury model.

Figure 1, Inset table.

Lobe	Animal	PO ₂					Density	Volume			Oxygen uptake	
		Mean	Max	Min	Delta	Delta		End-Inspiratory	End-Expiratory	Tidal	per breath	Proportion of total lung
		kPa				%	HU				mL/breath	%
Left	SL	45.6	53.6	37.5	16.1	16	-247.5	105.2	30.1	75.1	11.9	38
Caudal	C	29.8	36.4	26.8	9.6	9	-367.4	112.3	51.3	61.0	5.8	29
Left	SL	37.3	48.9	28.6	20.3	20	-450.1	97.1	50.6	46.5	9.3	30
Cranial	C	30.7	36.2	27.9	8.3	8	-548.3	95.9	52.0	43.9	3.6	18
Right	SL	52.0	55.0	50.3	4.7	5	-334.8	23.8	10.9	12.9	0.6	2
Accessory	C	30.8	36.7	27.2	9.4	9	-571.2	43.8	17.7	26.1	2.4	12
Right	SL	53.3	58.1	50.6	7.6	8	-153.6	59.5	15.3	44.3	3.3	11
Caudal	C	29.6	32.9	26.6	6.3	6	-341.4	104.2	46.6	57.6	3.6	18
Right	SL	51.1	57.0	47.9	9.1	9	-451.4	86.4	45.1	41.3	3.7	12
Cranial	C	26.4	29.4	24.4	5.0	5	-562.6	84.1	48.0	36.1	1.8	9
Right	SL	48.9	52.5	45.4	7.1	7	-472.6	75.9	40.1	35.8	2.5	8
Middle	C	31.9	35.4	29.5	5.9	6	-672.3	115.3	69.8	45.5	2.7	13



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Declarations of interest:

The authors declare that they have no conflicts of interest.

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OC39

The mediating role of endocrine factors in the positive relationship between fat mass and bone mineral content in children aged 9-11 years: The Physical Activity and Nutrition in Childhood Study

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Introduction: Increased fat mass may increase bone mass through greater biomechanical load on bones [1]. Further, fat mass is a regulator of endocrine function which may influence bone metabolism both positively and negatively [2]. The mediating role of endocrine factors in the positive relationship between fat mass and bone mass in pre- and early-pubertal children is unclear. The aim of this study was to examine the association between fat mass and bone mineral content (BMC), and to investigate whether this relationship is mediated by insulin, free leptin index, adiponectin, dehydroepiandrosterone sulphate (DHEAS), testosterone and estradiol in girls and boys aged 9 to 11 years.

Methods: We utilised cross-sectional data from the 2-year follow-up of the Physical Activity and Nutrition in Childhood study, an ongoing longitudinal study in a population sample of Finnish children (n = 396, 203 girls). The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. The parents or caregivers of the children provided their written informed consent, and the children provided their assent to participation. Total body less head (TBLH) BMC and fat mass were assessed with dual-energy X-ray absorptiometry. Endocrine factors were assessed from fasted venous blood samples. Serum insulin was measured by electrochemiluminescence immunoassay. Plasma leptin was analysed using a competitive radioimmunoassay. Plasma leptin receptor, serum high-molecular-weight adiponectin and serum DHEAS were analysed using enzyme linked immunosorbent assay kits. Testosterone and estradiol

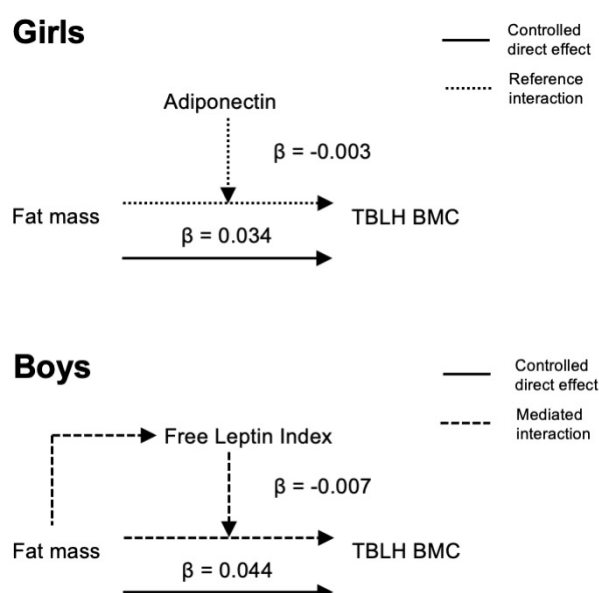
were measured using liquid chromatography-mass spectrometry. We applied the novel 4-way decomposition method [3] to analyse associations between fat mass, endocrine factors, and BMC, adjusting for age, stature, pubertal status, lean mass and baseline BMC. The 4-way decomposition method allows the total relationship between fat mass and BMC to be separated into four components: controlled direct effect, reference interaction, mediated interaction, and pure indirect effect.

Results: Fat mass had a positive controlled direct effect on BMC in girls and boys ($\beta = 0.033$ to 0.68 , $p < 0.001$). We observed a negative interaction between fat mass and adiponectin with BMC in girls ($\beta = -0.003$, $p = 0.033$). We observed a negative mediated interaction between fat mass and free leptin index with BMC in boys ($\beta = -0.007$, $p = 0.007$).

Conclusions: In children with greater levels of adiponectin and free leptin index, the relationship between fat mass and BMC became less positive, in girls and boys respectively. Fat mass primarily positively influenced BMC through pathways not related to the endocrine factors we assessed, likely through mechanical loading. As the relationship between fat mass and endocrine factors with BMC is likely moderated by weight status and pubertal stage, further research is needed to assess whether these observations extend to children and adolescents with overweight and obese weight status.

Figure 1. Summary of significant mediation and moderation effects from the 4-way decomposition, adjusted for age, stature, pubertal status, lean mass, and baseline TBLH BMC.

Girls: $n = 191$, Boys: $n = 181$.



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Remote teaching and learning in Higher Education: A cross-sectional survey on the impact on physical activity and health-related quality of life of staff and students.

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Introduction:

Physical activity (PA) is an important determinant of health. Interventions to increase PA have been greatly explored in different cohorts but there is a paucity of research focusing on PA in the academic community, particularly in the higher education institutions (HEI). With the current imposed remote teaching, there is an increase in prolonged sitting time, inactivity, screen time, risking the occurrence of musculoskeletal issues like back or neck pain. Thus, researching the levels of PA (and correlators) is

pivotal. This study examined the PA levels and associations with the health-related quality of life (HRQoL) of staff and students at a UK HEI.

Methods:

Eighty - eight staff (n = 44; mean age 47 ± 10 years; 82% male) and students (n = 44; mean age 21.4 ± 4.0 ; 80% male) participated in the online survey after giving consent. Self-reports of PA and HRQoL were examined using the International Physical Activity Questionnaire – Short Form (IPAQ-SF) and European Quality of Life 5 Dimensions (EQ5D). Linear regression examined the associations between PA and HRQoL against 95% level of significance ($p \leq 0.05$).

Results:

About 62% of the staff and 55% of students were minimally active. Staff, 18 %, and students, 23%, who performed health-enhancing physical activity (HEPA) had the median (range) of walking 676 (0 – 2772) and 528 (10 - 5544) (MET/week) respectively. Median moderate to vigorous physical activity (MVPA) was 200 (0 – 2940) for staff and 500 (0 – 3000) (MET/week) for students. The vigorous physical activity (VPA) of staff was 334 (0 – 2400) and student, 100 (0 – 3840) (MET/week). The total physical activity (TPA) was 480 (180 - 960) and 1116.0 (0 - 11472) (MET/week) for staff and students respectively. Although, the TPA was associated with self-care ($r = - 0.34$; $p < 0.001$), usual activity ($r = - 0.30$, $p = 0.03$), anxiety/depression ($r = - 0.28$, $p = 0.03$) in staff but it was not was not predictive of these. There was no association with pain/discomfort and mobility in the staff. The TPA was not associated with all the domains of HRQoL in the students.

Conclusion:

A high proportion of minimal activity with varying levels of physical activity was reported during remote teaching and learning in both staff and students of UK HEI. The variation in associations of physical activity with self-care, usual activity, and anxiety/depression could provide further insights on factors influencing health-related quality of life. Large-sample studies are required to validate these findings and provide strategies for promoting health and well-being for both students and staff.

Keywords: Physical activity, Health-related quality of life, remote teaching and learning, staff and students, higher education

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The effect of the 2020 COVID-19 lockdown measures on the diet, hydration and physical activity habits of Scottish adults

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Introduction: COVID-19 was declared a global pandemic due to the virus's spread and the danger posed to vulnerable individuals. Scotland introduced lockdown and social isolation measures, reducing time spent outdoors and in public spaces. Increased social isolation has been linked to poorer mental health and well-being, leading to increased alcohol consumption, reduced physical activity (PA) levels, and increased sedentary activities. This study aimed to examine the impact of the lockdown due to the COVID-19 pandemic on physical activity, diet, and hydration in Scottish adults.

Methods: A mixed-methods online survey, which utilised both open and closed questions, was used to examine these factors during lockdown from July 2020 to November 2020. Participants were asked about their physical activity, diet, and hydration habits and whether lockdown impacted these. The study was approved by the University of Stirling General University Ethics Panel (GUEP).

Results: A total of 297 adults (aged 18-72 y) living in Scotland completed the self-reported questionnaire. The results demonstrated that lockdown measures increased the consumption of healthy and unhealthy food and alcohol. Most participants reported that their PA levels had decreased, with only 22% meeting the guidelines for physical activity when the strength guidelines were included. The participants completed significantly less moderate PA than the guidelines recommend ($p < 0.001$). Further analyses revealed that there were not sufficient levels of strength-based activities completed, and sitting activity increased for nearly all the participants.

Conclusions: Hydration habits were not largely impacted during the lockdown; however, alcohol consumption increased amongst the female respondents. The percentage of participants meeting the PA guidelines was significantly low when accounting for strength-based activities. This observation underlines the importance of addressing the impact of social isolation on lifestyle factors such as PA and alcohol intake. The main limitations of this study are the sample size and the utilisation of a self-reported questionnaire; however, the results provide an indicator of the changes in diet, hydration and PA in the Scottish adults during the COVID-19 lockdown, another possible area of future research could include other methods such as the utilisation of accelerometers to quantify the PA of the participants and a 7-days weighed food/fluid diary to estimate the energy, fluids and macronutrients intake.

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Activation of Ano1 channels by Ca^{2+} signalling in interstitial cells of Cajal contributes to tone in the mouse and monkey lower esophageal sphincter

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Background

Myogenic tone in the lower esophageal sphincter (LES) prevents stomach acid refluxing into the esophagus body¹. LES tone has been proposed to be due to activation of Ca^{2+} -activated- Cl^- channels (CaCC) leading to the opening of voltage-dependent $\text{Cav}_{1.2}$ Ca^{2+} channels in LES smooth muscle cells (SMCs). LES-SMC are relatively depolarized, facilitating activation of $\text{Cav}_{1.2}$ channels to sustain contractile tone². In other regions of the gut, intramuscular interstitial cells of Cajal (ICC-IM) influence SMC by the activation of CaCC encoded by *Ano1*. In colon ICC-IM, Ca^{2+} transients activate *Ano1* channels and contribute to setting the resting membrane potential of colonic muscles^{3,4}. Thus, Ca^{2+} signalling is fundamental for ICC-IM function, yet little to nothing is currently known about Ca^{2+} signalling in LES ICC-IM or if they express *Ano1* channels. We hypothesized that ICC-IM exhibit intracellular Ca^{2+} signalling that activates *Ano1* channels, this then depolarizes electrically connected SMC, to set favorable conditions for activation of $\text{Cav}_{1.2}$ channels and contributes to myogenic tone of the LES.

Methods

Immunohistochemistry and isometric tension recordings were performed on strips of mouse and *Cynomolgus* monkey LES. LES SMCs and ICC-IM were isolated from mice expressing ICC and SMC fluorescent reporters (*Kit*^{+/copGFP} and *SmHC*^{+/eGFP} mice respectively). Ca^{2+} transients in LES ICC-IM were visualised *in-situ* in mice expressing GCaMP6f using Cre-LoxP, driven by a *Kit* promoter). Spatio-temporal map analysis was used to quantify Ca^{2+} transients⁵.

Results

Immunohistochemistry revealed that *Ano1* channels are highly expressed in ICC-IM (*Kit*⁺ cells) of the mouse (n=6) and monkey LES (n=8). qPCR data from enriched populations of LES ICC-IM and SMC from fluorescent reporter mice showed expression of enriched *Ano1* transcripts occurred almost exclusively in ICC-IM (n=17). ICC-IM imaged from LES tissues of *Kit*-GCaMP6f mice exhibited stochastic intracellular Ca^{2+} signaling *in situ*, firing hundreds of Ca^{2+} transients min^{-1} from multiple intracellular sites. Ca^{2+} transients were abolished by the SERCA pump inhibitor cyclopiazonic acid ($P < 0.001$, n=12). ICC-IM Ca^{2+} release relied on the $\text{IP}_3\text{R1}$ channel as ICC-IM had enriched expression of *Itpr1*, with little to no expression of *Itpr2/3* or *Ryr1-3*. Furthermore, ICC-IM Ca^{2+} transients were unaffected by the ryanodine receptor inhibitor tetracaine ($P > 0.05$, n=8). Ca^{2+} influx played a major role in sustaining Ca^{2+} transients, as they were reduced after 6 mins in Ca^{2+} free medium ($P < 0.001$, n=13). ICC-IM contained enriched transcripts for genes encoding proteins for store-operated- Ca^{2+} entry (SOCE) such as *Stim1/2* and all 3 variants of the plasma membrane bound Orai Ca^{2+} channel (*Orai1-3*), whereas ICC-IM showed minimal expression of *Cacna1c* (encodes $\text{Cav}_{1.2}$ channels). ICC-IM Ca^{2+} transients were unaffected by the $\text{Cav}_{1.2}$ channel inhibitor nifedipine (n=13, $P > 0.05$) but were reduced in frequency by an Orai channel antagonist (GSK 7975A, n=10, $P < 0.01$). Finally, mouse (n=11) and monkey (n=10) LES strips developed spontaneous tone that was reduced by ~ 60 % ($P < 0.001$) by the *Ano1* channel antagonist Ani9.

Conclusions

Ano1 channels, expressed in ICC-IM, are activated by dynamic Ca^{2+} signalling in ICC-IM, contributing to spontaneous tone in the mouse and monkey LES.

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Interaction of arrhythmia-associated calmodulin mutations with voltage-gated Ca^{2+} channel ($\text{Ca}_{v1.2}$) and Ca^{2+} /CaM-dependent protein kinase II delta ($\text{CaMKII}\delta$).

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) and Long-QT Syndrome (LQTS) are two major inherited lethal cardiac channelopathies responsible for sudden death predominantly among young population. Of late, mutations in the highly conserved calcium (Ca^{2+}) sensor protein calmodulin (CaM) have been linked to these cardiac arrhythmia syndromes in human patients. CaM regulates the activity of ion channels and several critical proteins in the heart either via direct binding or indirectly through the activation of Ca^{2+} /CaM-dependent protein kinase II delta ($\text{CaMKII}\delta$).

However, there is a major gap in understanding the precise molecular mechanism of CaM-associated cardiac conditions.

One of the ion channels regulated by CaM is the voltage-gated Ca^{2+} channel $\text{Ca}_{v1.2}$, which is essential for maintaining Ca^{2+} homeostasis in the cell. CaM binds $\text{Ca}_{v1.2}$ in the N-terminal (NSCaTE) and the C-terminal regions (IQ and C domains). The CaM binding site in CaMKII δ consists of the amino-acid region 294-315 (CaMKII $\delta_{294-315}$). Short synthetic peptide encompassing the CaM binding domains were used for the characterisation of CaM-target interactions. The binding kinetics of CaM with each peptide, in Ca^{2+} -free (5 mM EGTA) and Ca^{2+} -saturated conditions (5 mM CaCl_2), were measured using isothermal titration calorimetry (ITC). The atomistic details of binding were obtained through X-ray co-crystallisation experiments.

Using ITC, we showed that none of the peptides bind to CaM in Ca^{2+} -free conditions whereas all the peptides bind Ca^{2+} -CaM with CaMKII $\delta_{294-315}$ showing the highest affinity (23 ± 5 nM, $n = 6$). Amongst the $\text{Ca}_{v1.2}$ peptides, $\text{Ca}_{v1.2}\text{IQ}$ showed the highest affinity towards CaM-WT (415 ± 19 nM, $n = 10$) followed by $\text{Ca}_{v1.2}\text{NSCaTE}$ (2.0 ± 0.2 μM , $n = 4$) and $\text{Ca}_{v1.2}\text{C}$ (8.0 ± 0.4 μM , $n = 6$). We showed that the binding affinity of CaMKII $\delta_{294-315}$ for the CPVT-associated CaM variants is comparable to CaM-WT, whereas it is reduced by ~ 5 fold for the LQTS-associated variant. CPVT-associated mutations did not significantly affect the interaction of CaM with $\text{Ca}_{v1.2}\text{IQ}$ peptide, however K_d increased to 169 ± 4 nM ($n = 5$) for the LQTS variant when compared to wild-type. The LQTS-associated mutation induced a 65-fold reduction in affinity (134 ± 8.7 μM , $n = 5$) for CaM: $\text{Ca}_{v1.2}\text{NSCaTE}$, when compared to the wildtype. We showed that binding of CaM to the $\text{Ca}_{v1.2}\text{C}$ peptide can be reduced up to 5-fold by arrhythmia-associated mutations (40 ± 2 μM , $n = 5$). We obtained high resolution crystal structures for CaM variants in complex with CaMKII $\delta_{294-315}$ or $\text{Ca}_{v1.2}\text{IQ}$ peptides ($2.0 \text{ \AA} - 2.8 \text{ \AA}$). We did not observe noticeable structural difference between the CaM-WT and mutant peptide complexes.

For the LQTS-associated CaM variant, impaired binding to $\text{Ca}_{v1.2}$ could result in dysregulation of Ca^{2+} signalling and consequently, generate irregular heartbeats characteristic of the disease. In contrast, Ca^{2+} -CaM: $\text{Ca}_{v1.2}$ interaction is not altered in CPVT which suggests a distinct molecular mechanism.

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Small conductance Ca^{2+} -activated K^+ current (I_{SK}) may not be activated by $[\text{Ca}^{2+}]_i$ elevation in non-failing ventricular myocytes.

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Background: The small conductance Ca^{2+} -activated K^+ current (I_{SK}) may be enhanced in ventricle as a result of heart failure, but whether I_{SK} may be enhanced by $[\text{Ca}^{2+}]_i$ -elevation, in non-failing ventricle, is unknown. **Aim:** To investigate the effects of an I_{SK} blocker, ICA, on rabbit ventricular ion currents associated with $[\text{Ca}^{2+}]_i$ elevation produced by stimulation of the $\text{Na}^+/\text{Ca}^{2+}$ -exchanger current, $I_{\text{Na/Ca}}$. **Methods:** Ventricular myocytes were isolated from hearts excised from rabbits (anaesthetised with Na^+ -pentobarbital, 100 mg/kg I.V.). Ion currents and $[\text{Ca}^{2+}]_i$ (by Fura-2) were recorded by whole-cell ruptured-patch clamp, at 35-37°C, before and after acute superfusion of ICA at 1 μM ($\sim 2 \times \text{IC}_{50}$ for I_{SK}) or 10 μM (potentially non-specific for I_{SK}). $[\text{Ca}^{2+}]_i$ was progressively, transiently (thus avoiding contracture) increased to supra-physiological levels, by repetitively stimulating reverse-mode $I_{\text{Na/Ca}}$, using voltage pulses (from -100 mV to +100 mV, 250 ms duration, 2 Hz frequency) whilst inhibiting I_{CaL} (10 μM nifedipine), and sarcoplasmic reticular Ca^{2+} release (10 mM caffeine) and uptake (1 μM thapsigargin). **Results: 1)** In the absence of ICA, the $I_{\text{Na/Ca}}$ stimulation protocol caused a stepwise, progressive, and marked increase in $[\text{Ca}^{2+}]_i$ over the first ~ 20 voltage pulses, in each of 6 cells (from 2 rabbits) studied. The depolarising voltage change (to +100 mV) resulted in a peak $[\text{Ca}^{2+}]_i$ of $1.17 \pm 0.33 \mu\text{M}$ (mean $\pm$ SEM), associated with an outward current of $+4.95 \pm 0.53 \text{ pA/pF}$, and the repolarising voltage change (to -100 mV) resulted in a peak $[\text{Ca}^{2+}]_i$ of $0.67 \pm 0.09 \mu\text{M}$ and an inward current of $-1.72 \pm 0.18 \text{ pA/pF}$. **2)** In these 6 cells, ICA at 1 μM (started once the $[\text{Ca}^{2+}]_i$ -elevation had plateaued) had no significant effect ($P > 0.05$) on outward ($+4.86 \pm 0.49 \text{ pA/pF}$) or inward ($-1.73 \pm 0.18 \text{ pA/pF}$) current, nor on peak $[\text{Ca}^{2+}]_i$ ($1.16 \pm 0.33 \mu\text{M}$ at +100 mV, and $0.68 \pm 0.08 \mu\text{M}$ at -100 mV). However, subsequently-superfused (in 4 cells) NiCl_2 (10 mM; to inhibit $I_{\text{Na/Ca}}$) significantly and markedly decreased, compared with the ICA 1 μM values, both outward current (from $+4.92 \pm 0.76$ to $+2.44 \pm 1.16 \text{ pA/pF}$; a 50% decrease) and inward current (from -1.73 ± 0.28 to $-0.69 \pm 0.27 \text{ pA/pF}$; a 60% decrease), and $[\text{Ca}^{2+}]_i$ (from 0.82 ± 0.10 to $0.25 \pm 0.02 \mu\text{M}$ at +100 mV; a 70% decrease, and from 0.58 ± 0.06 to $0.19 \pm 0.01 \mu\text{M}$ at -100 mV; a 67% decrease). **3)** By contrast, ICA at 10 μM significantly reduced (vs control) both outward current (from $+8.24 \pm 1.53$ to $+5.38 \pm 1.64 \text{ pA/pF}$; a 35% decrease) and inward current (from -2.86 ± 0.33 to $-1.45 \pm 0.55 \text{ pA/pF}$; a 49% decrease), as well as peak $[\text{Ca}^{2+}]_i$ (significant at -100 mV: from 1.24 ± 0.23 to $0.28 \pm 0.03 \mu\text{M}$; a 77% decrease; 4 cells, 1 rabbit). Subsequently applied NiCl_2 ($n=3$ cells) had no significant effect (vs ICA 10 μM) on outward or inward current, nor on $[\text{Ca}^{2+}]_i$. **Conclusions:** In non-failing ventricular myocytes, I_{SK} (assessed as any response to 1 μM ICA) may not be activated by $[\text{Ca}^{2+}]_i$ -elevation. Furthermore, ICA at $\sim 20 \times \text{IC}_{50}$ for I_{SK} , may inhibit $I_{\text{Na/Ca}}$.

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Noradrenaline stimulates rabbit atrial L-type Ca^{2+} current via β_1 - and α_1 -adrenoceptors, attenuated by β_2 -activation, with a mixed and minor contribution from α_2 .

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Introduction: An increase in atrial L-type Ca^{2+} current (I_{CaL}) by noradrenaline (NA) may promote afterdepolarisations and atrial fibrillation (AF). However, the contribution of the individual adrenoceptor (AR) sub-types to such I_{CaL} -increase is poorly understood; including in rabbit, a species commonly used to study AF mechanisms. **Aim:** To investigate effects, on NA-stimulated I_{CaL} , of various broad-action and sub-type-specific α - and β -AR antagonists, alone or in combination, in rabbit atrial myocytes. **Methods:** I_{CaL} was recorded by whole-cell-patch clamp at 35-37°C in left atrial myocytes isolated enzymatically from rabbit hearts (anaesthetic: Na^+ -pentobarbital, 100 mg/kg I.V.). **Results: 1)** Noradrenaline (310 nM) increased peak (at 0 mV) I_{CaL} , from -14.38 ± 2.18 to -19.70 ± 2.94 pA/pF (i.e. by $39 \pm 10\%$; $P=0.025$ (1-way-ANOVA), $n=6$ cells, 3 rabbits. **2)** Broad-action β -AR antagonism of this I_{CaL} -response to NA was studied using propranolol ($\beta_1+\beta_2$ -AR antagonist; 0.2 μM), which decreased I_{CaL} in each of these 6 cells (reversible in each of 5 cells in which drug washout was studied), and on average by $55 \pm 5\%$ ($P<0.001$ vs NA). **3)** Broad-action α -AR antagonism of I_{CaL} -responses to NA was studied using phentolamine ($\alpha_1+\alpha_2$ -AR antagonist; 1 μM), either in the absence or presence of propranolol. Without propranolol ($n=9$ cells, 2 rabbits), phentolamine decreased I_{CaL} in each of these 9 cells (reversible in 8), on average by $43 \pm 5\%$ ($P<0.001$ vs NA). The degree of this I_{CaL} -reduction (i.e. by phentolamine alone) was similar ($P=0.143$, un-paired t-test) to that from propranolol alone. When applied after propranolol ($n=7$ cells, 4 rabbits), phentolamine produced a mixed I_{CaL} -response: decrease in 4 of these 7 cells (by 21, 29, 44 and 48%; reversible in 3 of those 4), and increase in the other 3 (by 16, 159 and 331%; reversible in each). There was no significant effect on average ($P=0.535$ vs NA+propranolol). **4)** β -AR-subtype-specific antagonism of I_{CaL} -responses to NA was investigated with CGP20712A (β_1 -antagonist; 0.3 μM : CGP) and ICI118551 (β_2 -antagonist; 0.1 μM : ICI). In each of 5 cells (4 rabbits) studied, CGP substantially decreased NA-stimulated I_{CaL} , on average by $64 \pm 4\%$ ($P=0.002$ vs NA). By contrast, ICI, in the continued presence of NA+CGP, increased I_{CaL} in each of these cells, reversibly, and on average by $33 \pm 9\%$ ($P=0.028$ vs NA+CGP). The degree of I_{CaL} -increase by ICI was not significantly different ($P=0.391$) to the degree of I_{CaL} -decrease by CGP. **5)** α -AR-subtype-specific antagonism of NA-stimulated I_{CaL} was studied with prazosin (α_1 -antagonist; 0.5 μM) and yohimbine (α_2 -antagonist; 10 μM). In each of 7 cells (3 rabbits) studied, prazosin decreased NA-stimulated I_{CaL} , on average by $48 \pm 6\%$ ($P<0.001$ vs NA). Yohimbine (still in the presence of NA+prazosin), decreased I_{CaL} further, in 6 of these 7 cells (in the other, there was a moderate, reversible, increase), with no significant effect on average ($P=0.237$ vs NA+prazosin). The degree of I_{CaL} -decrease by prazosin was not significantly different ($P=0.073$, Mann-Whitney) from that by CGP. **Conclusion:** Stimulation of I_{CaL} by NA in rabbit atrial myocytes is mediated, based on adrenoceptor sub-type-antagonist responses, substantially by activation of β_1 - and α_1 -ARs, attenuated by β_2 -activation, with either a contributing or attenuating (and, on average, negligible) action of α_2 -activation.

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Fast voltage-dependent sodium (Na_v) currents are functionally expressed in mouse corpus cavernosum smooth muscle cells.

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Voltage-gated sodium (Na_v) channels have been discovered in phasic smooth muscles exhibiting spontaneous electrical activities. However, they have not been discovered in corpus cavernosum, a phasic tissue type known to function as a syncytium. We report, for the first time, fast voltage-dependent sodium current in mouse corpus cavernosum smooth muscle (CCSM).

C57BL/6 mice were humanely euthanised in accordance with European Union legislation and the approval of Dundalk Institute of Technology Animal Use and Care Committee. CCSM cells were isolated using collagenase-proteinase mixture. Currents were recorded at room temperature using the whole cell patch-clamp technique. Isometric tension recordings were performed to study CCSM tissue activity. Six cells from 6 animals were used in each data set.

When cells were voltage clamped at -100 mV and subjected to depolarising pulses from -80 to +50 mV fast voltage-dependent inward currents were observed. These were determined to be sodium current, as evidenced by a 95% reduction in amplitude when external sodium concentration was reduced from 130 mM to 13 mM ($p < 0.05$, paired t-test).

At least two sub-types of Na_v currents were distinguished on the basis of TTX sensitivity: 'TTX-sensitive' and 'TTX-insensitive'. In the former, the mean IC_{50} was 10 nM (95% CI ranged from 7 to 15 nM), while in the latter, the concentration-response curve was clearly biphasic, with IC_{50} of 14 nM and 672 nM (95% CI range from 9 nM to 59 nM and 445 nM to 6 μM , respectively).

The two groups had different activation $V_{1/2S}$, at -28 ± 12 mV and -39 ± 1 mV for the TTX-sensitive and -insensitive currents, respectively ($p < 0.05$, extra sum of squares F test, Graphpad Prism). They also had different inactivation $V_{-1/2S}$, at -71 ± 1 mV and -78 ± 1 mV for the TTX-sensitive and -insensitive currents, respectively ($p < 0.05$, extra sum of squares F test, Graphpad Prism). These findings suggest that the two populations of Na_v current can be separated based on their steady state voltage-dependent activation and inactivation kinetics.

Although, veratridine 30 μM , an agonist of Na_v channels, reduced the peak current by 20% ($p < 0.05$, paired t-test), it slowed inactivation, resulting in a 7-fold increase in sustained current amplitude (-20 ± 12 to -145 ± 41 pA; $p < 0.05$, paired t-test). Under current clamp conditions, the mean duration of evoked action potentials was increased from 0.2 ± 0.1 s to 1.2 ± 0.3 s by veratridine, and this was reduced to 0.1 ± 0.02 s by 10 μM TTX ($p < 0.05$, ANOVA and Tukey's post hoc test).

Isometric tension experiments were performed in a drug cocktail to block the effects of endogenous neurotransmitters (phentolamine 3 μM , L-NOARG 100 μM , ab-methyleneATP 10 μM , and atropine 1 μM). Under these conditions, veratridine (10 μM) induced a series of phasic contractions in CCSM tissue strips that were abolished by TTX (100 nM, $p < 0.05$, ANOVA and Tukey's post hoc test).

These findings suggest that Nav channels could contribute to the mechanisms of detumescence, and potentially serve as a clinically relevant target for pharmaceutical intervention.

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In Alzheimer's disease, Amyloid Beta initiates small vessel disease through damage to resistance artery Ca^{2+} spark vasoregulation.

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Alzheimer's disease (AD) is increasingly viewed as a small vessel disease of the brain, as reduced cerebral blood flow not only predates diagnosis but also signifies a more severe phenotype. Blood flow into the brain is controlled by the pial arteries, which contract or dilate to ensure a consistent blood flow, a process known as autoregulation. One of the principle mechanisms of autoregulation is the activation of mechanosensitive, rapid, high amplitude and spatially restricted Ca^{2+} release events known as Ca^{2+} sparks in the smooth muscle cells (SMCs). These are functionally coupled to the large conductance Ca^{2+} activated K^+ (BK) channels that hyperpolarise the plasma membrane and promote vasodilation. This project sought to determine the effects of amyloid beta ($\text{A}\beta$) on Ca^{2+} sparks and cerebral diameter control, in the context of an extensively characterised AD mouse model: APP23, which has a 7-fold overexpression of amyloid precursor protein.

18 month old, male APP23^(+/-) and wild-type (Wt) littermates or 10-12 week C57/Bl6 mice were euthanized in adhered to the UK Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Pial cerebral arteries from APP23^(+/-) and Wt mice were loaded with the Ca^{2+} indicator Fluo-4-AM and pressurised to 60 mmHg. Arteries from the APP23^(+/-) mouse showed a significant reduction in Ca^{2+} spark frequency compared to Wt controls (9.9 ± 4.3 vs 30.8 ± 6.1 , $N = 5-7$, $P < 0.05$). This change in Ca^{2+} spark frequency was associated with a decreased spontaneous transient outward current (STOC) frequency (-30 mV) in freshly isolated SMCs for cerebral arteries from the APP23^(+/-) compared to Wt (1.0 ± 0.4 vs 2.7 ± 0.5 Hz, $N = 10-14$), measured using perforated patch electrophysiology. In pressure myography experiments, this loss in STOC activity translated to

an increased myogenic tone at 60 mmHg (27.6 ± 2.1 vs 36.1 ± 2.2 % myogenic tone, N = 25-29) and a reduced contraction to the BK channel inhibitor paxilline (30.5 ± 4.3 vs 12.7 ± 2.1 % constriction, N = 8-12). This pattern of damage to the Ca^{2+} spark vasoregulation was replicated in cerebral arteries from C57/Bl6 mice through exposure to $\text{A}\beta$. Pre-incubation with $\text{A}\beta_{(1-40)}$ (5 nM) for 30 minutes reduced Ca^{2+} spark frequency compared with arteries incubated with a scrambled control (3.6 ± 1.1 vs 7.8 ± 1.5 , N = 6-7). Consistent with these observations, the $\text{A}\beta_{(1-40)}$ peptide reduced STOC frequency (2.2 ± 1.2 to 0.7 ± 0.8 Hz, N = 6), whereas cells exposed to the scrambled control had no effect (1.5 ± 0.4 to 1.8 ± 0.4 , N = 7). Finally, incubated with $\text{A}\beta_{(1-40)}$ peptide reduced the constriction to paxilline (8.0 ± 1.1 vs 19.2 ± 4.5 % constriction, N = 5) in pressurised arteries.

Overall, our data provide the first detailed mechanistic explanation for the development of small vessel disease of the brain in AD and suggest exciting and novel opportunities for future intervention.

OC48

Extract of Black Sticky Rice Prevent Hyperglycemia-Induced Developmental & Motoric Impairment during Early Development of Zebrafish (*Danio rerio*)

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Introduction

Hyperglycemia, a major pathological condition of diabetes mellitus, that expose fetus become one factor contributing unfavorable effect of diabetes mellitus on pregnancy. Compelling evidence shows that the offspring subjected to uncontrolled hyperglycemia during gestation display developmental, behavioral, neurochemical, and cellular abnormalities. Oxidative stress plays important role in fetus developmental impairment due to hyperglycemia. Therefore, antioxidant substance such as Black Sticky Rice (*Oryza Sativa* Linn. var *Glutinosa*) extract which is anthocyanins-rich substance possibly inhibit hyperglycemia complications.

Objectives

In this study we focused on the effect of Black Sticky Rice extract preventing Hyperglycemia-Induced Developmental & Motoric Impairment during development using zebrafish embryos (*Danio rerio*) as a hyperglycemia model.

Methods

Zebrafish embryos were divided into 6 groups, consist of negative control group, 5% glucose exposure group, 0.6 $\mu\text{g/ml}$ Black Sticky Rice extract exposure group, and 5% glucose with Black Sticky Rice extract (0,15 ; 0,3 ; 0,6 $\mu\text{g/ml}$) co-incubation exposure groups. Embryo was exposed to glucose from 2 hpf (hourspost-fertilization) until 72 hpf and exposed to Black Sticky Rice Extract from 2 hpf

until 120 hpf. Stereo microscopy was used to image live embryo for observing mortality and characterizing the morphology of zebrafish larva, including head size, body length, and cardiac abnormality. Heart rate was counted using Danioscope software to detect the effect on the cardiac activity. Tactile response, motility, and swimming distance were observed using Ethovision XT 14 software to figure out the effect on the motoric function. The procedures used were approved by ethical committee in Faculty of Medicine Universitas Brawijaya.

Results

This study showed that mortality of embryos was increase while body length and head size were decrease in 5% glucose exposure group compared to control ($p<0,05$). Group of zebrafish embryo with Black Sticky Rice extract co-incubation (0,15 ; 0,3 ; 0,6 $\mu\text{g/ml}$) showed lower mortality in 24, 48 and 72 hpf ($p<0,05$); body length improvement in 72 and 96 hpf ($p<0.01$; $p<0.05$) ; and head size improvement in 72 and 96 hpf ($p<0,01$) compared to 5% glucose exposure group. Decreasing heart rate due to hyperglycemia condition in 72 and 96 hpf was improve significantly by Black Sticky Rice extract ($p<0.01$). Tactile response, motility, and swimming distance were decrease in 5% glucose exposure group compared to control ($p=0,037$; $p=0.05$; $p=0,009$). Black Sticky Rice extract co-incubation could improve these 3 indicators of motoric function ($p<0.05$).

Conclusion

This study identified that Black Sticky Rice extract could prevent hyperglycemia-induced developmental & motoric impairment during early development of zebrafish (*Danio rerio*).

Keywords: black sticky rice extract, hyperglycemia, development, motoric, zebrafish, embryo

OC49

The effect of acute exercise in humans on cancer cell growth *in vitro*: findings from a meta-analysis and crossover study

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Background

Regular physical activity (including structured exercise) reduces the risk of developing site-specific cancers, including breast, endometrial, and colon cancer. However, the underlying biological mechanisms are not fully understood. During exercise, skeletal muscle and other secretory organs release cytokines and other peptides into the circulation, which have the potential to influence key signalling pathways involved in cancer progression.

Aims

To determine the effect of exercise-conditioned serum on cancer cell growth *in vitro*, we: i) systematically reviewed and meta-analysed the available evidence, and ii) stimulated a colon cancer cell line with serum collected immediately pre- and post-exercise from adults with lifestyle risk factors for colon cancer.

Methods

Five literature databases were systematically searched for studies that assessed cancer cell growth after exposure to human serum collected before and immediately after an acute bout of exercise. Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were pooled using a three-level random-effects model. Meta-regressions were performed with participant age and disease status, exercise type, cell line phenotype, and serum incubation time as covariates.

In addition, we recruited physically inactive, overweight (BMI ≥ 25 kg/m²) males aged ≥ 50 years into a randomised, controlled, crossover study. Participants completed an acute bout of moderate-intensity aerobic interval exercise (6 x 5-min @ 60% heart rate reserve) and a non-exercise control condition (60-min rest) in a randomised, counterbalanced order. Serum samples were collected immediately before and after both conditions and used to stimulate a human colon cancer cell line (LoVo). Colon cancer cell growth was evaluated after 48 hours of incubation by the resazurin assay. The difference in cell growth was assessed using a multilevel linear model, with change from baseline as the dependent variable, baseline values as a covariate, condition (exercise or control) as a fixed effect, and participants as a random factor.

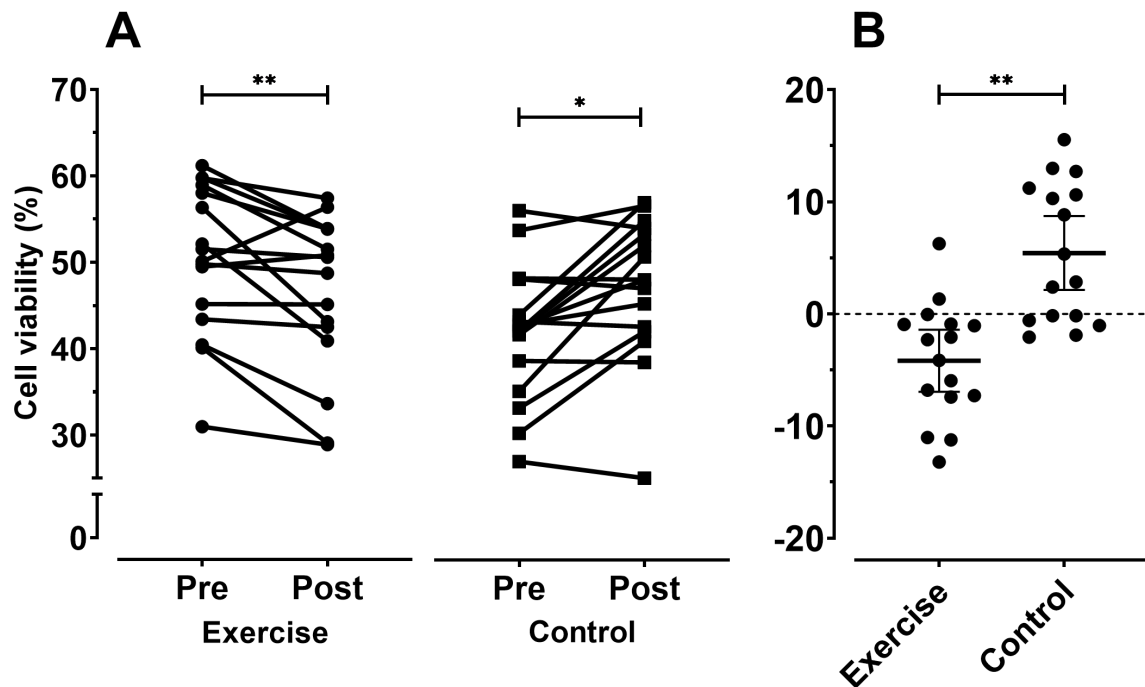
Results

Seven studies encompassing 98 participants were included in the meta-analysis. Studies used a range of cancer cell lines including breast, colon, lung, and prostate. Pooled analysis showed that exercise-conditioned serum reduced cancer cell growth compared with pre-exercise serum (SMD = -1.23, 95% CI: -1.96 to -0.50; $I^2 = 75.1\%$). However, the certainty of evidence was very low due to unexplained heterogeneity, high probability of publication bias, and risk of bias within individual studies.

Sixteen participants were recruited into the crossover study (age: 60.0 ± 8.0 years; BMI: 29.9 ± 2.4 kg/m²). The median heart rate reserve during aerobic interval bouts was 59.7% (IQR: 57.6 to 61.8%). Incubating colon cancer cells with exercise-conditioned serum reduced cancer cell growth compared with the non-exercise control condition (MD: -5.7%, 95% CI: -8.8 to -2.6%) (Figure 1).

Conclusions

The results of our meta-analysis and crossover study provide evidence that exercise-conditioned serum reduces cancer cell growth *in vitro*. This suggests that acute exercise-induced modulations in serum could contribute to the reported inverse associations between physical activity and cancer risk. However, many questions remain regarding the underlying mechanistic pathways, relevance of *in vitro* findings to *in vivo* physiology, and potential effect modifiers such as exercise intensity and cancer cell phenotype.



OC50

Carotid body modulation decreases weight gain and improves metabolic function in the rat by impacting feeding behaviour and hypothalamic satiety pathways

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Introduction: The carotid bodies (CBs), peripheral chemoreceptors defined as O₂ sensors, are also involved in energy homeostasis [1,2]. Moreover, CB dysfunction has been implicated in metabolic diseases genesis since the abolishment of its activity, via the resection or neuromodulation of its sensitive nerve, the carotid sinus nerve (CSN), prevents and reverses the pathological features in prediabetes and type 2 diabetes (T2D) animal models [3-5]. Considering that dysmetabolic states are deeply involved with the deregulation of feeding behavior and satiety pathways in the hypothalamus and that insulin, leptin, and dopamine that activate the CB, play important roles in these mechanisms we investigated whether the beneficial impacts of CSN denervation on metabolism include the modulation of feeding behavior and hypothalamic satiety pathways.

Material & Methods: Two different strains of rats were used: Wistar rats submitted either to control diet (CTL) or to a high-fat diet (HF, 60% enriched in lipids) and Zucker Diabetic fat (ZDF) rats (fa/fa) submitted to Purina 5008 diet, and its control group (Zucker Lean +/-). After 10 weeks, both groups were submitted to either CSN ablation or sham surgery and followed up during 9 and 7 weeks, respectively. Groups included 5-8 animals per group. Caloric intake, weight gain, whole-body insulin sensitivity, and glucose tolerance were evaluated. Hypothalamic expression of leptin receptors (ObR), dopamine type 2 receptors (D2R), insulin receptors (IR), AKT (protein kinase B), and tyrosine hydroxylase (TH) was evaluated by Western Blot technique. Laboratory care was in accordance with the European Union Directive 2010/63/EU. Experimental protocols were approved by NOVA Medical School Ethics Committee. The significance of the differences between the mean values and SD was calculated by one- and two-way ANOVA with Bonferroni multiple comparison test. Differences were considered significant at $p < 0.05$.

Results: HF animals and ZDF fa/fa animals show increased weight gain, insulin resistance and glucose intolerance. CSN denervation reversed all these pathological features in HF animals but only insulin resistance in ZDF fa/fa animals. HF diet and ZDF fa/fa animals exhibited increased caloric intake by 39.1%, and 100.4%, respectively, effects attenuated by 9.5% and 53.2% with CSN denervation (caloric intake CTL =206.39 Kcal/day; caloric intake lean=303.83 Kcal/day). In the hypothalamus, HF diet significantly decreased the expression of IR, AKT, ObR, and TH by 15.5%, 32.5%, 19.2%, and 28.9%, respectively, and increased the expression of D2R without producing significant changes. CSN denervation attenuated the decreased expression of IR, ObR, and D2R and increased the expression of AKT and TH. ZDF fa/fa animals show an increase in the expression of D2R and IR by 35.3% and 7.2% respectively. CSN denervation also produces a decrease in the expression of TH and AKT by 20.7% and 29.3% respectively, effects attenuated with CSN denervation.

Conclusions: CSN denervation positively impacts weight gain and metabolic function in different models of dysmetabolism. We can postulate that it may be in part due to the control of the dopaminergic and insulin pathways at the hypothalamus, and that the CB has a role in the regulation of feeding behavior and satiety.

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OC51

Erg channels control excitability in male murine anterior pituitary corticotrophs

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The control of electrical excitability of anterior pituitary corticotrophs is essential for coordinating the release of stress hormones in response to stress. A variety of ion channels are reported to be important for controlling corticotroph excitability however potassium channels that regulate spontaneous and secretagogue-evoked (CRH and AVP) activity, remain poorly understood. In several pituitary cells, members of ether-a-go-go related (Erg, Kv11.x) family of voltage dependent potassium channels control both spontaneous and hormone-induced regulation of excitability. In these studies, we used a pharmacological approach to test the role of Erg channels in both basal and CRH/AVP evoked excitability.

Corticotrophs from male BK-POMC-GFP mice on a C57BL6 background (age 2-5 months), were isolated and maintained in primary culture in accordance with UK Home office requirements. Current clamp and voltage-clamp recordings were performed using the perforated patch clamp approach with protocols controlled by Clampex and analysed using Clampfit. Pharmacological inhibition of Erg currents were conducted by bath exposure to E-4031 (5 μ M), a selective pharmacological inhibitor of Erg channels or vehicle control using a gravity perfusion system. Statistical analysis was performed using Mixed effect analysis ANOVA with further analysis by Sidak's and Tukey's multiple comparison tests with Mean $\pm$ SD.

E-4031 (n=9) significantly increased basal spike frequency (from 0.1 ± 0.2 Hz to 1.5 ± 1.0 Hz; $p < 0.05$). This E-4031-induced increase in basal spike frequency resulted in an apparent significant ($p < 0.01$) attenuation of the normal CRH/AVP evoked fold increase in spike frequency (CRH/AVP alone increased spike frequency 14.9 ± 6.2 fold but in the presence of E4031 the fold increase in CRH/AVP-induced spike frequency was only 2.0 ± 0.9 fold). E4031 also significantly reduced the normal CRH/AVP evoked transition to bursting activity in comparison to the vehicle control (n=7) treated corticotrophs (burstiness factor in presence of CRH/AVP was reduced from 0.4 ± 0.3 to 0.1 ± 0.1 ($p < 0.01$) and event duration from 206.9 ± 188.2 to 58 ± 45.9 ms ($p < 0.01$) in the presence of E4031). E-4031 did not change the spike amplitude or the resting membrane potential under basal or CRH/AVP treated conditions compared to the vehicle control. In voltage clamp recordings E-4031 sensitive currents were routinely recorded but the amplitude was highly variable between cells. CRH/AVP (n=6) had no significant effect on isolated Erg currents suggesting they are not a direct target for intracellular pathways activated by CRH/AVP in corticotrophs. RNA sequencing of FACS-purified corticotrophs from male POMC-GFP mice revealed highest expression of Erg 1 with a lower expression level of Erg 2 & Erg 3.

Our data reveal that while Erg channels are expressed in murine male corticotrophs and play a role in controlling basal and CRH/AVP evoked activity they are not direct targets for regulation of corticotroph excitability by CRH/AVP. Further studies are required to understand the role of Erg currents in controlling corticotroph physiology.

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OC52

Impact of carotid sinus nerve resection on glucose homeostasis and weight gain in mice: is there a role for β 2-adrenergic signalling?

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Obesity is a worldwide epidemic being the main cause of cardiovascular and metabolic disturbances (1). Modulation of carotid body (CB) activity, via resection or electrical modulation of carotid sinus nerve (CSN), restores insulin sensitivity and glucose tolerance and decreases weight gain and body fat mass, as well as, normalizes whole-body sympathetic nervous system activity (SNS) in the rat (2-5) and mice. Knowing that CB signals modulate SNS activity, herein we investigated the contribution of β 2-adrenergic signalling to the beneficial effects of CSN resection in dysmetabolic states.

Experiments were performed in 4 weeks male C57BL/6J and β 2-adrenergic receptor knockout (β 2-AR^{-/-}) mice (n=3-6) fed with high-fat diet (HF) (5.1Kcal/g) or with a standard diet (2.56Kcal/g) during 12 weeks. After this period, animals were submitted to bilateral CSN resection or to a sham procedure and kept under the respective diets for more 3 weeks. Insulin sensitivity, glucose tolerance, caloric intake and body weight were monitored. One- and two-way Anova with Bonferroni's multiple comparisons test were used. Data were present as mean $\pm$ S.E.M. Experimental protocols were approved by the local and national ethical committee (CIEPAL #2018082016528754).

Insulin sensitivity was not altered in β 2-AR^{-/-} mice (AUC β 2-AR^{-/-}_{control}=10968 $\pm$ 1330 mg/dl*min vs. Wild-type (WT)_{control}=11135 $\pm$ 584). However, HF diet induced insulin resistance in β 2-AR^{-/-} mice (β 2-AR^{-/-}_{HF} =22696 $\pm$ 1946mg/dl*min) ($p<0.05$), effects that were similar in the WT animals (WT_{HF}=22336 $\pm$ 2069mg/dl*min) ($p<0.0001$) and were restored 3 weeks after CSN resection. Moreover, glucose tolerance was not modified in β 2-AR^{-/-} mice and the HF diet decreased by 25 and 37% ($p<0.01$) the glucose tolerance in β 2-AR^{-/-} and WT mice, respectively, an effect that was improved 2 weeks after CSN resection. Additionally, body weight increase was similar between WT and β 2-AR^{-/-} mice both in control and in animals submitted to the HF diet. The CSN resection decreased by 115 ($p<0.0001$) and 122% ($p<0.0001$) the weight increase in WT and β 2-AR^{-/-} mice, respectively.

We conclude that the role of CB in the control of glucose homeostasis and the regulation of weight is not mediated by β 2-adrenergic receptors.

*Equally shared last authorship

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OC53

HIIT'ing or MISS'ing the optimal management of Polycystic Ovary Syndrome: A systematic review and meta-analysis of high- *versus* moderate-intensity exercise prescription.

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Introduction: Polycystic Ovary Syndrome (PCOS) is the most common endocrinopathy in young females, affecting 4-18% of the premenopausal population. PCOS is a metabolic disorder associated with increased cardiovascular disease risk. Exercise is an effective treatment strategy to manage symptoms and reduce cardiovascular disease risk factors in PCOS. High-intensity interval training (HIIT) has been suggested as a more efficient exercise modality in PCOS; however, the impact of HIIT compared to usual care moderate intensity steady state exercise (MISS) is hampered by small patient numbers and inconsistency in the methods employed.

Methods: We synthesized the available data through a systematic review and meta-analysis to compare the effectiveness of HIIT to MISS exercise without concurrent diet or pharmacological interventions. Our primary outcome measures assessed the impact of HIIT and MISS on cardiorespiratory fitness (CRF) and insulin resistance (IR), using measures of VO_{2max} and HOMA-IR respectively. Secondary analyses compared the influence of exercise intensity on body composition and hormone and lipid profiles.

Results: A total of 17 studies ranging from 8 to 24 weeks in duration were included in the meta-analyses, with HOMA-IR assessed in 219 women (HIIT = 60; MISS = 159) and VO_{2max} assessed in 222 (HIIT = 28; MISS = 194) women. MISS exercise significantly improved CRF ($\Delta = 1.081$, $p < 0.001$), whereas HIIT resulted in no improvement ($\Delta = 0.641$, $p = 0.128$). Both MISS ($\Delta = -0.341$, $p = 0.078$) and HIIT ($\Delta = -0.257$, $p = 0.374$) exercise showed no significant effect on IR. Fasting insulin was significantly reduced following MISS ($p = 0.009$) but not HIIT exercise. Body composition was significantly reduced by MISS exercise [BMI ($p < 0.001$); waist circumference ($p < 0.001$) and waist-to-hip ratio ($p = 0.022$)], whereas HIIT resulted in no significant differences. Neither HIIT nor MISS exercise had a significant impact on lipid profile..

Discussion: This is the first meta-analysis to investigate the impact of HIIT and MISS exercise intervention in PCOS. Surprisingly, our results contrast with previous literature in healthy and diseased cohorts following HIIT exercise, with a significant improvement in CRF only evident following MISS exercise and not HIIT. Additionally, there was a trend towards improvement in HOMA-IR following MISS exercise, which was not evident following HIIT. Therefore, in isolation, HIIT exercise may not provide superior outcomes in CRF and IR compared with MISS. The available data included in the meta-analysis was from low- and moderate-quality studies. There is therefore a lack of high-quality evidence to support these conclusions. High publication bias was also evident within the MISS exercise interventions, but was not present from HIIT intervention studies. Our analysis focused on isolated exercise interventions as opposed to combined interventions with pharmacological agents and/or diet which may explain some of the disparate outcomes in this analysis.

In conclusion, the small number of studies and participants, and low quality evidence highlight the need for larger randomised controlled trials to establish the true effect of isolated exercise interventions, especially HIIT, in the management of PCOS.

OC54

A hypothalamic-hindbrain pathway that suppresses feeding following acute stress

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Acute stressors elicit behavioural and physiological adaptations that promote survival, including increased vigilance, reduced exploratory behaviour, and decreased appetite. Complex neural circuits contribute to these processes, with the caudal nucleus of the solitary tract (cNTS) and its resident glucagon-like peptide-1 (GLP1) neurons playing a key role¹. GLP1 neurons are activated following stress^{2,3} and mediate stress-induced suppression in feeding⁴, but the inputs which drive their activation in response to stress are unknown. To identify stress-activated direct inputs to GLP1 neurons we performed monosynaptic retrograde tracing. Glu-cre/tdRFP mice (n=9, 4 females, 5

males) were anaesthetised using isofluorane (1.5-3%) and stereotactically injected with a 1:1 cocktail of AAV8-CAG-FLEX-RabiesG and AAV5-EF1a-TVA:mCherry (200nl) targeted to the cNTS followed three weeks later by injection of EnvA-RABV-deltaG-GFP (400nl). Seven days later mice were exposed to 30mins restraint stress (or left undisturbed) and transcardially perfused (4% formaldehyde) after 90mins. Coronal sections (40µm) were immunolabelled for GFP and cFOS to identify recently activated, RABV-infected neurons. The paraventricular nucleus of the hypothalamus (PVN) provided dense monosynaptic input to GLP1 neurons, and acute stress increased the number of activated RABV-GFP-positive neurons by 27.1 percentage points [95% confidence interval (95%CI): 16.1, 47.8 percentage points; $p=0.036$, Student's T-test). To establish whether PVN input is necessary for stress-induced activation of GLP1 neurons, we used an intersectional approach to selectively inhibit cNTS-projecting PVN neurons. Wildtype C57BL/6 mice ($n=13$ males) were injected with rgAAV-hSyn-Cre into the cNTS followed by AAV8-hSyn-DIO-hM4Di:mCherry ($n=5$) or AAV1-CAG-FLEX-EGFP ($n=8$) into the PVN. Injection of the ligand, CNO (2mg/kg), significantly increased 2h dark-onset food intake in hM4Di-expressing mice by 0.27g (95%CI: 0.19, 0.326; $p=0.029$), but not in control mice [0.154g (95%CI: 0.324, 0.00625); $p=0.11$; Sidak multiple comparisons]. Furthermore, while acute stress significantly suppressed food intake in control mice by 0.265g ($n=5$ males; 95%CI: 0.16, 0.36; $p=0.005$), mice in which cNTS-projecting PVN neurons were inhibited ($n=4$) displayed no reduction in chow intake [effect size: 0.055 (95%CI: -0.01, 0.135g); $p=0.57$; Sidak multiple comparisons]. As a terminal procedure, mice were injected with CNO (2mg/kg), exposed to acute stress, and transcardially perfused 90mins later. Coronal brain sections (35µm) were immunolabelled for cFOS, GLP1, and GFP or mCherry. Chemogenetic inhibition of cNTS-projecting PVN neurons significantly attenuated the number of cFOS-immunoreactive neurons in the cNTS ($p=0.0041$, Student's T-test) as well as the percentage of cFOS-immunoreactive GLP1 neurons following acute stress ($p=0.033$, Student's T-test). Finally, we determined the molecular phenotype of these cNTS-projecting PVN neurons using RNAscope *in situ* hybridization and immunolabelling. Approximately half of identified cNTS-projecting PVN neurons expressed *Crh* mRNA ($49.0\pm1.4\%$), while only $3.9\pm1.1\%$ expressed oxytocin ($n=3$). We also confirmed *Crh* expression by a subset of RABV-labeled PVN neurons that provide synaptic input to GLP1 neurons. These findings reveal a hypothalamic-hindbrain pathway that mediates stress-induced activation of cNTS neurons and feeding suppression.

The experimental protocols were approved by the FSU Institutional Animal Care and Use Committee, and were consistent with the US Public Health Service's Policy on the Humane Care and Use of Laboratory Animals and the NIH Guide for the Care and Use of Laboratory Animals.

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OC55

β-alanine protects the brain tissue from ischemic damage

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Brain stroke is one of the leading causes of death worldwide, with ischemic mechanism (blood flow obstruction) generating up to 90% of stroke cases. Oxygen-glucose deprivation (OGD), the main effect of ischemia, leads to depletion of intracellular ATP, hence loss of the ATP-dependent glutamate uptake by glutamate transporters. This, in turn, induces excitotoxicity due to excessive amounts of glutamate in extracellular space. Thus, the obvious treatment strategy is hyperactivation of inhibitory receptors, e.g. GABA_A receptors (GABA_ARs) and downregulation of excitatory glutamate receptors, first of all those of NMDA type (NMDRs). However, the use of GABA_AR and NMDAR selective ligands with high activity is associated with deleterious side effects such as impaired functional recovery after stroke¹ and symptoms of psychosis². Hence, there remains a pressing demand for medications that counteract ischemia-induced excitotoxicity with lowered side effects. In our study we tested β-alanine (Ala) as a perspective protector from neural cell ischemic damage. Ala induces inhibition via action at glycine receptors and GABA_ARs, and suppresses excitation by competing (as a partial agonist) with glycine at its co-agonist binding site on NMDARs³.

We have tested Ala's effects on two types of neurons: cerebellar granule cells (CGCs) and Purkinje cells (PCs). To reproduce ischemic damage, murine cerebellar slices were perfused with solution containing 10 mM sucrose (to replace 10 mM glucose required for normal perfusion solutions) and gassed with 95%N₂/5%CO₂ (to produce low O₂ insult). To quantify the effect of Ala on neural cell functioning we used whole-cell patch-clamp to register the amplitude of anoxic depolarization of cell membrane (AD) which follows OGD development. We found that application of 1 mM Ala reduces significantly the AD amplitude. In mV, for CGCs: from 12.12±0.79 (n=12) to 7.56±0.58 (n=11), P=1.4*10⁻⁴; in PCs: from 39.25±2.71 (n=14) to 24.1±1.77 (n=14), P=1.1*10⁻⁴, Student's t-test for both comparisons. To test the impact of Ala on cell survival, we used imaging of fluorescence generated by propidium iodide (marks dead cells) in a course of OGD in cerebellar CGC layer. To analyze experimental data, we used two-way repeated measures analysis of variance for time (factor 1) and experimental action (control conditions, OGD, OGD+Ala – factor 2). We found that factor 2 and the combination of factor 1 ´ factor 2, but not factor1, cause a significant impact: factor 1, F_(1,62),

$_{11.04})=1.202$, $P=0.33$; factor 2, $F_{(2, 12)}=6.72$, $P=0.011$; factor 1 $\times$ factor 2, $F_{(46, 157)}=1.56$, $P=0.023$. Tukey test on factor 2: $P<1*10^{-4}$ for Control vs. OGD and for OGD vs. OGD+Ala, $P=0.075$ for Control vs. OGD+Ala.

The traditional approach to developing novel pharmacological therapies involves designing drugs that display a single activity profile at a very high potency, thus creating a unidirectional "silver bullet" that interacts with a single drug target. However, the simultaneous modulation of several drug targets at lower potency may provide a superior therapeutic index for multi-mechanism conditions like ischemic stroke⁴. This makes Ala, which reduces significantly ischemic damage of neural cells via simultaneous suppression of excitatory receptors and upregulation of inhibitory receptors, a promising candidate for ischemic stroke treatment.

Reference 1 :- Clarkson et al. (2010). Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature* **468**, 305.

Reference 2 :- Wu & Tymianski (2018). Targeting NMDA receptors in stroke: new hope in neuroprotection. *Mol Brain* **11**, 15.

Reference 3 :- Tiedje et al. (2010). Beta-alanine as a small molecule neurotransmitter. *Neurochemistry international* **57**, 177-188.

Reference 4 :- Morphy & Rankovic (2009). Designing multiple ligands - medicinal chemistry strategies and challenges. *Curr Pharm Des* **15**, 587-600.

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OC56

Psychological stress alters voiding behaviour and enhances activity in both low and high threshold bladder afferent nerves in female mice

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Recent experimental evidence shows that bladder overactivity occurs in female mice following water avoidance stress exposure, with accompanying alterations in detrusor contractile responses (West et al, 2021). The storage and micturition reflexes of the bladder are controlled by information from the afferent nerves and changes in their activity known to result in bladder dysfunction. Stress has been shown to affect activity of the afferent nerves in the gut (Yu et al, 2010) and it has been postulated that similar sensitization may also occur in bladder afferents, explaining the overactive voiding phenotype seen in rodent models. However, to date there is no direct evidence to support this. Therefore, the aim of this study was to investigate the effects of water avoidance stress on bladder afferent nerve activity in response to distension (bladder filling) in mice.

Adult (12-14 weeks) female C57BL/6J mice were allocated to either stress or control groups, (n=7 per group). Stressed animals were exposed to water avoidance stress (WAS) for 1hr/day for 10 days. Unstressed controls were age-matched and housed normally. Voiding behaviour was measured using void pattern analysis before starting and on completion of the WAS treatment period. Mice were sacrificed 24-h after final stress exposure and whole bladders isolated to measure afferent nerve activity and intravesical pressure during bladder distension. Data are presented as mean $\pm$ SEM and differences between control and stress groups were determined using unpaired Student's t-test.

Water avoidance stress caused bladder overactivity in adult female mice evident as an increase in urinary frequency from 6.7 ± 1.5 in controls to 13.1 ± 1.0 voids per 4 hour period in stressed mice ($p=0.009$), without change in urine output. As bladder volume increased, both intravesical pressure and afferent nerve activity increased (Figure 1A&B), with bladder compliance unaffected by stress. The afferent nerve activity recorded during bladder distensions was significantly increased after stress at low bladder pressures (2, 4-7mmHg) relevant to normal physiological filling (Figure 1B). While at higher pressures >25 mmHg afferent nerve activity was significantly lower in stressed animals (Figure 1 B). The number of individual nerve fibres contributing to the nerve activity was similar between control and stressed groups and the ratio of low and high threshold nerves was also unchanged by stress. The low threshold nerves, responsible for filling sensations, were significantly more active in stressed animals at low pressure (5mmHg) ($p<0.01$), however at the higher pressures (35 and 40mmHg) the low threshold nerves in stressed animals were significantly less active than controls ($p<0.05$). The high threshold nerves, responsible for pain sensations, were more active in the stressed animals than in controls across all pressure points from 10-35mmHg ($p<0.05$).

These results indicate that WAS causes enhanced activity of individual afferent nerve fibres in response to bladder distension. The enhanced activity was seen in both low and high threshold nerves suggesting that stressed animals may experience enhanced bladder filling sensations at lower bladder volumes as well as increased pain sensations, both potentially contributing to the increased urinary frequency seen after stress.

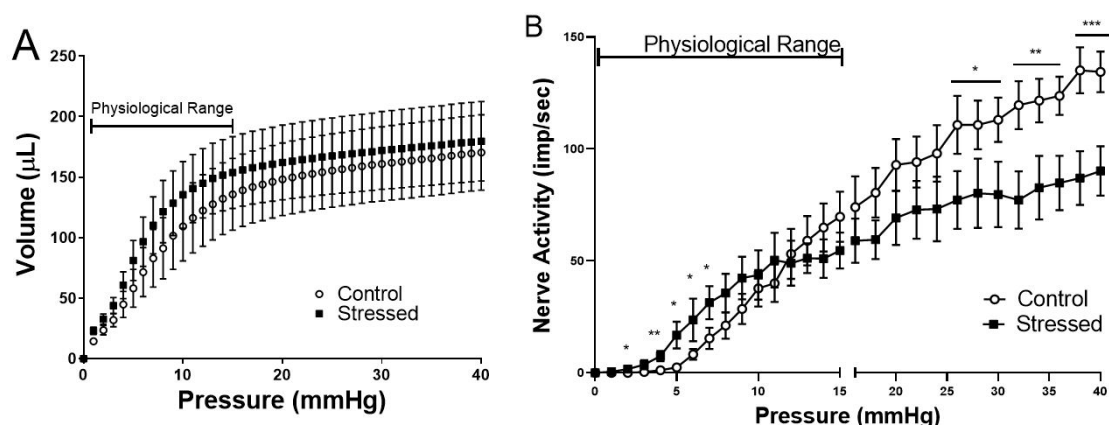


Figure 1: The effect of 10-days water avoidance stress on A) the volume-pressure relationship and B) afferent nerve activity during bladder distension.

Reference 1 :- West EG, Sellers DJ, Chess-Williams R, McDermott C. (2021) Bladder overactivity induced by psychological stress in female mice is associated with enhanced bladder contractility. *Life Sci.* 2021 Jan 15;265:118735.

Reference 2 :- Yu, Y. B., Yang, J., Zuo, X. L., Gao, L. J., Wang, P. & Li, Y. Q. (2010) Transient receptor potential vanilloid-1 (TRPV1) and ankyrin-1 (TRPA1) participate in visceral hyperalgesia in chronic water avoidance stress rat model. *Neurochem Res*,35, 797-803.

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OC57

Graded calcium spikes differentially signal neurotransmitter input in cerebrospinal fluid contacting neurons of mouse spinal cord

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The action potential and its all-or-none nature is fundamental to neural communication, typically initiated once voltage-gated Na⁺ (Nav) channels are activated. In contrast, here we show that cerebrospinal fluid contacting neurons (CSFcNs) in spinal cord do not use Nav channels, but rather two different types of voltage-gated Ca²⁺ channel, enabling spikes of different amplitude.

Spinal cord slices (300 μ m) were obtained under terminal anaesthesia (100mg.kg⁻¹ sodium pentobarbital (i.p.) from C57/Bl6 mice (P30-P52) in which GCAMP6f was expressed under the vesicular GABA transporter promoter. Imaging was performed on a custom built 2-Photon laser scanning microscope. Drugs were applied via microinjection. Analysis was performed in Igor Pro (Wavemetrics). Spike amplitudes were measured as the difference between the peak $\Delta F/F$ signal occurring in a 333ms window around the spike time and the mean signal in the preceding 166ms. All data are expressed as mean $\pm$ SEM.

Spontaneous activity occurred at a low frequency in CSFcNs (0.16 Hz; IQR = 0.12 - 0.23 Hz; 61 CSFcNs, N=7 mice). The distributions of inter-spike-intervals decayed exponentially and had coefficients of variation close to 1. Spikes in individual CSFcNs displayed distinct low and high amplitude events with multi-modal amplitude distributions. The frequency and amplitude of spontaneous activity of CSFcNs was unaffected by the voltage-gated sodium channel blocker tetrodotoxin (1 μ M; n=33 cells, 3 slices; Wilcoxon test, p=0.2011 and p=0.396 respectively). Bath application of the high voltage activated Ca_v channel blocker cadmium (Cd²⁺, 100 μ M) reduced the frequency and amplitude of spontaneous Ca²⁺ events in CSFcNs (to 56 and 54% of control respectively, n=31 cells, Wilcoxon test, p=>0.0001 and p=0.0012 respectively), although low amplitude events persisted in 30 out of 31 CSFcN. Cd²⁺ changed the event amplitude histograms from multi-modal to unimodal, selectively attenuating the larger amplitude event. Bath application of the selective T-type blocker ML218 (3 μ M)

dramatically reduced the frequency of Ca^{2+} events in all CSFcNs (to 10% of control, $n=27$ cells, 4 slices, $N=4$ animals, Wilcoxon test, $p=>0.0001$) with complete inhibition observed in 20 of 27 spontaneously active CSFcNs. Bath application of Cd^{2+} ($n=15$ CSFcNs) or ML218 ($n=27$ CSFcNs) also significantly reduced the amplitude of K^{+} -evoked Ca^{2+} events to 30% and 25% of control respectively Cd (Wilcoxon test, $p<0.0001$). The amplitude of focally applied ACh-evoked Ca^{2+} events ($n=68$ CSFcNs) was larger than ATP-evoked Ca^{2+} events ($n=138$ CSFcNs, Mann-Whitney test, $p=>0.0001$). The amplitude of ACh-evoked Ca^{2+} responses were reduced by the nicotinic Ach receptor antagonist MCA ($50\mu\text{M}$, $n=22$ CSFcNs), but not ML218 ($3\mu\text{M}$, $n=68$ CSFcNs; Kruskal-Wallis test, $p=<0.0001$ and $p=>0.9999$ respectively). The amplitudes of ATP-evoked Ca^{2+} responses were reduced by ML218 ($n=43$ CSFcNs, Wilcoxon test, $p=<0.0001$).

In CSFcN therefore, T-type Ca^{2+} channels are required for spontaneous spiking and generate lower amplitude spikes, whereas large amplitude spikes require high voltage activated Ca^{2+} sensitive Ca^{2+} channels. These different amplitude spikes signal input from different transmitter systems; purinergic inputs evoke smaller T-type dependent spikes while cholinergic inputs evoke large T-type independent spikes. Different synaptic inputs to CSFcNs can therefore be signalled by the spike amplitude.

OC58

Vectorcardiographic, attractor-based analysis for adaptive real-time arrhythmia detection

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Introduction: Detection of intermittent arrhythmic episodes via long-term cardiac monitoring is important. For instance, atrial fibrillation may be life-threatening in the event of carotid artery embolization and stroke. Several machine-learning models exist to this end, but most are based on single ECG lead-input which may omit important diagnostic information and encumber translation into a hospital setting, where 12-lead ECG is standard. Dimensionality reduction may be achieved via vectorcardiographic (VCG) analysis, whereby the 12-channel ECG signal is converted into three orthonormal leads. Most tools also rely on extracting temporal waveform features, potentially precluding accountancy for long-term physiological variations in heart rate. Generating a 2D 'attractor', in which all the data pertaining to the signal is represented in a single bounded space, may cope with these changes. Here, aberrant waveform events are reflected in attractor shape (illustrated in Figure 1) based on their relative, rather than absolute timing within the cardiac cycle and baseline variation is removed without waveform distortion.

Aims: Using traces from two online databases, I aimed to combine VCG and attractor analysis to develop a novel tool for real-time, adaptive detection of a range of arrhythmic beat-types, test its performance and then to investigate methods of improving accuracy.

Methods: The following is an outline of the proposed programme: Matrix transformation followed by application of Pythagoras' theorem are applied to convert 12-lead ECG to a single VCG maximum amplitude signal. Pan-Tompkins' algorithm extracts cycle periods from which 2D attractor coordinates are derived. Spectrograms are computed from these values and then used to extract time-frequency moments (instantaneous frequency and spectral entropy). These 'features' are employed to train a bidirectional long-short-term memory neural network which classifies single cycles as being arrhythmic or normal.

Results: Low training and testing times support the model's suitability for beat-by-beat analysis and 'on-the-fly' re-configuration of network parameters for personalised arrhythmia detection. When trained and validated on patient-specific data, an average classification accuracy of 82.9% (n = 13; SD:14) was achieved. When trained on signals from multiple patients, the model yielded a relatively low accuracy of 63.73% (n = 23; SD: 20.7). However, accuracy was still high for conditions which were better represented in training, including atrial fibrillation (n = 2; mean: 71.45%; SD: 5.59) and paroxysmal supraventricular tachycardia (n = 3; mean: 76.76%; SD: 6.52). When additional data pertaining to Wolff-Parkinson-White syndrome (WPW), an arrhythmia type that had relatively poor representation in training, were added to the training dataset, there was a significant increase in mean cycle classification accuracy in all WPW patients from 53.20% to 77.29% ($p < 0.05$).

Conclusions: This novel amalgamation of VCG and attractor analysis therefore has the capacity to learn meaningful information about ECG signals and has potential utility in automated arrhythmic detection in clinical and day-to-day contexts. The high performance of the bespoke tool suggests its applicability to continuous monitoring of patients with an established risk for further arrhythmic episodes. With more training data, the generically-trained model may be employed for diagnosing arrhythmia in individuals to which the network is naïve.

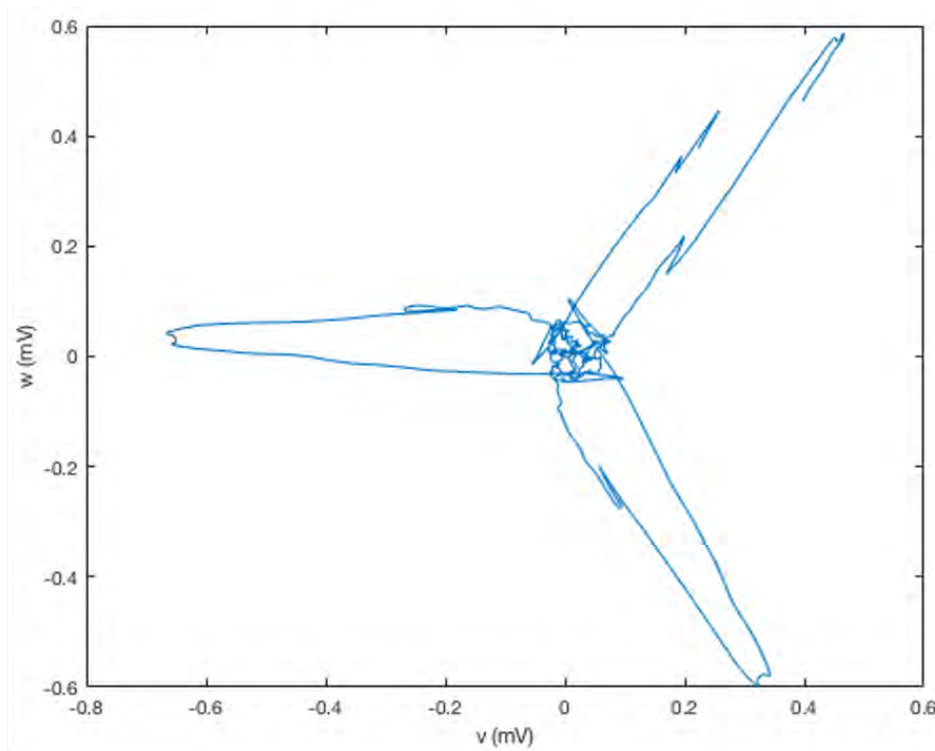


Figure 1: Attractor representing a single normal cardiac cycle, plotted in the 2D reconstructed 'phase-space' wherein all possible states of the system are signal are represented, with each state corresponding to a particular point. The dense central core region is produced when none of the delay co-ordinates are located on the R peak, which occurs for the majority of the cycle period. The long narrow arms are produced when one of the three points quickly traverses an R peak.

Acknowledgements :- N/A.

OC59

The Middle Cerebral Artery Blood Velocity Response to Moderate and Heavy Intensity Cycling In Healthy Adults

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Introduction The kinetic response of the middle cerebral artery blood velocity (MCAv) response to steady-state moderate-intensity exercise on a recumbent stepper has been modelled previously in healthy adults, using a mono-exponential function with a time delay. However, no data exist regarding the MCAv kinetic response to whole body upright cycling, nor heavy-intensity exercise, which may require a more complex approach due to hyperventilation-induced hypocapnia. Data from incremental exercise suggest that moderate-intensity exercise elicits the greatest increases in MCAv, though this has not been explored during constant work-rate exercise.

Aim The aim of this study was to compare the amplitude and time-based parameters of the MCAv response to moderate and heavy-intensity cycling in adults using an exponential model.

Method Seventeen healthy adults (23.8 ± 2.4 years, 8 males) completed a ramp incremental test to exhaustion on a cycle ergometer to determine maximal oxygen uptake and the gas exchange threshold (GET). Participants then completed three 6-minute transitions at a moderate-intensity (90% GET) and three 6-minute transitions at a heavy-intensity (40%Δ) in a counterbalanced order, all on separate visits. Bilateral MCAv was measured throughout using transcranial Doppler ultrasonography. MCAv mean data were exported as 1-sec averages and time aligned to exercise onset. For each participant, left and right MCAv data from each corresponding repeat transition were obtained and pooled, resulting in six sets of MCAv data per person that were subsequently averaged. The MCAv response to each intensity were analysed using a mono-exponential model with a time delay for each participant. Differences in kinetic parameters between moderate and heavy-intensity exercise were explored using paired t-tests.

Results Baseline MCAv before each trial was similar between intensities (69.4 ± 9.1 vs. 70.8 ± 8.4 $\text{cm} \cdot \text{s}^{-1}$, $P=0.16$). At exercise onset, consistent, rapid fluctuations in MCAv were observed within both intensities for ~ 25 s, before MCAv increased in an exponential-like fashion. The time constant of the MCAv kinetic response was similar between moderate- and heavy-intensity cycling (25.4 ± 9.7 vs. 26.0 ± 7.7 s, $P=0.82$), as was the time delay (29.3 ± 11.3 vs. 29.1 ± 9.6 s, $P=0.95$). The amplitude of the MCAv increase from baseline was significantly greater during heavy (23.9 ± 10.0 $\text{cm} \cdot \text{s}^{-1}$, $34.1 \pm 14.4\%$) compared to moderate (12.7 ± 4.4 $\text{cm} \cdot \text{s}^{-1}$, $18.7 \pm 7.5\%$) intensity cycling (both $P < 0.01$). After attaining a peak or steady state, MCAv decreased as the exercise bouts progressed within each intensity. This occurred significantly earlier during heavy-intensity exercise (164 ± 43 vs. 248 ± 90 s, $P < 0.01$), and MCAv decreased by a significantly greater magnitude during heavy-intensity exercise, compared to moderate (9.5 ± 6.9 vs. 2.8 ± 3.8 $\text{cm} \cdot \text{s}^{-1}$, $P < 0.01$). Nevertheless, MCAv at the end of the bout remained elevated during heavy-intensity exercise, compared to moderate (85.2 ± 9.6 vs. 79.3 ± 7.7 $\text{cm} \cdot \text{s}^{-1}$, $21.1 \pm 12.7\%$ vs. $14.9 \pm 7.8\%$, $P \leq 0.01$).

Conclusion These findings indicate that the MCAv kinetic response to moderate and heavy-intensity cycling in adults can be modelled using an exponential function. This is the first study to show that increases in MCAv are greater during constant work-rate heavy-intensity cycling, compared to moderate, but that the time constant and time delay of the responses are similar between intensities. These novel analysis techniques form an important area to explore cerebrovascular responses and regulation during exercise.

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Molecular basis for targeting CamKII in Nav1.5- and Ca²⁺-mediated arrhythmic syndromes

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Introduction: Cytosolic kinases in cardiomyocytes, particularly Ca²⁺/calmodulin-dependent kinase II (CaMKII), have been implicated in the regulation of cellular ionic currents, specifically Na⁺ and Ca²⁺ currents, and thus may play a crucial role in promoting cardiac arrhythmias. Importantly, CaMKII levels have been shown to increase in conditions of compromised cardiac function such as heart failure. CaMKII effects on cellular currents have been assumed to be restricted to post-translational modification. However, CaMKII possesses a nuclear localisation signal which allows it to influence gene transcription via effects on transcription factors and epigenetic chromatin accessibility. The *Scn5a* gene encodes the cardiac sodium channel Nav1.5 responsible for action potential activation and propagation and hence plays a crucial role in regulating cardiac electrophysiological function. As such, disrupted Nav1.5 expression and function have been implicated in a variety of inherited arrhythmic syndromes such as Brugada syndrome (BrS) and Long QT syndrome 3 (LQT3), as well as following cardiac structural or energetic dysfunction. CaMKII phosphorylation of Nav1.5 at multiple sites has been shown to increase arrhythmic tendency and promote BrS and LQT3 phenotypes. Nav1.5 also appears to be inhibited by intracellular Ca²⁺. CaMKII can control the activity of the sarcolemmal L-type Ca²⁺ channel Cav1.2 regulating cellular Ca²⁺ homeostasis which is known to be important in arrhythmogenesis. However, the mechanisms underlying CaMKII-Nav1.5 and CaMKII-Ca²⁺ relationships are poorly understood. Specifically, we hypothesise modification by CaMKII at the transcriptional level. **Aim:** To explore the effects of CaMKII inhibition on (1) *Scn5a* gene transcription and Nav1.5 protein expression, (2) *Cacn1ac* gene transcription, and Cav1.2 protein expression, and (3) cellular Ca²⁺ flux. **Methods:** This utilised induced pluripotent stem cells (iPSC) derived cardiomyocyte populations (N = 5, with 50000 cells per sample). CaMKII inhibition was achieved via incubation with KN93 inhibitory peptide for 24 hours at a concentration of 10µM. KN92 peptide was used in controls for the same concentration and time period. *Scn5a* and *Cacn1ac* gene expression was investigated using SYBRgreen qPCR, whereas Nav1.5 and Cav1.2 protein expression was investigated using both western blotting and immunofluorescence. Fluo 4-AM Ca²⁺ dye and Clariostar microplate reader were used to investigate Ca²⁺ flux. **Results:** Interestingly, CaMKII inhibition significantly increased *Scn5a* transcription by 160% (± 10%) (P = 0.001) and significantly increased *Cacn1ac* transcription by 140% (± 10%) (P = 0.007). Western blots and immunofluorescence did not reveal significant differences. Finally, CaMKII inhibition completely abolished cellular Ca²⁺ flux. **Conclusions:** Thus, the findings elucidate molecular mechanisms underlying arrhythmogenesis by providing insight into the CaMKII-Nav1.5 and CaMKII-Cav1.2 relationships. This demonstrated an important regulatory role of CaMKII in the transcription of *Scn5a* and *Cacn1ac* genes. Additionally, the results show the importance of CaMKII in mediating cellular Ca²⁺ flux. Hence, pathological conditions elevating CaMKII

could result in significant reductions of sarcolemmal Na^+ and Ca^{2+} currents as well as dramatic increases in cellular Ca^{2+} flux. Together these promote arrhythmic triggers and substrates e.g. alternans and afterdepolarisations from increased Ca^{2+} flux and slowed conduction velocity from reduced Na^+ current. This highlights CaMKII as a novel potential antiarrhythmic pharmacological target.

OC61

Effects Of Cardiac Geometric Remodeling During Heart Failure On Cardiac Function

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Introduction

Heart failure with preserved ejection fraction (HFpEF, $\text{EF} \geq 50\%$) and heart failure with reduced ejection fraction (HFrEF, $\text{EF} \leq 40\%$) are two main types of heart failure (HF) with similar prevalence. 88% of HFpEF [1] and 80% of HFrEF [2] undergo cardiac remodeling. Here we develop a myocardial numerical model to investigate how these geometric changes benefit or impede heart function. We further show that geometric changes can skew the EF measure, making it ineffective in the diagnosis of HFpEF, and propose a new corrected EF measure to improve it.

Methods

CMRI of 5 HFpEF porcine models (aortic banding), 10 HFrEF porcine models (circumflex ligation), and 6 sham/healthy are obtained before intervention (baseline) and at the 28th- or 42nd-day time point (termination). Cardiac anatomic and strains are measured. A numerical model of the LV is reconstructed as a discretized, idealized prolate shape, based on measured dimensions. It can undergo specific magnitudes of spatially uniform and incompressible strains, and allow the calculation of the consequent stroke volume and EF function.

Results

HFpEF and sham groups have EF above 50%, while HFrEF group has EF less than 40%. HFpEF experiences a concentric hypertrophy (higher LVM and wall thickness), while HFrEF experiences

eccentric hypertrophy with dilation (decreased SI and unchanged RWT). Both HF models have decreased strains.

With the numerical LV model, increasing wall thickness with the same strain (Fig. 1A) was found to increase EF but not SV, suggesting that higher wall thickness artificially increased EF when there is no change to flow function. Increasing dilation at the same strain (Fig. 1B) was found to increase SV but decrease EF, suggesting that dilation enhances the conversion of strain into flow function, but EF is not a successful indicator of this. Interestingly, wall thickening is found to increase the epi-to-endo spatial variability, while dilation reduces the difference.

Due to radial strain, the epi- and endo- surfaces have different strains from the mid-wall layer. The epi- and endo- surfaces are thus not good locations for quantifying strain or volumetric flow function. We thus propose a novel marker, the corrected ejection fraction (CEF), which is calculated using the mid-wall layer rather than the endocardial layer:

$$CEF = SV/Vol_{ED,mid} = \alpha \times EF \quad (1)$$

$$\alpha = Vol_{ED,endo} / (Vol_{ED,endo} + LVM/2\rho_{LV}) \quad (2)$$

When applied to the porcine data, CEF can successfully differentiate between healthy and HFpEF hearts, while EF cannot (Fig. 2), and is shown to be free of biases due to geometric changes to the heart.

Conclusions

From our modeling, we find that dilation to be advantageous to cardiac flow function, but not wall thickening. We further find that EF is an imperfect indication of function because it can be modulated by the cardiac geometry changes and cannot distinguish some HF phenotypes from normal because of a flawed reliance on measurements based on the endocardial boundary rather than the mid-wall layer. We proposed a corrected EF measure, the CEF, which we show can remove this geometric dependency and enable a better representation of the cardiac contractile function.

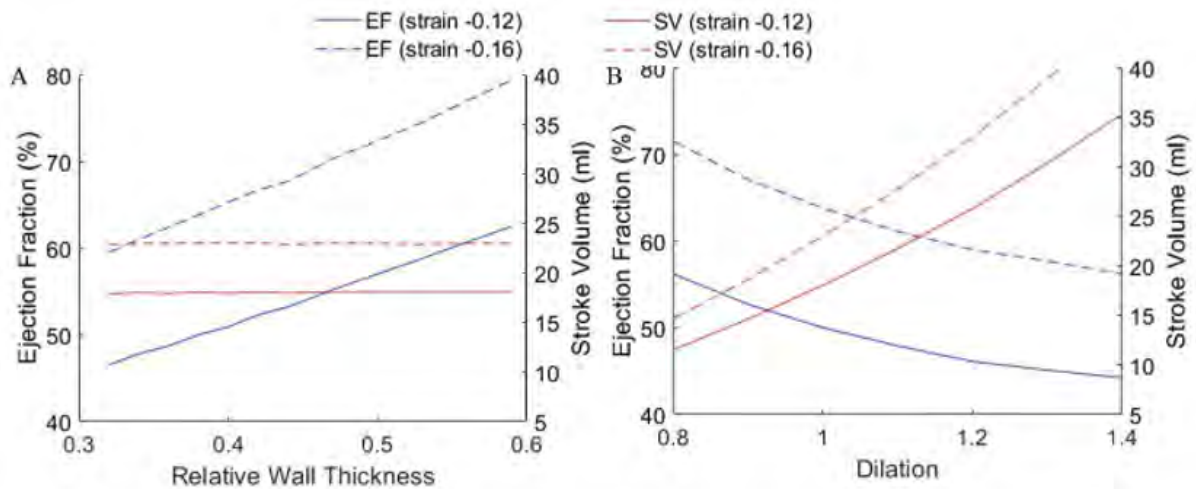


Figure 1: The dependence of EF and SV on (A) relative wall thickness and (B) dilation.

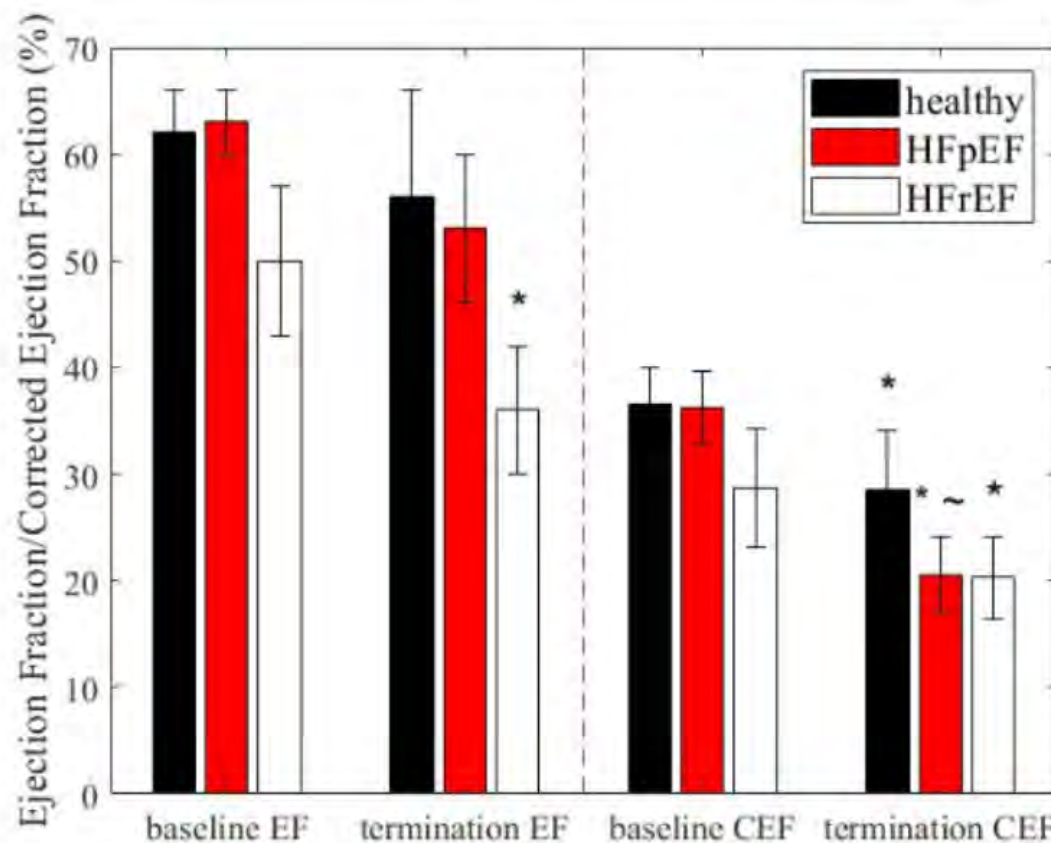


Figure 2. EF and CEF of healthy, HFpEF and HFrEF porcines hearts at baseline and termination time points. * $p < 0.05$ with baseline. ~ $p < 0.05$ with healthy termination

Reference 1 :- Katz et al. (2013), Am J Cardiol, 112, 1158-1164.

Reference 2 :- Nauta et al. (2020), Eur J Heart Fail, 22, 1147-1155.

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OC62

Regulation of blood–brain barrier integrity by microbiome-associated dietary methylamines

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Introduction

Communication between the gut microbiota and the brain is primarily mediated *via* soluble microbe-derived metabolites, but the details of this pathway remain poorly defined. Methylamines produced by microbial metabolism of dietary choline and L-carnitine have received attention due to their proposed association with vascular disease, but their effects upon the cerebrovascular circulation have not hitherto been studied.

Aim

Here we use an integrated *in vitro/in vivo* approach to investigate how physiologically relevant concentrations of the dietary methylamine trimethylamine (TMA) and its host-derived oxidation product trimethylamine *N*-oxide (TMAO) affect blood-brain barrier (BBB) integrity.

Methods

hCMEC/D3 human immortalised cerebromicrovascular endothelial cells grown under polarising conditions on 0.4µm transwell filters were used to model BBB barrier function *in vitro*, assessing permeability to a 70kDa FITC-dextran tracer, transendothelial electrical resistance (TEER), and gene expression by microarray, n=3-4 independent experiments. Male wild-type C57Bl/6 mice were used to assess *in vivo* effects of acute and chronic TMAO exposure and interactions with lipopolysaccharide (LPS)-induced inflammatory BBB disruption, monitoring BBB integrity *via* Evans blue dye extravasation and brain gene expression by RNAseq, n=5-8 mice. All experiments were conducted under UK Home Office licence approval and reviewed by the QMUL Animal Welfare Ethical Review Board. Statistical analysis was by one- or two-way ANOVA as appropriate, with $P < 0.05$ taken as statistically significant. Gene expression data was corrected for multiple testing using the Benjamini-Hochberg procedure and $P < 0.1$ was taken as statistically significant.

Results

In vitro studies revealed a clear distinction between the effects of TMA and TMAO; TMA dose-dependently impaired BBB permeability barrier function, whilst TMAO exhibited a biphasic response, physiologically relevant concentrations reducing and supra-physiological doses enhancing permeability. Microarray analysis indicated that TMA activated pathways characteristic of cellular stress responses, while TMAO upregulated a number of pathways associated with cytoskeletal rearrangement and actin bundle formation. Notably, TMAO upregulated expression of the major tight junction regulator annexin A1. Further analysis of this pathway using hCMEC/D3 cells stably expressing shRNA sequences targeting annexin A1 showed this protein to be a major mediator of TMAO actions.

Acute treatment of mice with TMAO (1.8mg/kg body weight, i.p.) enhanced BBB integrity within 2 hours, and was able to restore the BBB permeability defect induced by LPS administration (3mg/kg i.p., assessed 4h post-LPS, TMAO administered 2h post-LPS). Whole brain RNAseq analysis of TMAO-treated animals identified upregulation of multiple genes involved in cellular or axonal growth pathways. Chronic treatment of mice for two months with TMAO *via* the drinking water (0.5mg/l)

reduced signs of BBB integrity damage caused by long-term sub-acute LPS treatment (0.5 mg/kg/week, i.p.).

Conclusions

We show that physiologically-relevant concentrations of the dietary methylamine TMAO can beneficially modulate BBB integrity, providing direct mechanistic evidence for a positive role of this microbiome-associated metabolite and emphasising the BBB as an interface in the gut-brain axis. Notably, our findings stand in contrast to previous work describing deleterious effects of TMAO exposure at high concentrations or under non-physiological conditions, emphasising the importance of taking a holistic approach to understanding gut microbiota-host interactions.

Acknowledgements :- This work was funded by Alzheimer's Research UK Pilot Grant No. ARUK-PPG2016B-6. PREDEASY™ efflux transporter analysis kits were generously provided through the SOLVO Biotechnology Research and Academic Collaborative Transporter Studies (ReACTS) Program. This work used the computing resources of the UK MEDical BIOinformatics partnership – aggregation, integration, visualisation and analysis of large, complex data (UK MED-BIO), which was supported by the Medical Research Council (grant number MR/L01632X/1). SF was supported by Fundación Alfonso Martín Escudero. TS was supported by a bursary from the Imperial College London Undergraduate Research Opportunities Programme.

OC63

Associations between physical activity and sedentary time with endothelial function, arterial elasticity, arterial stiffness and clustered cardiometabolic risk in children: The ALSPAC study

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Introduction: The recently updated UK physical activity (PA) and sedentary time (ST) guidelines recommend an average of at least 60 minutes of moderate to vigorous PA (MVPA) per day across the week and to minimise ST to reduce cardiovascular disease risk in 5-18 year olds. However, these guidelines are based primarily on evidence surrounding measurement of traditional cardiovascular disease risk factors (e.g., adiposity and blood biomarkers) rather than direct measures of arterial structure and function. Literature examining associations between PA or ST and vascular health in paediatric populations is of low quality due to few objective measures of PA and ST, small sample sizes and a lack of control of confounding variables. **Aim:** To examine associations between objectively measured PA and ST with vascular outcomes (endothelial function, arterial elasticity and arterial stiffness) and clustered cardiometabolic risk in children. **Methods:** Cross-

sectional analysis of data from 4,277 children (2,226 girls) aged 10.6 ± 0.2 y from the Avon Longitudinal Study of Parents and Children. At age 9, a clustered cardiometabolic risk score was determined, vascular outcomes (flow mediated dilation for endothelial function, distensibility coefficient for arterial elasticity, pulse wave velocity for arterial stiffness) were measured age 10, and light PA, MVPA and ST measured via ActiGraph accelerometers age 11. Multiple linear regression analysis was combined with compositional data analysis for the accelerometer data to examine the associations between light or MVPA or ST as the predictor variable with the vascular variables or clustered cardiometabolic risk as the outcome. Missing data in covariates was replaced using multiple imputation. Models were adjusted for the following covariates: age, sex (group models only), age in years from peak height velocity, mother's social class, baseline vessel diameter (flow mediated dilation models only), time between vascular outcome measurement and accelerometer measurement (or time between clustered cardiometabolic risk and accelerometer measurements when cardiometabolic risk was the outcome), cardiorespiratory fitness scaled to lean body mass, lean mass index, clustered cardiometabolic risk (except where the cardiometabolic risk score was the outcome) and family history of cardiovascular disease. Results: Neither light PA, or MVPA or ST (relative to the remaining activity behaviours) were significantly associated with any of the vascular outcomes. The proportion of time spent in MVPA and ST (relative to the remaining activity behaviours) was inversely ($\beta = -0.126$; 95% CI = -0.202 to -0.050; $P = 0.001$) and positively ($\beta = 0.136$; 95% CI = 0.026 to 0.246; $P = 0.016$) associated with clustered cardiometabolic risk in the group analysis and the relationships were sex dependent. Although MVPA was negatively associated with clustered cardiometabolic risk in both boys ($\beta = -0.144$; 95% CI = -0.255 to -0.034; $P = 0.011$) and girls ($\beta = -0.110$; 95% CI = -0.211 to 0.009; $P = 0.032$), only girls had a positive association between ST and clustered cardiometabolic risk ($\beta = 0.199$; 95% CI = 0.060 to 0.339; $P = 0.005$). Conclusion: In childhood, MVPA should be maximised, and ST limited for cardiometabolic benefits. Longer exposure to cardiometabolic risk factors may be required to establish relationships between activity behaviours and vascular outcomes in youth. This highlights the need for additional high quality prospective research following individuals throughout adolescence and into early adulthood.

Acknowledgements :- We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

OC64

Nordic hamstring exercise metrics: how valid are they?

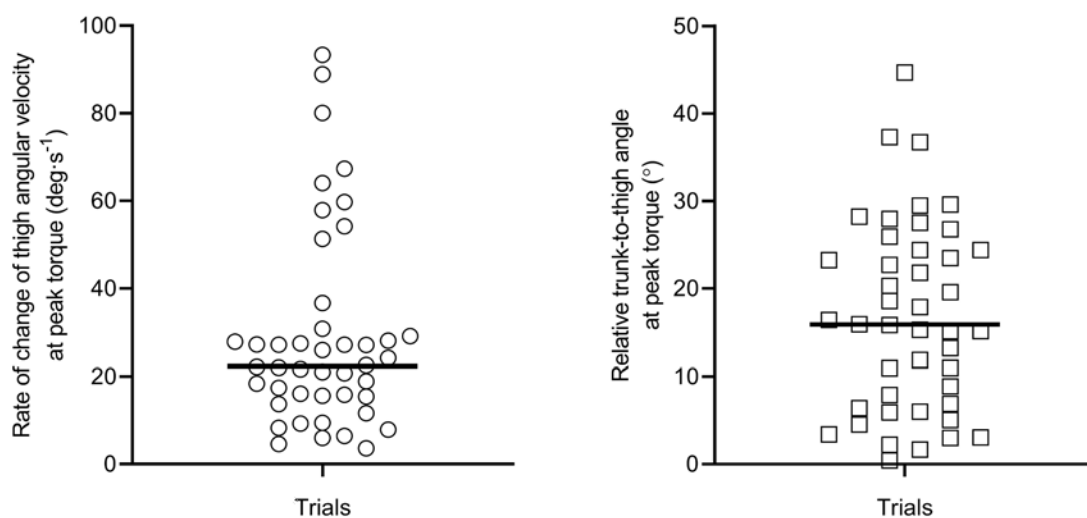
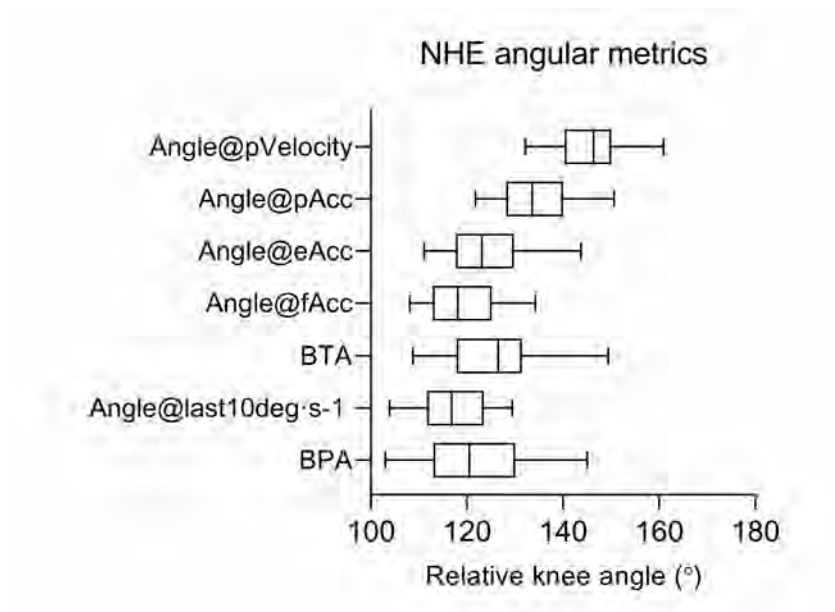
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Introduction: There has been a large amount of research focused on hamstring strain injuries in the last decade. As these types of injuries have frequent reoccurrence within 2 years (14-63%) (Brukner, 2015), prevention of initial injury is critical. Nordic hamstring exercise training has attracted much interest in the literature, due its success in reducing hamstring strain injuries, by up to 51% (Van Dyk et al., 2019) when employed as a component of preventative training programmes (Petersen et al., 2011; Van Der Horst et al., 2015). Following the development of a novel hamstring training device (NordBord) by Opar *et al.*, (2013), much of the subsequent research has centred on using Nordic eccentric knee flexor strength as a kinetic measure. There has been a rise in the use of kinematic variables, sometimes in place of force measurement, to provide a biomechanical analysis of the Nordic hamstring exercise action. However, variation in the methods used and a lack of clarity when defining terminology has made comparison between similar exercise studies difficult. **Aim:** We aimed to assess the utility of kinetic and kinematic metrics to assess Nordic hamstring exercise technique by comparing several metrics collected concurrently. **Methods:** Ethical approval for the study was approved by the Sheffield Hallam University Ethics Committee. Kinetic metrics collected were peak force, peak torque and peak torque normalised to body mass. Kinematic metrics collected included break-point angle, break-torque angle, relative trunk-to-thigh angle, angular velocity of the knee joint, first acceleration, angle of first acceleration, acceleration elbow angle at acceleration elbow, peak acceleration, angle at peak acceleration, peak velocity, angle at peak velocity and angle at last instance of $10 \text{ deg}\cdot\text{s}^{-1}$. 18 recreational male participants completed 3 bilateral Nordic hamstring exercise repetitions on a hamstring device equipped with in-line strain gauge load cells, integrated with a 3-dimensional motion tracking system. **Results:** Mean break-point angle occurred after the angle at first acceleration ($121.5\pm 10.4^\circ$ vs. $119.2\pm 7.1^\circ$) whereas break-torque angle occurred later in the Nordic hamstring exercise action ($126.0\pm 9.8^\circ$) showing highest correlation to the angle at greatest acceleration ($123.9\pm 7.9^\circ$, $r=0.85$). Future research should consider movement quality as the angular velocity of the knee joint at break-torque angle demonstrated large variation ($\text{range}=3.6\text{-}93.4 \text{ deg}\cdot\text{s}^{-1}$), and there was high intrasubject variability of relative trunk-to-thigh angle at peak-torque ($\text{range}=0.4\text{-}44.7^\circ$). **Conclusions:** This report proposes standardisation of methods and terminology used to define the Nordic hamstring exercise, providing a range of definitions, and allowing simpler comparison between kinematics research. Measuring break-torque angle is recommended to represent the proxy muscle length point at which hamstring muscle failure occurs, providing a specific metric relative to the proposed injury mechanism during high-speed running.

Table 1 Mean $\pm$ SD for each metric considered in the study. Variability and interquartile ranges (IQRs) also reported for every metric.

Metrics	Mean $\pm$ SD	Range (Min-Max)	IQR (Q1-Q3)
Kinetics			
Peak force (N)	249.4 $\pm$ 116.8	84.1-527.9	164.4-333.3
Peak torque (Nm)	149.7 $\pm$ 70.1	50.5-316.7	98.6-200.0
Peak torque/kg (Nm/kg)	1.6 $\pm$ 0.7	0.4-3.2	1.0-2.2
Kinematics			
BPA (°)	121.5 $\pm$ 10.4	103.0-145.0	113.0-130.0
BTA (°)	126.0 $\pm$ 9.8	108.8-149.4	117.8-131.5
AVK (deg·s ⁻¹) at BTA	29.2 $\pm$ 22.6	3.6-93.4	15.5-30.3
RTA (°) at BTA	16.7 $\pm$ 10.8	0.4-44.7	6.5-24.4
fAcc (deg·s ⁻²)	21.1 $\pm$ 10.0	6.7-52.8	14.4-26.8
Angle@fAcc (°)	119.2 $\pm$ 7.1	108.1-134.3	112.9-125.2
eAcc (deg·s ⁻²)	54.1 $\pm$ 27.8	21.0-121.9	34.1-76.9
Angle@eAcc (°)	123.9 $\pm$ 7.9	111.1-143.8	117.7-129.8
pAcc (deg·s ⁻²)	222.5 $\pm$ 61.8	87.9-340.9	194.4-267.6
Angle@pAcc (°)	134.0 $\pm$ 7.6	121.8-150.7	128.2-140.0
Angle@last10deg·s ⁻¹ (°)	117.3 $\pm$ 6.8	103.9-129.4	111.7-123.5
pVelocity (deg·s ⁻¹)	101.0 $\pm$ 24.0	40.1-155.8	82.9-119.1
Angle@pVelocity (°)	145.6 $\pm$ 7.1	132.2-160.9	140.4-150.0



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OC65

Heart rate variability diverse metrics as summary indexes of physiological interconnectedness and adaptability: examples of response to high-altitude trekking and during listening to Mozart music

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Measures which are robust indicator of overall biological functioning may be intended as summary parameters emergent from the physiological network complexity. Meaningful and valid whole-system indicators, obtained by feasible methods, can be of crucial importance and interest. As a matter of fact, heart rate variability (HRV) has been largely used as a sign of overall system adaptability and health (1). However, the specific response of HRV as an emergent property of interdependent regulatory systems operating at different time scales to adapt to environmental and psychological stressors and stimuli (2) has been poorly addressed. We used time-domain, frequency-domain and non-linear metrics of HRV in two diverse studies; the acquisition system consisted of a customized lightweight chest strap, based on a sensor with a sampling frequency of 500 Hz; ECG was acquired during 5 or 7 minutes in the supine position; both studies adhered to ethical standards and participants provided their written, informed consent. The Himalayan study was part of the "Kanchenjunga Exploration & Physiology" project: six Italians, five men and one woman (44 ± 15 years, 25.81 ± 3.25 kg/m²), lowland dwellers, and six Nepalese, all males (30 ± 8 years, 24.36 ± 4.70 kg/m²), lowland dwellers and altitude porters, completed 300 km of a Himalayan trek. ECG analysis was conducted at *baseline*, and before (*bHA*) and after (*aHA*) the high-altitude circuit (up to 5,143 m). SDNN, pNN50 and CVI were lower at *baseline* than at *bHA* (47.8 ± 24.0 vs 74.6 ± 37.6 ms, $p=.010$; 11.6 ± 14.0 vs 33.1 ± 26.4 %, $p=.003$; 3.08 ± 0.40 vs 3.51 ± 0.52 , $p=.008$). Higuchi fractal

dimension (HFD) showed higher basal values in the Nepalese group (1.60 ± 0.04 vs 1.55 ± 0.03 , $p=.041$), and a tendency for highest values at *bHA*. It may be argued that, before the trek started, participants had greater arousal and stress. Our results also indicated a better cardiovascular resilience of porters. The musical study was a cross-over intervention study part of the "HRV & Breath Tune" project: 25 European university students, 21-to-34 years, whose 68% females and 32% males, listened - in diverse days randomly ordered - to the Mozart Sonata in A minor, K. 331 (*Mozart*) or the same piece consisting only of beat (*Destructured*), plus *Control*. Tendencies were found for both SD1 ($p=.092$, $\eta^2_p=0.13$) and SD2 ($p=.130$, $\eta^2_p=0.11$) - with lower values during *Mozart* listening - and HFD, lower during *Destructured* listening (1.60 ± 0.04 vs 1.62 ± 0.07 and 1.62 ± 0.08 , $p=.117$, $\eta^2_p=0.13$). We argued that *Control* was the most relaxing condition, and *Mozart* allowed to maintain a bit of arousal, supporting that the "Mozart effect" acts through mood (3). We suggest using non-linear metrics, such as HFD (4), to evaluate the effect of several stressors on a plethora of populations. All in all, HRV metrics represent an intriguing hallmark of stress, disease and ageing; however, caveats should be highlighted about the use of HRV diverse metrics for feasible application, as large misuse and overuse flaw such indicators.

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Reference 3 :- Thompson WF, Schellenberg EG, Husain G. Arousal, Mood, and The Mozart Effect. *Psychol Sci.* 2001;12(3):248–51

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Acknowledgements :- The Himalayan study was supported by the Department of Psychological, Health and Territorial Sciences, University "G. d'Annunzio" of Chieti-Pescara. The music study was supported by the "Departments of Excellence 2018–2022" initiative of the Italian Ministry of Education, University and Research for the Department of Neuroscience, Imaging and Clinical Sciences (DNISC) of the University of Chieti-Pescara.

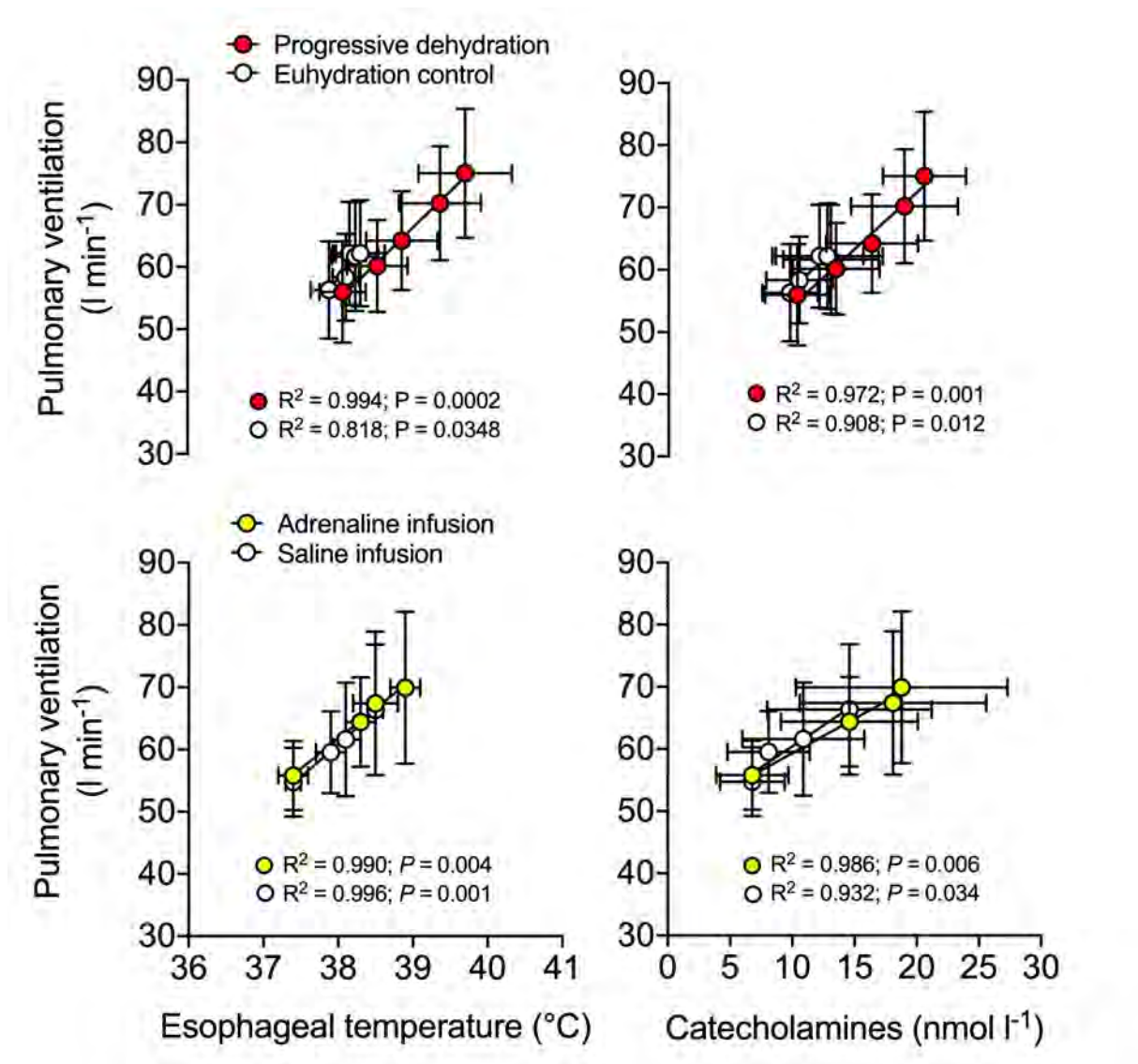
OC66

Pulmonary ventilation and gas exchange during prolonged exercise with distinct hydration and circulation levels: influence of dehydration, hyperthermia and sympatho-adrenergic activity

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Ventilation increases during prolonged heavy exercise in humans (1), but the impact of dehydration and hyperthermia on pulmonary ventilation and gas exchange remains unclear. In a series of human studies, we sought to: 1) characterise the influence of dehydration and hyperthermia, and the associated blunted pulmonary and systemic circulation, on the ventilatory and pulmonary gas exchange responses to endurance exercise; 2) isolate the contribution of dehydration and hyperthermia on the observed hyperventilation; and 3) discern the influence of sympatho-adrenergic activity in the ventilatory response to prolonged exercise. **Methods:** Following ethical approval, twenty-nine endurance-trained males took part in four studies. In study 1, on separate days, seven participants performed two submaximal exercises in the heat at a constant workrate initially eliciting $61 \pm 2\% \dot{V}O_{2\max}$. On the first day, they cycled until volitional exhaustion (135 ± 11 min), while developing progressive dehydration and hyperthermia (DE-HY; $3.9 \pm 0.7\%$ body mass loss and oesophageal temperature (T_{oes}) $39.7 \pm 0.6^\circ\text{C}$). On the second day (control), they cycled for the same duration maintaining euhydration and stabilising T_{oes} at $38.2 \pm 0.3^\circ\text{C}$. In studies 2 and 3, different participants ($n=7-8$) completed a series of submaximal cycling bouts that induced a state of either isolated hyperthermia, isolated dehydration, combined dehydration and hyperthermia, or euhydration (control). In study 4, while cycling for 120 min at a constant workrate initially eliciting $65\% \dot{V}O_{2\max}$, seven participants were intravenously infused, from 30 min onward, with equal volumes of either an adrenaline solution ($0.1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) or a saline solution (control). Ventilatory responses, pulmonary inspiratory and expiratory gases, $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) were measured in all the studies. Arterial blood gases were obtained in study 1. Statistical significance was determined using repeated measures ANOVA. **Results:** In study 1, at rest and after 20 min of exercise, ventilation ($\dot{V}E$), blood gases, $\dot{V}CO_2$ and $\dot{V}O_2$ were similar in both trials (all $P > 0.05$). Thereafter, $\dot{V}E$ increased $19 \pm 8 \text{ l}\cdot\text{min}^{-1}$ in DE-HY, but $6 \pm 2 \text{ l}\cdot\text{min}^{-1}$ in control (both $P \leq 0.006$). Thus, at exhaustion, $\dot{V}E$ was $13 \pm 8 \text{ l}\cdot\text{min}^{-1}$ higher in DE-HY. Hyperventilation in DE-HY was accompanied by a lower P_aCO_2 ($4 \pm 3 \text{ mmHg}$) and higher respiratory frequency ($7 \pm 4 \text{ breaths}\cdot\text{min}^{-1}$), arterial partial pressure of O_2 ($11 \pm 6 \text{ mmHg}$), and mixed-venous arterial CO_2 and O_2 content differences ($23 \pm 11 \text{ ml}\cdot\text{l}^{-1}$ and $32 \pm 7 \text{ ml}\cdot\text{l}^{-1}$, respectively) (all $P \leq 0.006$). Despite a lower cardiac output in DE-HY ($3.3 \pm 1.5 \text{ l}\cdot\text{min}^{-1}$; $P=0.001$), $\dot{V}O_2$ remained unchanged and $\dot{V}CO_2$ was elevated. The increased $\dot{V}E$ during exercise in both conditions was closely related to the rise in T_{oes} and circulating catecholamines (Fig. 1). Hyperthermia independently increased $\dot{V}E$ to a similar extent as combined dehydration and hyperthermia (5.7 ± 2.3 versus $5.0 \pm 2.2 \text{ l}\cdot\text{min}^{-1}$; $P=0.886$), whereas preventing hyperthermia in dehydrated individuals restored $\dot{V}E$ to control levels ($0.8 \pm 3.5 \text{ l}\cdot\text{min}^{-1}$; $P=0.719$). Adrenaline infusion caused hyperthermia and increased $\dot{V}E$ ($4.9 \pm 3.2 \text{ l}\cdot\text{min}^{-1}$; $P=0.006$; Fig. 1). **Conclusion:** Hyperthermia is the main stimulus increasing $\dot{V}E$ during prolonged exercise with distinct hydration and circulation levels. Sympathoadrenal discharge is a contributing factor to hyperthermia-induced hyperventilation. Adjustments in pulmonary gas exchange during endurance exercise with dehydration and hyperthermia ameliorate metabolic and acid-base disturbances in the face of a markedly blunted pulmonary and systemic circulation.



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OC67

Cardiovagal modulation is acutely reduced following high-intensity interval exercise but not moderate-intensity continuous exercise in physically active young and older male adults

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INTRODUCTION: Aerobic exercise attenuates age-related declines in resting cardiovagal modulation, as measured by heart-rate variability (HRV) and cardiac baroreflex sensitivity (BRS), which could reduce cardiovascular events and all-cause mortality risk. However, endurance exercise intervention studies in older adults produced equivocal findings. In this respect, the acute exercise model is advantageous as it allows for greater experimental control of exercise and confounding variables, and may be useful in investigating mechanisms of the exercise response. However, whether ageing alters HRV and BRS responses to acute exercise of different intensities remains unknown.

PURPOSE: We compared the response pattern of HRV and BRS at 10 and 60 min following an acute bout of high-intensity interval exercise (HIIE) and moderate-intensity continuous exercise (MICE) in physically active young and older adults.

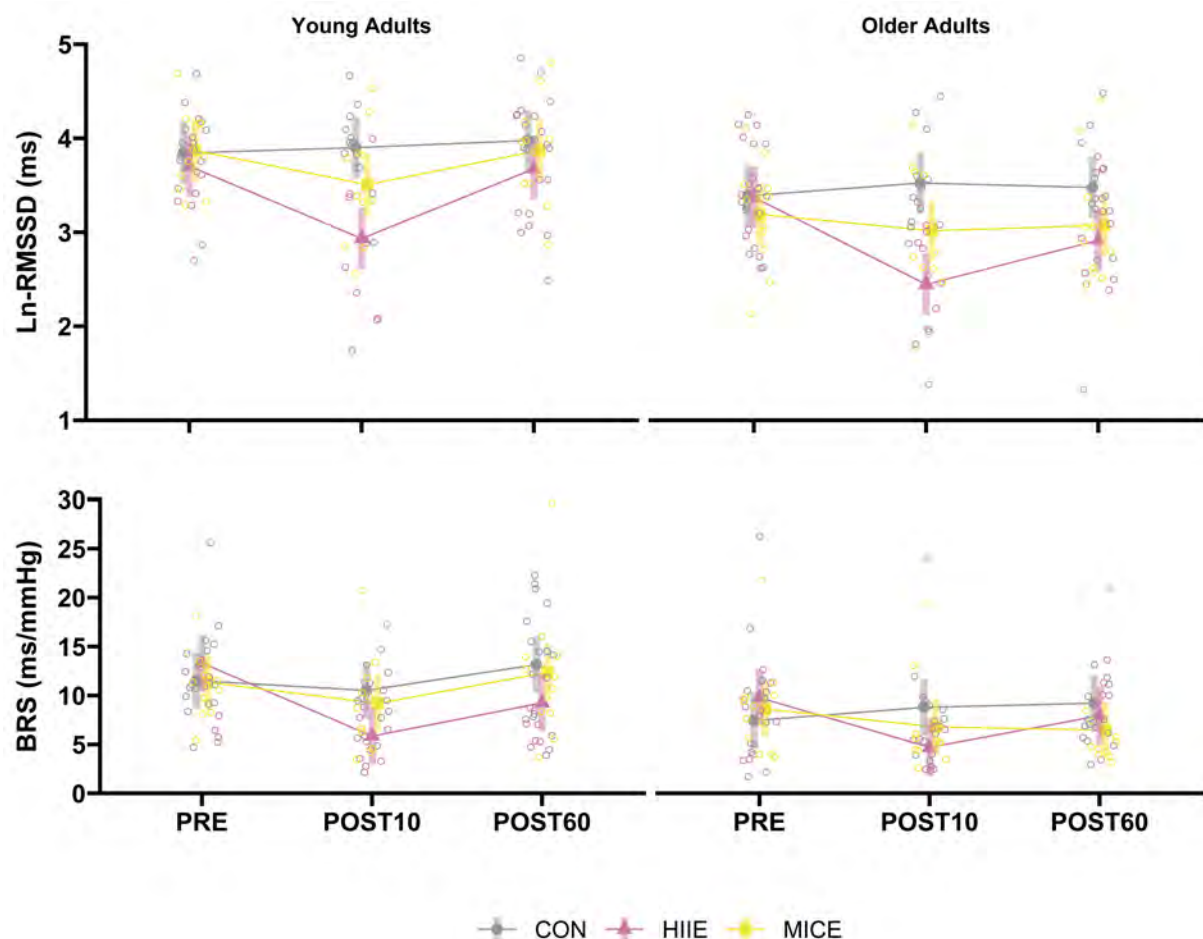
METHODS: Twelve young (20-40 years) and older (57-76 years) healthy and physically active male adults performed an isocaloric acute bout of HIIE, MICE, or a non-exercise condition in a randomized order. Exercise intervention intensity was defined by the maximum oxygen uptake determined during a maximal cardiopulmonary exercise test. HRV time and frequency domain indices and BRS measured at rest, 10 and 60-min following exercise were analysed offline over 2-min time-bins using a software built-in Matlab. Beat-by-beat systolic blood pressure and R-R intervals were recorded in a supine position using finger plethysmography and 5-lead ECG, respectively. BRS was estimated by the spontaneous sequence method, and the time-frequency domain analysis was conducted using the Daubechey-12 discrete wavelet algorithm. Pre-and-post condition changes in outcomes were analysed with linear mixed models.

RESULTS: Ln-standard deviation of normal-to-normal intervals ($d = -0.53$; 95% CI: -0.77 to -0.30 ms, $p < 0.001$), Ln-root mean square of successive differences ($d = -0.85$; 95% CI: -1.09 to -0.61 ms, $p < 0.001$), Ln-high-frequency power ($d = -1.60$; 95% CI: -2.11 to -1.10 ms²; $p < 0.001$), and BRS ($d = -6.28$; 95% CI: -8.91 to -3.64 ms/mmHg, $p < 0.001$) decreased following HIIE in young and older adults, but not in MICE (Figure 1). Indices returned to baseline 60 min into recovery. Overall group differences suggested that older adults had lower Ln-root mean square of successive differences ($d = -0.54$; 95% CI: -0.93 to -0.15 ms, $p = 0.009$) and Ln-high-frequency power ($d = -1.16$; 95% CI: -1.90 to -0.42 ms², $p = 0.004$), while BRS between young and older adults was not different ($d = -2.98$; 95% CI: -5.96 to 0.00 ms/mmHg; $p = 0.05$).

CONCLUSION: We found no evidence of age-associated response patterns in HRV or BRS to a single bout of HIIE or MICE in physically active participants. HIIE reduced cardiovagal modulation in physically active young and older adults, returning to baseline values 60 min into recovery. This might increase the likelihood of cardiovagal supercompensation towards resting vagal predominance on

the long run without increasing the risk for acute cardiovascular events in physically active young and older adults given the fast cardiovagal reactivation observed.

ETHICS: This study was aligned with the Declaration of Helsinki and approved by the Ethical Review Board of the Faculdade de Motricidade Humana, Universidade de Lisboa (10/2020). Informed consent was obtained from all studied participants.



OC68

Regulation of Phase Reset Mechanism in Mammalian Circadian Rhythms by Oligonucleotide Therapeutics

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In the COVID-19 pandemic, a lot of countries implemented lockdown policies to socially distancing.

The European CBT-I Academy has issued a statement addressing this policy's potential not to induce sleep disorders by increased stress and decreased afternoon activity. Getting high-quality sleep is an effective way to establish a daily rhythm and reduce to health risks.

One of the most common causes of sleep disorders has been recently stayed up late at night and exposed to strong Blue-Light from smartphones and other devices. Blue-Light stimulates melanopsin (OPN4)⁽¹⁾, a photoreceptor in retinal cells, which projects directly to the Suprachiasmatic Nucleus (SCN), and results in a Phase Shift in the mammalian circadian rhythms.

In this study, we screened DNA aptamers that specifically bind to OPN4 to regulate the phase of the central clock in one cell. The following experiments are committed to the policy of the Toyohashi University of Technology.

As a result, we obtained 15 kinds of OPN4 (melanopsin) DNA aptamers (Melapts) by the Cell-SELEX methods⁽²⁾. We performed a functional screening for the effect of Melapts on mammalian circadian rhythm by observing the phase shifts of *Period2* (*Per2*)⁽³⁾ gene expressional rhythms with 24 hours period. Circadian rhythms are driven by the transcriptional and trans-activational feedback loop of the clock gene (*Per2* and so on).

We have the *Per2*-ELuc cell line, mouse fibroblast cells with *Period2*-promoter region followed by Emerald Luciferase (ELuc) from the Brazilian' Pyrearinid beetle (*Pyrearinus termitilluminans*), expressed OPN4 stably on the cell membrane. OPN4-expressing *Per2*-ELuc cell⁽⁴⁾ can be used to measure the phase shift of the single-cell clock after input of Melapt and light stimulation. *Per2*-ELuc cell line added Melapts and photo-stimulated by Blue-Light for 15 min at subjective dawn at the CT22 (Circadian Time 22) or subjective afternoon at the CT8

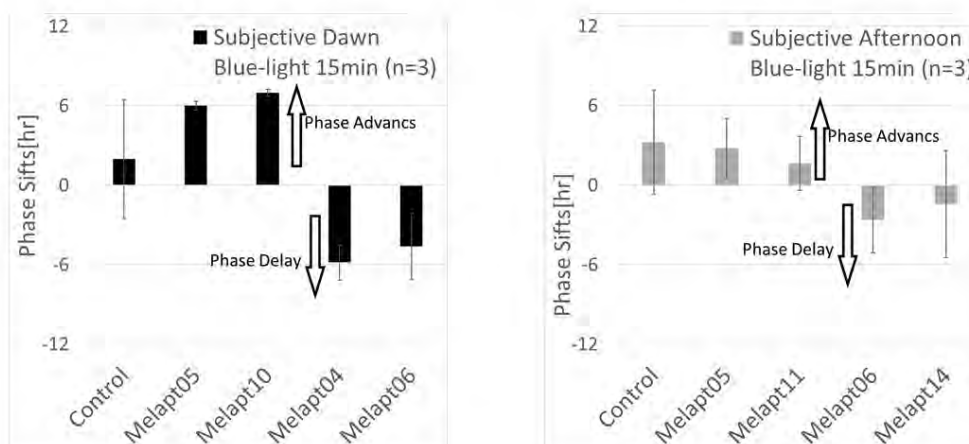
When photo stimulation at subjective dawn induces the Phase Advance, we obtained Melapt 05 and 10, making the clock phase more Phase-Advance. Melapt 04 and 06, which make the clock phase to Phase-Delay.

On the other hand, when photo-stimulation at subjective afternoon induces the Phase-Delay, we obtained Melapt 06 and 14 for more Phase-Delay and Melapt 05 and 11 Phase-Advance.

For example, it is supported that adding Melapts binds to OPN4 and inhibits transcription of the *Per2* clock gene by photo stimulation at the CT8 afternoon when the transcriptional of the *Per2* clock gene begins to decrease. The final effect of Melapt may result in either Phase-Advance (Melapt05,11) or Phase-Delay (Melapt06,14), depending on the timing of adding Melapt.

This study suggests that Melapt 05 helps you wake up earlier in the morning, and Melapt 06 enables you to sleep earlier at night. These Melapts may help to shift the phase of circadian rhythms.

Fig.1 The phase of circadian rhythms in *Per2*-ELuc cells by Melapts.



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OC69

Application of molecular dynamics to elucidation of the mechanism of glucose transport via GLUT1

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Owing to the uncertainties of transport kinetics, the mechanism of glucose transport via GLUT1 remains unclear, however extended atomistic molecular dynamics simulations of GLUT1 embedded in a symmetrical bilayer of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) in the fluid state at

323.15 K, or in the gel state at 308.15 K, surrounded by a physiological salt solution have resolved some ambiguities.

An outstanding question is: does net glucose transport require conformational changes that alternately expose the central high affinity ligand binding site to externally and internally facing solutions; or does glucose transit by random jumps between adjacent sites, aided by small fluctuations that intermittently open and close the tunnels and cavities along the central cleft?

By using a “flooding protocol,” equivalent to 50 mM glucose, in comparison with another without glucose, atomistic molecular dynamics simulations reveal a large number of amino acids, whose fluctuations alter with raised glucose concentration. Both protocols are applied to the fluid and gel membrane states: the gel state reduces transporter fluctuations [1].

The glucose-dependent changes are most evident in the extra-membranous zones. In the fluid state, during 2 μ s simulations, these glucose-amplified fluctuations permit glucose and water permeation from both external and cytosolic solutions along the length of the central pore. However, in the gel state, glucose penetration is confined to the extramembranous regions.

With increasing glucose proximity (< 5 Å) to the bottlenecks that normally occlude the internal and external openings of GLUT1's central pore, the frequency of opening events increases by 2-20-fold. These bottlenecks are formed by hydrophobic side chains, held together by van der Waals interactions. Rotamer changes occasionally alter the minimal external bottleneck radius from ≈ 1.0 Å to 2.2 Å and at the internal barrier from 1.2 Å to 2.3 Å. Thus, close glucose proximity allows spontaneous unsteered glucose flows through these apertures. No glucose penetration into the intramembranous regions occurs in the gel state.

Furthermore, in the fluid flooded state, glucose proximity (< 6 Å), increases the probability of wider separations occurring in 12/17 salt bridges between lysine or arginine and glutamate or aspartate within the extramembranous loops.

Glucose-dependent opening of the extramembranous tunnels in the fluid-flooded condition permits several observed glucose traversals through the GLUT1's central region, considered to be the high affinity binding site, without any large accompanying transmembrane helical conformation changes.

This structural expansion caused by multiple simultaneous H-bonding interactions between glucose and GLUT1 accounts for the higher spontaneous intramolecular glucose mobility seen in these simulations than with any reported previously.

These findings are consistent with the temperature sensitivity of glucose transport seen in reconstituted lipid DPPC vesicles [2] and in human erythrocytes [3] and the glucose-dependent decrease in activation energy of L-sorbose transport across human erythrocyte GLUT1(4).

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OC70

Pharmacological inhibitors of the cystic fibrosis transmembrane conductance regulator exert off-target effects on store-operated calcium entry and epithelial sodium channel function

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Introduction: The cystic fibrosis transmembrane conductance regulator (CFTR) channel and the epithelial Na⁺ channel (ENaC) play essential roles in transepithelial ion and fluid transport in various tissues, dysfunction of which leads to diseases such as cystic fibrosis, secretory diarrhoea and kidney disease. In the field of epithelial transport, two drugs assumed to be relatively selective CFTR inhibitors, CFTR_{inh}-172 and GlyH-101 (Ma *et al.*, 2002; Muanprasat *et al.*, 2004), have been staple tools for studying the role of CFTR *in vitro*. However, the potential off-target effects of these inhibitors on cation channels present in epithelial cells have not previously been addressed.

Aims: To investigate the effects of CFTR_{inh}-172 and GlyH-101 on store-operated calcium entry (SOCE) through calcium-permeable channels, as well as on ENaC function.

Methods: Calu-3 and HEK293 epithelium-derived cells were loaded with the Ca²⁺-sensitive fluorescent dye, Fura-2-AM, and SOC channels activated by depleting intracellular Ca²⁺ stores with 200nM thapsigargin, in Ca²⁺-free conditions. SOCE was measured by tracking changes in cytosolic Ca²⁺ using a repeated extracellular Ca²⁺ addback protocol, alternating between Ca²⁺-free solution and superfusate containing 1mM Ca²⁺. Western blot and qPCR were performed to detect CFTR protein and mRNA expression, respectively, in each cell line. *Xenopus* oocytes were purchased from the European *Xenopus* Resource Centre and micro-injected with cRNA to express human $\alpha\beta\gamma$ -ENaC or $\delta\beta\gamma$ -ENaC. ENaC-expressing oocytes were subjected to two-electrode voltage-clamp (-60 mV holding potential), and changes in transmembrane currents (*I_M*) in response to amiloride (100 μ M) and CFTR-inhibitors were recorded. All summary data are presented as mean $\pm$ SEM, and n denotes the number of independent experiments.

Results: Pre-treatment of Calu-3 cells with 20 μ M CFTR_{inh}-172 irreversibly reduced the SOCE amplitude (24.2 $\pm$ 12.3% for 10-min, p=0.0258 and 44.2 $\pm$ 10.0%, p<0.0001 for 30-min treatment, n=4-14, Holm-Sidak multiple comparisons vs. vehicle-treated) and rate (52.0 $\pm$ 16.4%, p=0.0043 for 30-min

treatment, n=4-14, Holm-Sidak multiple comparisons vs. vehicle-treated) in a time-dependent manner (n=4-14, $p < 0.05$, chi-square test). Pre-treatment of HEK293 cells with 20 μ M CFTR_{inh}-172 or 10 μ M GlyH-101 also significantly inhibited the amplitude ($50.7 \pm 10.3\%$, $p = 0.0030$ and $38.5 \pm 8.0\%$, $p = 0.0098$, respectively; n=5-6, Holm-Sidak multiple comparisons vs. vehicle-treated) and rate ($67.5 \pm 10.5\%$, $p = 0.0015$ and $65.7 \pm 7.3\%$, $p = 0.0015$, respectively; n=5-6, Holm-Sidak multiple comparisons vs. vehicle-treated) of SOCE. This was surprising as HEK293 cells expressed negligible levels of CFTR mRNA or protein compared to Calu-3 cells. It was also notable that CFTR inhibitors affected ENaC function. Amiloride-sensitive I_M (ΔI_{ami}) in *Xenopus* oocytes expressing $\alpha\beta\gamma$ -ENaC were reduced by 20 μ M CFTR_{inh}-172 ($17.5 \pm 3.0\%$, $p = 0.0004$, n=9, Student's paired t-test) and 10 μ M GlyH-101 ($49.6 \pm 4.0\%$, $p = 0.0005$, n=9, Student's paired t-test). While GlyH-101 also inhibited ΔI_{ami} in $\delta\beta\gamma$ -ENaC expressing oocytes ($43.6 \pm 2.3\%$, $p < 0.0001$, n=9, Student's paired t-test), CFTR_{inh}-172 had a small stimulatory action ($8.2 \pm 1.1\%$, $p = 0.0019$, n=9, Student's paired t-test). Water-injected control oocytes did not respond to amiloride, CFTR_{inh}-172, or GlyH-101.

Conclusion: The putative specific CFTR inhibitors, CFTR_{inh}-172 and GlyH-101, exerted CFTR-independent inhibition of SOCE in human epithelial cell lines, and altered ENaC-mediated currents differentially based on subunit composition. Our data indicate that caution is needed when interpreting results using these inhibitors with the intention of dissecting the involvement of CFTR in biological processes.

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OC71

Droplet Electro-transfection by Electrostatic Field without Contact Charge

Electrophoresis Droplet Electro-transfection by Electrostatic Field without Contact Charge

Electrophoresis

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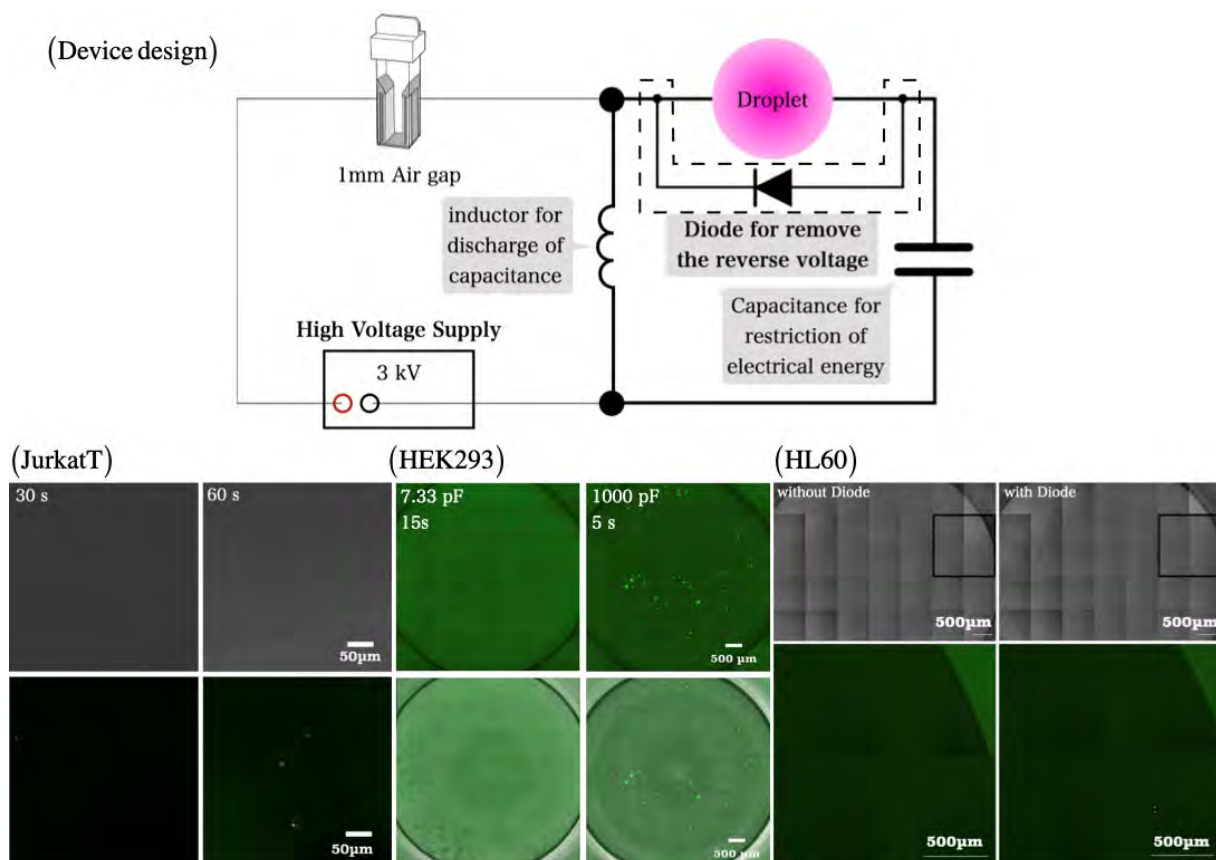
Electroporation (EP) is a method of introducing foreign substances, such as nucleic acids and proteins, into cells by controlling the permeability of the cell membrane using a high-energy electric

field. EP does not require external reagents and can be used for a wide range of applications. In addition, it is easy to operate and can be used even at a low biosafety level. However, EP is highly cytotoxic because it applies high-energy electrical pulses to cells. Previously, we reported water-in-oil droplet electroporation (w/oEP), in which electrostatic charges, a low-energy pulsed electric field, are applied to cells to reduce the cytotoxicity of EP [1]. However, since w/oEP uses contact charge electrophoresis (CCEP) [2], the droplets need to move through the oil. This unstable droplet motion results in a decrease and instability for transfection efficiency. In this work, we have developed a device to realize the electrical energy condition of w/o EP without CCEP.

A device was designed to apply an electric field similar to that of w/oEP. The electrical energy applied to the droplet was limited by connecting a capacitance in series with the droplet. The capacitance was calculated by regarding the droplets in oil as spheres of conductors in the insulator. A coil was connected in parallel to the droplet and capacitor to apply a periodic pulsed electric field. The plasmid vector (pCMV-EGFP) was introduced into adherent cells (HEK293 cells) and floating cells (Jurkat T cells) using this device. 4 μ L droplets containing 50-1000 ng/ μ L plasmid DNA and 4000 cells were subjected to the voltage. The circuit parameters were as follows: inductance of the coil was 47 μ H, capacitance of the capacitor was 7.33 pF or 1000 pF, and the processing time was 5-60 s. When the capacitor discharges, an electric field of reverse polarity is applied to the droplet, but no reverse voltage is applied to the w/o EP. Furthermore, the cytotoxicity of the bipolar voltage had been experimentally elucidated by conventional methods. Therefore, we eliminated the reverse voltage with a parallel diode. Using this device, we introduced pCMV-EGFP into HL60 cells. Two days after transfection, cells were observed by fluorescence microscopy and the number of cells with fluorescence signal was counted by ImageJ.

EGFP-positive Jurkat T cells were observed for 30 s and 60 s. EGFP was not observed in HEK293 cells at any application time under the condition of 7.33 pF capacitance. However, EGFP was observed in HEK293 cells treated for 5 s under the condition of 1000 pF electrostatic capacity. The HL60 cell transfection was performed according to the conditions described previously. Two days after the gene transfection, EGFP-positive cells were found only in the condition with connected the diode. This suggests that the application of bipolar voltage may have had a negative effect. These results suggest that this novel method can be applied to various types of mammalian cells by examining the conditions.

Time [sec]	Inductance [μH]	Capacitance [pF]	Diode	ImageJ counts	Number of Cells	Efficiency %
30	5	2000	○	216	100000	0.22
30	5	2000	×	42	100000	0.04
30	5	3000	×	23	100000	0.02
30	5	3000	○	15	100000	0.02
30	20	3000	×	14	100000	0.01
30	20	3000	○	11	100000	0.01
30	20	2000	×	9	100000	0.01
30	20	2000	○	5	100000	0.01



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OC72

Nutraceutical targeting of the bile acid receptor, farnesoid X receptor, for intestinal disease.

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Introduction: Bile acids, classically known for their roles in dietary fat absorption are now recognised as hormones critical to regulating intestinal and metabolic function, including mucosal immune responses, epithelial proliferation/apoptosis, and transepithelial transport and barrier function. Downregulation of the nuclear bile acid receptor, farnesoid X receptor (FXR), in intestinal epithelium occurs in inflammatory bowel disease and colorectal cancer, whereas FXR activation prevents disease progression in pre-clinical models. Thus, strategies to upregulate epithelial FXR expression have been proposed to treat such conditions. Previous studies suggest that some plant-derived phytochemicals have the capacity to modulate FXR activity. Plant sources have been reported to contain these phytochemicals, such as KFS1, which are proposed to have FXR modulatory activity.

Aim: The aim of the current study was to investigate the potential for developing plant extracts, rich in FXR-modulating phytochemicals, as a novel nutraceutical-based approach to treat and prevent intestinal diseases.

Methods: T₈₄ human colonic epithelial cells and primary cultures of murine colonic epithelial enteroids were employed to examine the effects of a common-dietary phytochemical, denoted here as KFS1, and a KFS1-rich plant extract on FXR expression and FXR signaling *in vitro*. *Ex vivo* studies were carried out on human colonic tissue obtained during endoscopy with ethical approval from Beaumont Hospital. *In vivo* studies in C57BL/6 mice were carried out and proximal colonic tissue were examined with ethical approval from the HPRC. Expression of FXR and FGF19/15, an established marker of FXR activation, was assessed by qRT-PCR, ELISA and immunoblotting. Data are expressed as mean $\pm$ SEM for a series of *n* experiments.

Results: KFS1 treatment (5 μ M; 24hrs) increased FXR mRNA and protein expression in T₈₄ cells by 4.2 $\pm$ 0.4 (*p*<0.05; *n* = 4) and 1.7 $\pm$ 0.1 fold (*p*<0.05; *n* = 7), respectively. KFS1 also enhanced FGF-19

protein expression, an established marker of FXR activity, in response to the FXR agonist, GW4064 (5 μ M), by 4.1 ± 0.5 fold ($p < 0.001$; $n = 7$). Similarly, KFS1 significantly upregulated FXR expression by 2.1 ± 0.4 fold in human biopsies ($p < 0.05$; $n = 6$). Oral KFS1 administration to mice had a tendency to upregulate colonic epithelial FXR signalling *in vivo* and increased FXR expression and GW4064-induced FGF15 mRNA by 2.3 ± 0.2 ($p < 0.01$; $n = 4$) and 2.2 ± 0.1 fold ($p < 0.01$; $n = 4$) in murine epithelial colonic enteroids. Finally, a methanolic KFS1-rich plant extract, denoted QE1, verified by LC/MS to contain KFS1, increased FXR protein expression in T₈₄ cells by 1.8 ± 0.2 fold ($p < 0.05$; $n = 5$) and enhanced GW4064-induced FGF19 protein release by 1.9 ± 0.1 fold ($p < 0.01$; $n = 9$).

Conclusion: In conclusion, our data demonstrate that KFS1-containing plant extracts modulate expression and activity of the nuclear bile acid receptor, FXR, in the intestinal epithelium. Given the critical role of FXR in maintenance of gut health, such extracts have significant therapeutic and commercial potential to be developed as a novel first-in-class “FXR-targeted nutraceutical” for treatment and prevention of common intestinal disorders.

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OC73

Protective effect of Phoenixin-14 on acetic acid-induced oxidative gastric injury in rats: role of the mucus layer and mast cells

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Introduction: Peptic ulcer occurs due to an imbalance between factors that attack gastric mucosa and those protecting from damage. Phoenixin (PNX), a newly discovered peptide with PNX-14 and PNX-20 isoforms, is widely expressed in the brain and gastrointestinal tract(1,2). Apart from its role in reproductive functions and the regulation of food intake, it is considered as a regulatory peptide of the gut-brain axis(3). The aim of the study was to investigate the possible antiulcer efficacy of PNX-14.

Methods: Ethical approval was obtained from Marmara University Animal Care and Use Committee (59.2020.mar). Female Sprague Dawley rats were randomised into “treatment plus pre-treatment” and “treatment without pre-treatment” groups. In the pre-treated ulcer groups ($n=16$), PNX-14 (50

$\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) or saline was injected intraperitoneally for 3 days prior to ulcer induction. On the 4th day, under ketamine and chlorpromazine anaesthesia (100 and 10 $\text{mg}\cdot\text{kg}^{-1}$, intraperitoneally) acetic acid (80%, v/v) was applied for 1 min on serosal surface of stomachs, while control rats (n=8) had sham-surgery. Post-surgically, PNx-14 or saline treatment was continued for 3 consecutive days. In the “treatment without pre-treatment” groups (n=16), PNx-14 ($50 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) or saline was administered for 3 days only after the ulcer-inducing surgery, and control rats (n=8) were treated with saline for 3 days following sham-surgery. On post-operative 3rd day, gastric serosal blood flow was measured under anaesthesia via laser Doppler flowmetry, and rats were then euthanised by exsanguination via cardiac puncture. Gastric levels of malondialdehyde (indicating lipid peroxidation), antioxidant glutathione and myeloperoxidase activity (indicating neutrophil infiltration) were measured by spectrophotometric methods. Gastric samples were stained by haematoxylin-eosin (injury scoring), toluidine blue (mast cells) and periodic acid Schiff (PAS; mucus-secreting cells and mucous secretion). Using GraphPad Prism 9.0 software, statistical analyses were made by one-way ANOVA and Student’s t-tests and expressed as mean $\pm$ SEM.

Results: In saline-treated ulcer groups, gastric MPO activity ($p<0.05$ - 0.01), malondialdehyde levels ($p<0.01$ - 0.001) and microscopic damage scores ($p<0.001$) were elevated with respect to control groups, while gastric glutathione levels were reduced ($p<0.01$). The number of mast cells in submucosal and muscular layers were increased and PAS-positive surface /neck mucous cells were severely depleted in saline-treated ulcer groups. PNx-14 had no effect on myeloperoxidase activity, but PNx-14 given either as treatment or treatment plus pre-treatment reduced malondialdehyde levels ($p<0.05$) and histological damage scores ($p<0.05$), while depletion in gastric glutathione was abolished in PNx-pre-treated ulcer group ($p<0.05$). Both modalities of treatments depressed the numbers of mast cells and increased the counts of PAS-positive cells. Gastric blood flow, which was not significantly altered in saline-treated ulcer groups, was decreased with PNx-14, reaching to statistical significance with its prolonged administration ($p<0.05$).

Conclusions: Despite its inhibitory action on gastric blood flow, PNx-14 alleviated gastric mucosal damage by supporting mucosal defence through the maintenance of mucus layer and by reducing mast cell infiltration, but had no impact on neutrophil-mediated inflammation.

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OC74

Characteristics of the inhibitory action of the novel small-molecule blocker of TRPC1/4/5 channels Pico145 on the muscarinic cation current in murine intestinal myocytes

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Evaluation of the functional roles of canonical TRPC1/4/5 channels in native cells has for a long time been hampered by the lack of highly potent, subtype-selective modulators of these channels. However, with the recent discovery of the direct and potent agonist of these channels, (-)-englerin A (EA) [1], as well as their subtype-specific small-molecule blocker termed Pico145 [2], there is now a good prospect for our better understanding of both the physiological significance and therapeutic potential of these receptor-operated channels, which are widely expressed in various cell types. We have previously extensively characterised biophysical and pharmacological properties of the muscarinic cation current (mI_{CAT}), which is activated through synergy between M2 and M3 muscarinic receptors in gastrointestinal myocytes, whereby it underlines cholinergic excitation-contraction coupling [3]. Since mI_{CAT} is predominantly mediated by TRPC4 channels [4] we now aim to characterise its inhibition by Pico145.

Ileal myocytes that were freshly isolated from two month-old male BALB/c mice were used for patch-clamp recordings of whole-cell currents. These were carried out at room temperature (20-22 °C) using symmetrical Cs^+ containing (125 mM) solutions with $[Ca^{2+}]_i$ 'clamped' at 100 nM using 10 mM BAPTA/4.6 mM $CaCl_2$ mixture, as necessary for mI_{CAT} isolation [4]. The steady-state I-V relationships of mI_{CAT} were measured by slow (6 s duration) voltage ramps from 80 to -120 mV, which were applied every 30 s. Values are means $\pm$ S.E.M.

Carbachol (50 μ M)-induced mI_{CAT} was already strongly inhibited by Pico145 at 1 pM. However, considering that the inhibition developed rather slowly, thus overlapping with current desensitisation, for a more reliable quantitative characterisation of this effect we used intracellularly applied GTP γ S (200 μ M) to induce mI_{CAT} bypassing muscarinic receptors, in which case no or very little desensitisation was present. The IC_{50} value for the inhibitory effect of Pico145 on this current was found to be 3.1 pM. The inhibition developed rather slowly, with the mean time constant of 140 ± 6 s ($n=9$), which was surprising for such a high affinity blocker. Moreover, voltage steps to -120 mV relieved the inhibition, while it was rapidly re-established upon stepping to 80 mV with the mean time constant of 108.0 ± 10.3 ms ($n=6$). Notably, EA (10 nM)-induced current was much more resistant to the inhibitory action, with 100 pM Pico145 causing current inhibition by only 43 ± 15 % ($n=6$). Finally, functional assessment of the Pico145 effect using *in vitro* tensiometry showed significant concentration-dependent suppression of 50 μ M carbachol-induced ileal contractions by Pico145 applied cumulatively at 0.1-100 pM. These properties of Pico145 are generally consistent with those reported for overexpressed TRPC4 channels (including its reduced potency at elevated EA concentration as well as some voltage dependence) [2], yet we note substantially higher (by 1-2 orders) affinity of Pico145 in the case of native TRPC4-mediated mI_{CAT} .

All animal studies using BALB/c mice were carried out in accordance with the recommendations of the EU Directive 2010/63 on the protection of animals used for scientific purposes and approved by the Institutional Ethics Committee (No. 04/20).

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OC75

Small molecule inhibition of CARM1 activity in skeletal muscle

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Coactivator-associated arginine methyltransferase 1 (CARM1) catalyzes the methylation of arginine residues of target proteins. CARM1 expression and activity are elevated during skeletal muscle atrophy. Importantly, the muscle-specific knockout of CARM1 partially attenuates the loss of muscle mass and the expression of atrophy-related genes during periods of neurogenic muscle disuse. Therefore, inhibition of CARM1 may be a therapeutic target for mitigating skeletal muscle atrophy. The purpose of this study was to determine the effectiveness of EZM2302 (EZM; Epizyme, Inc.), a novel, orally bioactive, specific CARM1 inhibiting compound, at repressing CARM1 activity in skeletal muscle. We utilized 3-month-old male and female mice (n = 3/sex) that were housed and cared for in accordance with Canadian Council for Animal Care guidelines. Mice were treated with either 150 mg/kg EZM or vehicle (Veh) via oral gavage BID for either 2, 4, or 8 days. Tissues were collected 6 hours following the final dose and muscle mass was recorded. Western blotting was performed to evaluate protein expression in the tibialis anterior (TA) muscle and liver. To assess CARM1 activity, we investigated the arginine methylation status (i.e., the methylated form of the protein relative to its total amount) of known CARM1 substrates BAF155 and PABP1, as well as the myocellular level of arginine methylated proteins preferentially targeted by CARM1. At this time, we observed similar outcomes between males and females so the data were collapsed by sex (n = 6). EZM significantly reduced BAF155 methylation status in the TA muscle by 72-80% between 2-8 days of treatment relative to the Veh-treated group. EZM also attenuated (p < 0.05) muscle PABP1 methylation status by 65% and 58% after 4 and 8 days of treatment respectively. Similarly, arginine methylation of CARM1-specific substrates across all muscle proteins was reduced by ~50% (p < 0.05) between 2-8 days of EZM administration. The attenuation of BAF155 and PABP1 methylation status, as well as arginine methylated CARM1-specific substrates, was greater in liver as compared to muscle. Skeletal muscle mass, including the TA, quadriceps, triceps, and extensor digitorum longus, in the EZM-

treated mice were similar to Veh-treated mice at all time points. Collectively, these results suggest that EZM is effective at significantly inhibiting CARM1 activity in skeletal muscle. Future work in our laboratory will examine the efficacy of EZM in attenuating the loss of muscle mass and function during atrophy-inducing conditions.

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OC76

The NAD⁺ dependent Poly ADP-ribose polymerase PARP-1 governs differentiation and energy metabolism of mouse skeletal muscle.

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Introduction. The NAD⁺ (Nicotinamide Adenine Dinucleotide) dependent PARP1 (poly-ADP-ribose polymerase1) enzyme is confined to the nuclear and cytoplasmic compartments. Activation of PARP1 cleaves the cofactor NAD⁺, yielding salvageable nicotinamide and ADP-ribose that is post-translationally applied to target proteins altering their biological activity (known as PARylation). Well established within the DNA Damage Repair Response, PARP1 inhibition has clinical utility in the treatment of a variety of cancers and it has been assumed that in absence of DNA damage PARP1 was basally inactive. However, emergent data indicate PARP1 may target additional fundamental cellular pathways governing energy metabolism and cellular identity. In parallel, our knowledge surrounding the molecular drivers of skeletal muscle cell differentiation is currently incomplete. Declines in myogenic capacity, muscle fiber contractility and metabolic adaptation all currently underpin the loss of physiological performance recorded in aged humans.

Aims. Here we asked whether modulation of the cells major NAD⁺ consumer PARP1 influences the progress of skeletal muscle differentiation or the metabolic capability of newly formed myotubes. **Methods + Results.** Analysis of C2C12 (murine myoblast) differentiation demonstrates basally detectable levels of both PARP-1 and PARylation (using Western immunoblotting (n=6)). Cellular levels of PARylation rise to a peak on day 1 of differentiation, coinciding with reduction in serum concentrations and PARylation trends downwards from days 2 through 6 with PARP1 also declining. PARP1 inhibitor treatment during differentiation significantly reduces levels of PARylation detectable by western blotting (n=6, 0.6 fold decrease ± 0.08 SEM, $p < 0.005$). Unbiased LC-MS evaluation (n=9) of these lysates showed PARP1 inhibition significantly altered the abundance of proteins regulating skeletal muscle development (5.85 Fold Enrichment, Pvalue 2.27×10^{-11}), muscle contraction (15.4 Fold Enrichment, Pvalue 1.14×10^{-11}) and myofibre assembly (18.35 Fold

Enrichment, Pvalue 4.57×10^{-09}). These data support the hypothesis that PARP1 controls the myogenic trajectory of skeletal muscle differentiation. RNAseq analysis (n=5) of SiRNA PARP1 in differentiating C2C12 muscle cells significantly altered the expression of 275 genes (165 Upregulated and 110 Downregulated (p<0.005)). Pathway enrichment of these genes was found not only to dysregulate well established PARP1 regulators like Base excision repair and RNA transport, but also overrepresentation of novel PARP1 associated pathways governing muscle folate metabolism (Pvalue 9.77×10^{-06}), protein folding events (Pvalue 7.76×10^{-06}) and glycogen formation (Pvalue 3.16×10^{-03}). Additionally, we show glucose deprivation of C2C12 myotubes elevates levels of PARylation in a concentration dependent manner emphasising the potential importance of PARP1 to muscle cell energy homeostasis (n=3, Pvalue 0.05). **Conclusions.** These data show that PARP1 mediated PARylation is dynamic during muscle differentiation and plays hitherto unanticipated roles in muscle physiology. Moreover, it suggests that cellular NAD⁺ availability may be of underappreciated importance to the inherent processes of differentiation, cellular architecture and energy metabolism.

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OC77

Chronic caffeine intake ameliorates adipose tissue function by improving angiogenesis, weight gain and metabolism

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Background and aims: Caffeine, a non-selective adenosine receptor antagonist, is one of the psychotropic substances most consumed in the world (1). Chronic caffeine intake improves weight gain (2,3), insulin sensitivity and glucose tolerance in animals (3,4) and humans (5) contributing to reduced risk of type 2 diabetes. Knowing that caffeine positively impacts metabolism, and that angiogenesis contributes to its improvement, we investigated if the beneficial effects of chronic caffeine intake on diet-induced insulin resistant animals involve improvements in lipid metabolism and angiogenic mechanisms in the adipose tissue.

Materials and Methods: Male Wistar rats aged 8-12 weeks were submitted to 3 and 19 weeks of high-fat diet (HF, 60% of lipids) or to control diet. In the last 2 and 6 weeks, respectively, groups were divided, and half submitted to caffeine treatment (1g/L) in drinking water. At the end of diet period insulin-sensitive tissues were collected. Proteins related to lipid metabolism: ATP citrate lyase (ACL) and hormone-sensitive lipase (HSL); to angiogenesis: platelet endothelial cell adhesion molecule (CD31) and vascular endothelial growth factor (VEGF); and to insulin signaling pathways: protein Kinase B (Akt) and insulin receptor (IR) were evaluated. Hematoxylin-eosin staining was performed to evaluate adipocyte perimeter and immunohistochemistry for CD31 and VEGF expression analysis.

Laboratory care followed the European Union Directive 2010/63/EU and was approved by NOVA Medical School Ethics Committee. Data was analysed using graphpad prism software using t-test student and ONE or TWO-Way ANOVA with Turkey's multiple comparison test. p-values<0.05 were considered significantly different.

Results: 3 weeks of HF diet increased weight gain (182.3%, p<0.0001), visceral fat (49.7%, p<0.05), adipocytes perimeter (35.9%, p<0.05) and HSL expression (51.99%, p<0.05); and decreased ACL (33%, p=0.06) in comparison to control group. 19 weeks of HF diet increased weight gain (191.3%, p<0.05), visceral fat (40.6%, p<0.01) and adipocytes perimeter (66.1%, p<0.0001); and decreased the expression of HSL (18.91%, p<0.05) in comparison to control group. Caffeine treatment, in 3 and 19 weeks of HF diet respectively, decreased weight gain (80.8%, p<0.01 and 66.4%, p<0.05), visceral fat (62.8%, p<0.05 and 58.3%, p<0.001) and adipocytes perimeter in the 19 weeks group (56.4%, p<0.0001). Also, caffeine treatment tendentially decrease the expression of ACL (18.2%, p=0.67) in 3 weeks of HF diet without alterations for 19 weeks and HSL expression (18.29%, p=0.61) in 3 weeks of HF diet and increased (28.9%, p=0.42) for the 19 weeks of HF diet. Insulin signaling pathway markers were not altered by HF diet or caffeine treatment in both groups. 3 weeks of HF diet tendentially decreased CD31 expression (55.83%, p<0.05) and VEGF expression (35.30%, p=0.52), and caffeine treatment increased CD31 (53.23%, p<0.05) and VEGF (68.07%, p=0.79) expression. The 19 weeks diet group showed a tendency to decrease CD31 expression (56.77%, p=0.13) effects not altered by caffeine treatment.

Conclusion: Caffeine administration in HF diet animals impacts weight gain, fat accumulation and adipose tissue signaling pathways, due to the contribution of increased lipolysis and angiogenesis markers. These results open new doors for a caffeine-based therapeutic approach for metabolic diseases related with adipose tissue dysfunction.

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Acknowledgements :- Fundação para Ciência e Tecnologia: BFM: PD/BD/128336/2017; FOM: CEECIND/04266/2017.

OC78

Thapsigargin blocks Electromagnetic fields-elicited intracellular Ca²⁺ increase in stress-response *in vitro* model

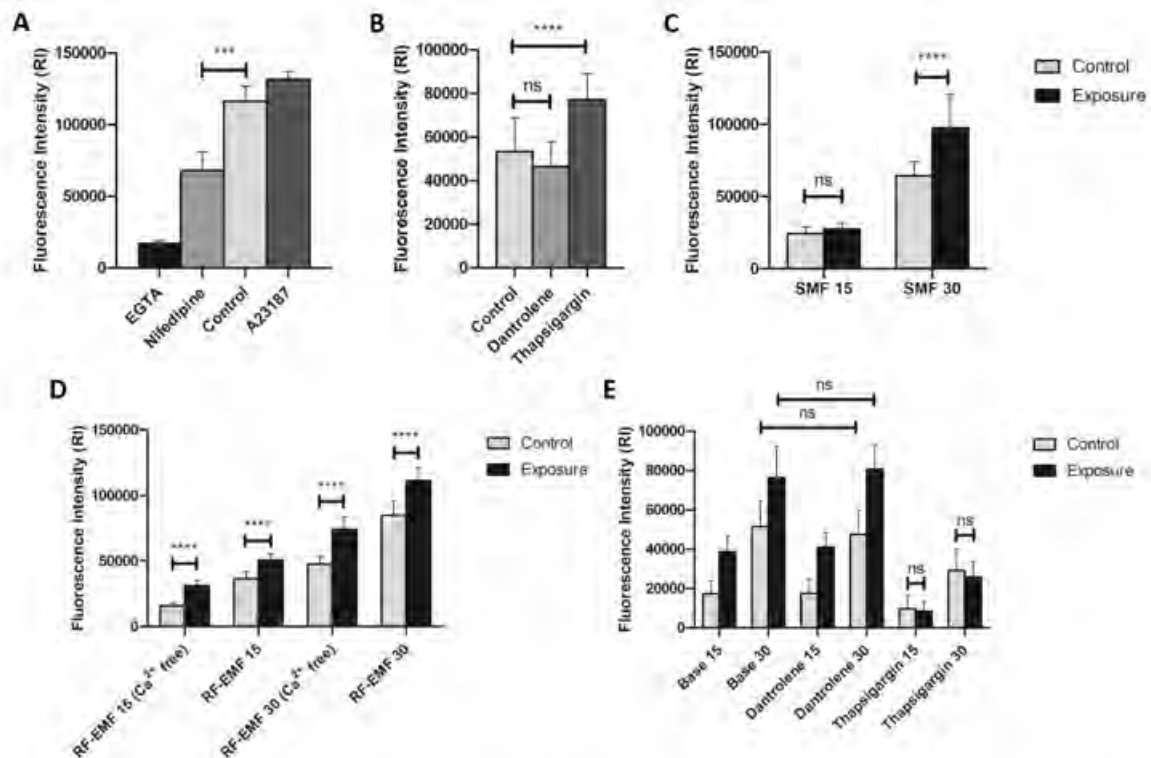
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A wide literature exists regarding the biological effects of various frequencies and intensities of electromagnetic fields (EMFs). These effects range from cellular proliferation to changes in expression and conduction of diverse types of ion channels. However, the major effect elicited by EMFs seems to be directed towards Ca^{2+} homeostasis. This is particularly remarkable since Ca^{2+} acts as a central modulator in various signalling pathways, including, but not limited to cell differentiation and survival. Despite the many evidence collected, however, the biological role of EMFs has yet to be unravelled and results reported in literature are often controversial due to variability of experimental parameters used, such as field intensity, level of exposure and model studied. Amongst the numerous pathways modulated by Ca^{2+} , a particularly interesting role is played by stress response. This pathway is initiated within the endoplasmic reticulum (ER) and is critical for cell survival. ER stress is accompanied by alterations in Ca^{2+} homeostasis, and the depletion of internal Ca^{2+} store can induce cellular stress and apoptosis. Here, we assessed the effect of EMFs on the stress-elicited increase in intracellular $[\text{Ca}^{2+}]$, by activating HEK 293 ER stress response and exposing the cells to the radio-frequency electromagnetic fields (RF-EMFs) produced by a phone and to static magnetic fields (SMFs) generated by a magnetic coil.

We detected a constant and time-dependent increase in intracellular $[\text{Ca}^{2+}]$ when medium changes were performed by aspirating and replacing fresh medium (stop-flow) as previously found in literature. Strikingly, this increase resulted to be significantly higher in the exposed group, already after 15 minutes of exposure. Similar results were obtained incubating cells with Ca^{2+} -free solutions, suggesting the involvement of the ER intracellular Ca^{2+} stores in the detected increase. In line with this thinking, we targeted Ca^{2+} cytoplasmic enrichment and withdrawal using respectively Ryanodine receptor (RyRs) blocker Dantrolene and sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPases (SERCAs) blocker Thapsigargin. Dantrolene treatment was proven to have no effect on this increase, consistent with the extremely low level of endogenous RyRs expression in HEK cells as previously proven by Western Blotting analysis. However, treatment with 5 μM Thapsigargin was sufficient to null the differences between exposed and control groups.

The current results shed a light on the involvement of ER stress response on the modulation of EMFs effects. The internal $[\text{Ca}^{2+}]$ regulation by SERCAs is key for cell survival and, in fact, the blockage of SERCAs lead in short time to severe depletion of ER stores and cell death. The initial Ca^{2+} increase detected is in line with the blockage of Ca^{2+} withdrawal as with the activation of non-selective Ca^{2+} -permeable cation channels triggered by depletion of intracellular stores. However, the rapid depletion of ER internal stores is enough to null the differences in intracellular $[\text{Ca}^{2+}]$ increase between the control and exposed groups, highlighting a direct modulation of ER Ca^{2+} efflux as a major effect of EMF exposure.



EMFs enhance the stress related Ca^{2+} increase in HEK 293 cells. (A): shows the fluorescence intensity in baseline conditions. The intracellular $[\text{Ca}^{2+}]$ is lowered by the addition of $10 \mu\text{M}$ nifedipine. Ca^{2+} chelator EGTA and calcium ionophore A23187 report respectively minimum and maximum response. (B): Baseline after incubation with $5 \mu\text{M}$ Dantrolene and $5 \mu\text{M}$ Thapsigargin. The block of SERCAs operated by Thapsigargin raise impairs Ca^{2+} withdrawal in the ER. The effect of dantrolene is not significant in line with the poor functional expression of RyRs in HEK 293 cells. (C): SMFs lead to similar increase as already found with RF-EMFs. However, the increase is delayed with respect to what observed with RF-EMFs. (D): RF-EMF exposure increase stress-related Ca^{2+} raise both in standard and Ca^{2+} -free conditions. (E): Thapsigargin blocks the EMFs related increase. (F): shows the average fluorescence level for each condition in RF-EMFs experiments. The baseline difference between control and exposed groups is not significative. $N=45$. $P\text{-value} < 0.001$.

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OC79

The zinc finger homeobox 3 transcription factor at the interface of regulating circadian rhythms and feeding behaviour

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Food intake is timed to primarily occur within the active phase of the day; whether that be during the light for diurnal or the night for nocturnal animals. The suprachiasmatic nucleus of the hypothalamus, the “master clock” within the brain, is essential for timing of the animal’s physiology to be aligned with the external light/dark cycle. Forced disruption to activities so that they no longer lie in the light/dark cycle, such as shift work and jet lag, predisposes the individual to metabolic disease and its complications. Zinc finger homeobox 3 (*Zfhx3*) is a transcription factor that is essential for the development of the mouse brain, but it is also expressed in discrete regions in the adult mouse brain including the suprachiasmatic nucleus. *Zfhx3* expression is crucial for time-keeping in absence of a light/dark cycle: global and conditional knock-out of *Zfhx3* renders the mice arrhythmic in the absence of external cues. In addition to a role in circadian timekeeping, *Zfhx3* is also highly expressed in brain regions that are important for the regulation of energy balance – such as the arcuate hypothalamic nucleus and the nucleus of the solitary tract - though its role in these areas is not known.

We hypothesised that global conditional knockdown of *Zfhx3*, in addition to its impact on arrhythmia in free-running conditions, would also alter feeding behaviour and energy homeostasis due to its expression in hypothalamic and hindbrain regions. To investigate this, we examined the metabolic phenotype of tamoxifen-induced knock-down of *Zfhx3* in *Zfhx3^{Flox/Flox};UBC-Cre⁺* mice compared to littermate controls using indirect calorimetry (males: n = 3-5, females: n = 4-6). Interestingly, we found that the knock-down mice gained significantly more weight in the 4-6 weeks post-tamoxifen treatment than their wild-type littermates (Males: +4.41g ± 0.19 SEM, *p* = 0.04, females: +3.05g ± 0.44 SEM, *p* = 0.05). Echo-MRI indicated that this was due to increases in both fat and lean mass. Weight gain was most pronounced in the male *Zfhx3*-knockdown mice, which could be attributed in part to their slightly higher food consumption (+1.49g ± 1.48 SEM, Genotype*Time: *p* = 0.018) during the indirect calorimetric experiment, which was not observed in female mice. Not only did the male *Zfhx3*-knockdown mice eat more food, they also expressed an abnormal rhythm of food intake in the light/dark cycle. This was accompanied by reduced amplitude and advanced phase in both activity (Amplitude: -10.59 AUC ± 1.76, *p* = 0.036; Phase: +2.52 hours ± 0.45 SEM, *p* = 0.018) and energy exchange (Amplitude: -7.3 AUC ± 0.87 SEM, *p* = 0.036; Phase: +2.5 hours ± 0.38 SEM, *p* = 0.036); neither of which were observed in the female knockdown mice. We can conclude that *Zfhx3* is involved in regulating energy homeostasis given the increased weight gain in both male and female knockdown animals. These results also suggest that, not only is expression of *Zfhx3* in adult mice important for maintaining physiological circadian rhythmicity even when external cues are present, but there is also a sexually dimorphic effect.

Reference 1 :- doi.org/10.1177/0748730417722631

Reference 2 :- <http://dx.doi.org/10.1016/j.cell.2015.06.060>

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Neuroprotective effect of plasminogen activator inhibitor-1 antagonist in the rat model of mild traumatic brain injury

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Introduction: Plasminogen activator inhibitor-1 (PAI-1) antagonists are known for their neuroprotective effects. Demonstration of a role for PAI-1 in the pathophysiology of inflammation as well as cerebrovascular injury drew considerable attention to the development of PAI-1 antagonists and no studies are testing the possible anti-inflammatory actions of PAI-1 antagonists during the secondary brain injury after TBI.

Aims/objectives: In this study, it was aimed to investigate the possible protective effects of PAI-1 antagonists in a rat mild traumatic brain injury (TBI) model.

Method: Sprague Dawley male rats were grouped as sham (n=7), TBI (n=9), TBI + PAI-1 antagonist (5 and 10 mg/kg TM5441 and TM5484; n=6-7). All experimental procedures used in this investigation were reviewed and approved by the Marmara University Animal Care and Use Committee (12.2019.mar). Animal care and all experiments were conducted in concordance with the principles of the World Medical Association's Declaration of Helsinki. Under anesthesia, TBI was induced by dropping a metal 300-gram weight from a height of 1 meter on the skull. Before and 24-hour after trauma neurological examination, tail suspension, Y-maze, novel object recognition tests were performed. Twenty-four hours after TBI, the rats were decapitated and activities of myeloperoxidase, nitric oxide release, luminol- and lucigenin-enhanced chemiluminescence were measured. Also, interleukin-1b, interleukin-6, tumor necrosis factor, interleukin-10, tumor growth factor-b, caspase-3, cleaved caspase-3, and PAI levels were measured with the ELISA method in the brain tissue. Brain injury was graded histopathologically following hematoxylin-eosin staining. Western blot and immunohistochemical investigation for low-density lipoprotein receptor, matrix metalloproteinase-3 ve nuclear factor-kB were also performed. Data were analyzed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) and expressed as means $\pm$ SEM. Values of $p < 0.05$ were considered to be statistically significant.

Results: Higher levels of myeloperoxidase activity in the TBI group ($p < 0.05$) were found to be suppressed in 5 and 10 mg/kg TM5441 treatment groups ($p < 0.05$ - $p < 0.01$). The tail suspension test score was increased in the TBI group ($p < 0.001$), and decreased in all treatment groups ($p < 0.05$ -

0.001). The histologic damage score was increased statistically significantly in the cortex, dentate gyrus, and CA3 regions in the TBI group ($p<0.01-0.001$), decreased in the treatment groups in the cortex and dentate gyrus ($p<0.05-0.001$).

Conclusion: PAI antagonists, especially TM5441, has antioxidant and anti-inflammatory properties against mild TBI in the acute period. Behavioral test results were also improved after PAI antagonist treatment after mild TBI.

OC81

Brain activity of men and women during simple visual Choice Reaction task

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It is well known phenomenon in psychology that men and women usually make decision/choice using different mind strategies. The existence of such phenomenon is obvious due to different ratio of determinant sex steroids that cause very powerful impact on brain development and functioning. However, the neurobiological aspects of men's and women's decision making strategies are still ambiguous and require more data. The knowledge of sex differences of brain activity potentially could provide better rehabilitation for men and women with various brain injuries.

Decision making implies execution of basic cognitive processes which are based on choice reaction. Thereby, visual choice reaction task with 2 simple stimuli was used in this study.

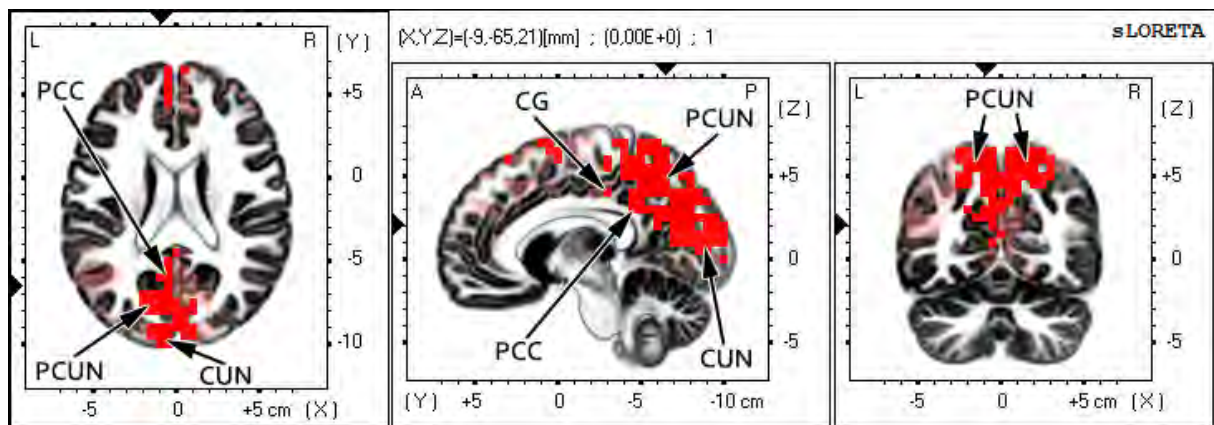
The aim of the study was to investigate whether there are any differences in brain activity and choice reaction time between male and female subjects during choice reaction task performance.

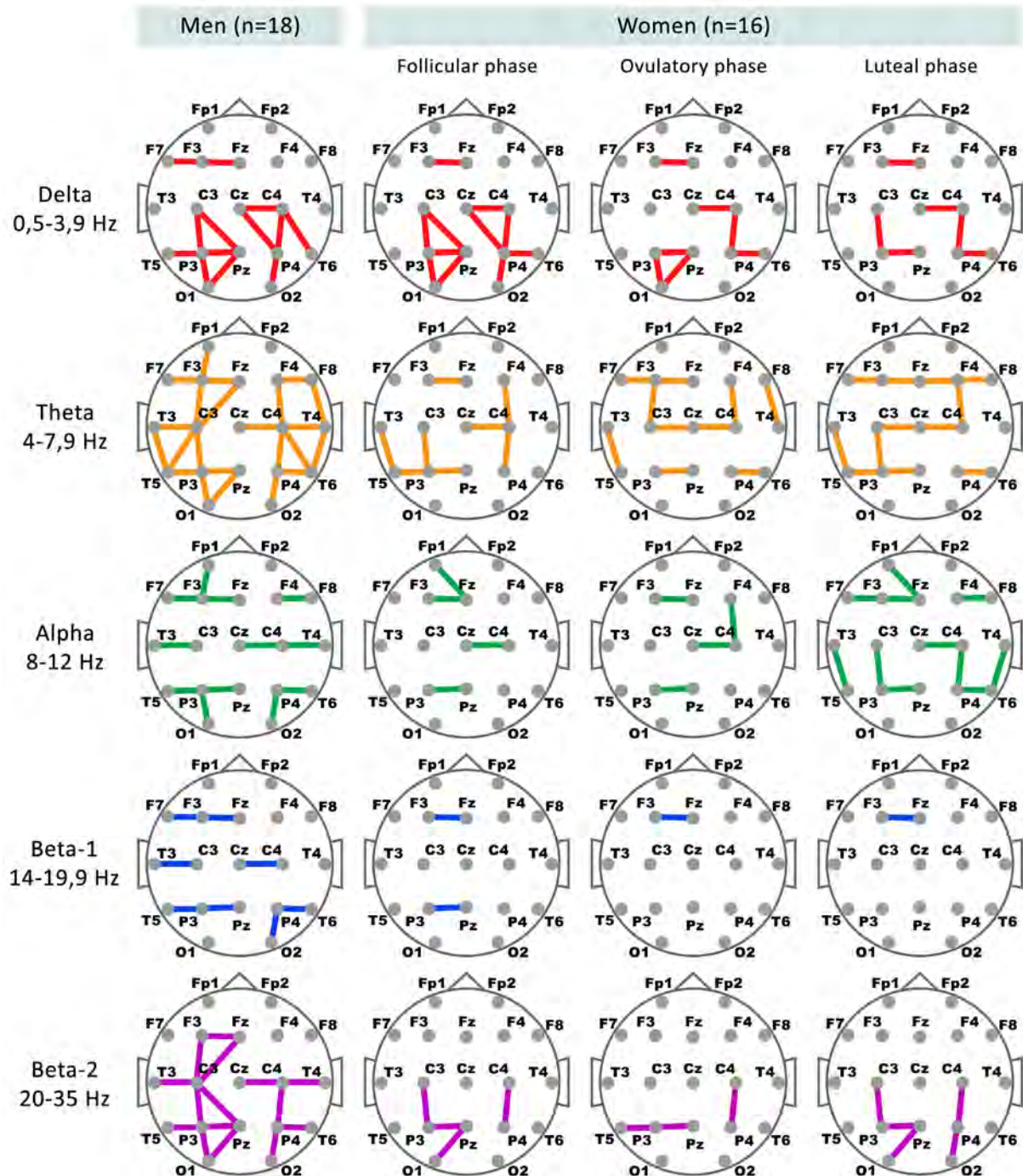
The study involved 18 male volunteers, right-handed, $21,4\pm0,97$ y.o., and 16 female volunteers, right-handed, $19,6\pm0,96$ y.o.. All participants were students of Taras Shevchenko National University of Kyiv and have no health complaints, reported brain injuries or psychiatric disorders. Each woman underwent testing procedure three times: in follicular, ovulatory and luteal phases of menstrual cycle. Phase of menstrual cycle was identified by calendar method and method of saliva crystallization.

In the study choice reaction time (CRT) of motor responses made by right (rhCRT) and left hands (lhCRT) were detected. Mean CRT was evaluated too. CRT data is presented below as Median [Lower Quartile (25%); Upper Quartile (75%)]. EEG was recorded during choice reaction task performance. EEG was done with 19 leads placed on the scalp according to the International 10-20 System. Localization and statistical analysis of 3D distribution of the EEG dipoles were performed applying the LORETA KEY software package v.20201109 [1,2]. Coherence analysis was performed for all possible coupled pairs of leads in delta (0,5-3,9 Hz), theta (4-7,9 Hz), alpha (8-12 Hz), beta-1 (14-19,9 Hz) and

beta-2 (19-35 Hz) bands applying the Neuron-Spectrum software. Significant level of coherence value was established equal or greater than 0,7 [3].

Compared to men women in follicular phase had larger mean CRT (402 [392; 429] ms vs. 434 [409; 493] ms ($p=0,023$)) and lhCRT (406 [389; 421] ms vs. 436 [420; 491] ms ($p=0,007$)). Women in ovulatory phase also had larger lhCRT than men (429 [412; 456] ms vs. 406 [389; 421] ms ($p= 0,042$)). Furthermore, women in ovulatory phase demonstrated greater lhCTR compared to their rhCRT (429 [412; 456] ms vs. 418 [384; 446] ms ($p= 0,039$)). There was no difference between men and luteal women in their CRTs. The only significant difference in EEG dipoles distribution was found between women in follicular and ovulatory phases. In comparison with follicular phase women in ovulatory phase demonstrated greater activation of parietal and occipital cortical zones within left hemisphere: cingulate gyrus (BA31), cuneus (BA 18, 19), posterior cingulate gyrus (BA 23) and precuneus (BA 7).





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National University of Kyiv, Ukraine. We would like to appreciate help of all students of Taras Shevchenko National University of Kyiv, who participated in the research

OC82

A G-protein dependent mechanism through adenosine A₁ receptor-activated GIRK channels is needed for synaptic plasticity processes in dorsal hippocampus

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In the dorsal hippocampal CA1 neurons, A₁ adenosine receptor-mediated GIRK (G-protein-gated inwardly rectifying potassium) channels conductance is constitutively active contributing to the resting membrane potential and preventing from any excitation excess. GIRK channel-dependent signaling disruption has been linked to the etiology of many diseases that involve neural excitability alterations, such as Alzheimer's disease, suggesting an important role of GIRK channels for cognitive processes that depend on hippocampal neuronal activity.

The main aim of the present work was to explore the role of A₁ adenosine receptor-mediated GIRK basal activity in the induction and maintenance of synaptic plasticity processes that support dorsal hippocampus-dependent cognitive functions.

For this purpose, we pharmacologically modulated basal GIRK channel conductance in the dorsal hippocampus by using A₁ adenosine receptor modulators (agonists: 2'-MeCCPA and CPA; antagonist: DPCPX) or by directly manipulating channel activity using ML297, a selective GIRK opener, and Tertiapin-Q, a specific GIRK blocker, and we examined its involvement in controlling synaptic plasticity processes at different levels of complexity.

On the one hand, using dorsal hippocampal slices, we studied pharmacological A₁ receptor and GIRK channel activity modulation effect on the induction and maintenance of long-term synaptic plasticity processes induced in CA1 after Schaffer collateral pathway high frequency stimulation. On the other hand, using an *in vivo* approach, we performed acute intracerebroventricular injections of GIRK selective modulators to study their contribution to CA3-CA1 synapse electrophysiological properties, synaptic plasticity, and non-associative learning and memory capabilities during an open field habituation task.

Our data shows that induction and maintenance of long-term synaptic plasticity processes in dorsal hippocampus involves a G-protein dependent mechanism through A₁ adenosine receptor-activated GIRK, as both A₁ receptor and GIRK channel activity modulation modified LTP/LTD induction threshold *ex vivo* (Vehicle (A₁ receptor), n = 10, 160 ± 2.4% of baseline, *p* < 0.001; 2'-MeCCPA, n = 5, 95 ± 3.5%, *p* = 0.806; CPA, n = 5, 109 ± 2.1%, *p* = 0.216; DPCPX, n = 6, 61 ± 4.1%, *p* = 0.003; Vehicle (GIRK channel), n = 21, 156 ± 1.7%, *p* < 0.001; ML297, n = 12, 73 ± 2.6% of baseline, n = 12, *p* < 0.001; TQ, n = 13, 95 ± 2.6%, *p* = 0.007) and *in vivo* (Vehicle, 184 ± 9% of baseline; n = 9, *p* = 0.003; ML297, n = 6, 75 ± 8%, *p* = 0.963; TQ, n = 6, 58 ± 6%, *p* = 0.508), even switching HFS-induced LTP into LTD. Also, the disruption of such mechanism leads to hippocampal plasticity-dependent learning and memory deficits as shown during the open field habituation task (ML297, n = 8, vs vehicle, n = 10: *p* = 0.032, TQ, n = 7, vs. vehicle: *p* = 0.012).

Altogether, these results support the contention that A₁ adenosine receptor-mediated GIRK basal activity governs hippocampal synaptic plasticity direction and that its dysregulation is detrimental for neural processes underlying cognitive function.

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OC83

Multistable systems: a new general theory in pathophysiology

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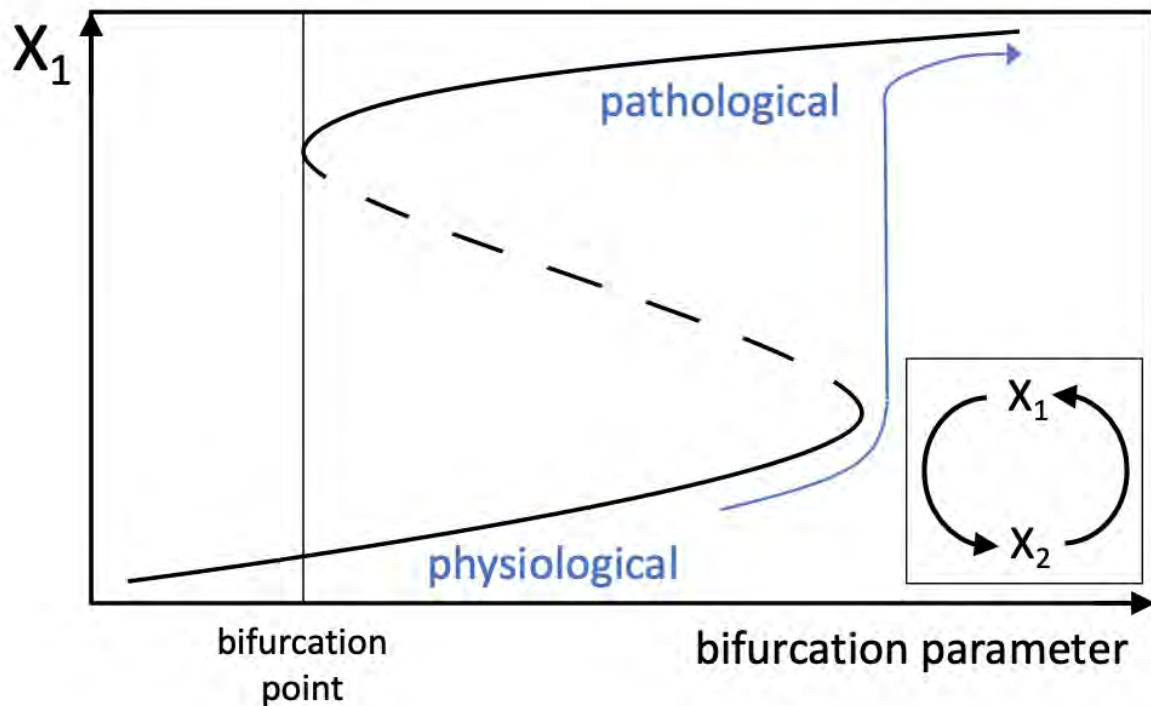
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It has been proved that different life processes can be modeled in terms of the dynamics of positive loop systems, i.e. closed chains of interactions with an even number of inhibitory steps, or none. These processes include regulatory mechanisms of gene expression, and cellular activities like division, differentiation, and migration. A generalization of this complex of evidence has led to the Loopomics paradigm, which assumes that all functional elements of a living being are involved in variously connected loop systems [1]. This allows to assume that the whole organism is governed by a restricted set of rules, which nevertheless give rise to a multitude of highly diverse epiphenomena, collectively known as biodiversity (cells, tissues, organisms, etc.). Accordingly, any change occurring in a living being can be reconducted to the activity of a multistable positive loop system switching among different steady states [2]. This is extremely relevant in pathophysiology, as the insurgence of a disease in a healthy organism starts from a primary event that converts the system from a physiological to a pathological status. Bistable switches have been actually used to describe different

diseases, including immunological disorders, neurodegenerative and neurological problems, infections, and cancer [3, 4].

Hence, a wide set of data argues for a new theory, based on the idea that any disease affecting a healthy human involves a change that can be reconducted to the activity of a positive loop generating a multistable system, typically a bistable one. In this kind of systems, the transition from monostability to multistability can be driven by the variation of bifurcation parameters, allowing the system to switch from a “physiological” to a “pathological” steady state. An exemplificative representation (Figure 1) shows a bifurcation diagram of a positive loop (inset) describing a schematic pathogenic process involving two functional agents, X_1 and X_2 , which may represent cells (e.g. lymphocytes), enzymes (e.g. kinases), or signal molecules (e.g. interleukins). The diagram shows the trajectories of the steady states of X_1 as a function of a bifurcation parameter, where continuous branches represent stable equilibrium points and the dashed branch unstable equilibrium points. The arrow indicates a possible path followed by the system in the transition from physiological to pathological status. The system dynamics are characterized by hysteresis, thereby opening the possibility of irreversible transitions for a strengthening of the interactions among the loop functional agents.

This theory assumes that the huge diversity of pathologies that can possibly affect a healthy human can be reduced to a unique theoretical model, consisting of a positive loop system representing functional relationships among cells, molecules, or biochemical agents. In this context, the physical entities that correspond to bifurcation parameters should be considered best therapeutic targets aiming at achieving full control of the disease mechanisms. The application of this new idea to pathophysiological studies could lead to overcome the disappointing impasse that is currently characterizing many approaches to unsolved diseases. Recent models of neurological diseases could provide a basis for a validation of the theory [5].



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PC001

Angiotensin I receptor clustering and hypoxic remodelling in O₂ sensitive cells

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Introduction: The carotid body (CB) contains specialised O₂ sensitive cells located at the carotid bifurcation that monitor the blood P_aO₂ supplying the brain to initiate corrective cardiovascular respiratory reflexes. O₂ sensitivity of the CB is modulated by G-protein-coupled-receptor signalling (1). The angiotensin 1 receptor (AT₁R) is expressed in the CB and angiotensin II causes chemoexcitation. In pathology, changes in AT₁R signalling promote CB hyperactivity. However, little is known about the single molecule distribution of AT₁R in CB type I cells or PC12 cells- a closely associated cell line, which may underpin signalling microdomains. Furthermore, it is not clear if AT₁R membrane expression can be altered by exposure to hypoxia. This study aimed to reveal if membrane AT₁R are clustered and if expression is altered by chronic hypoxia.

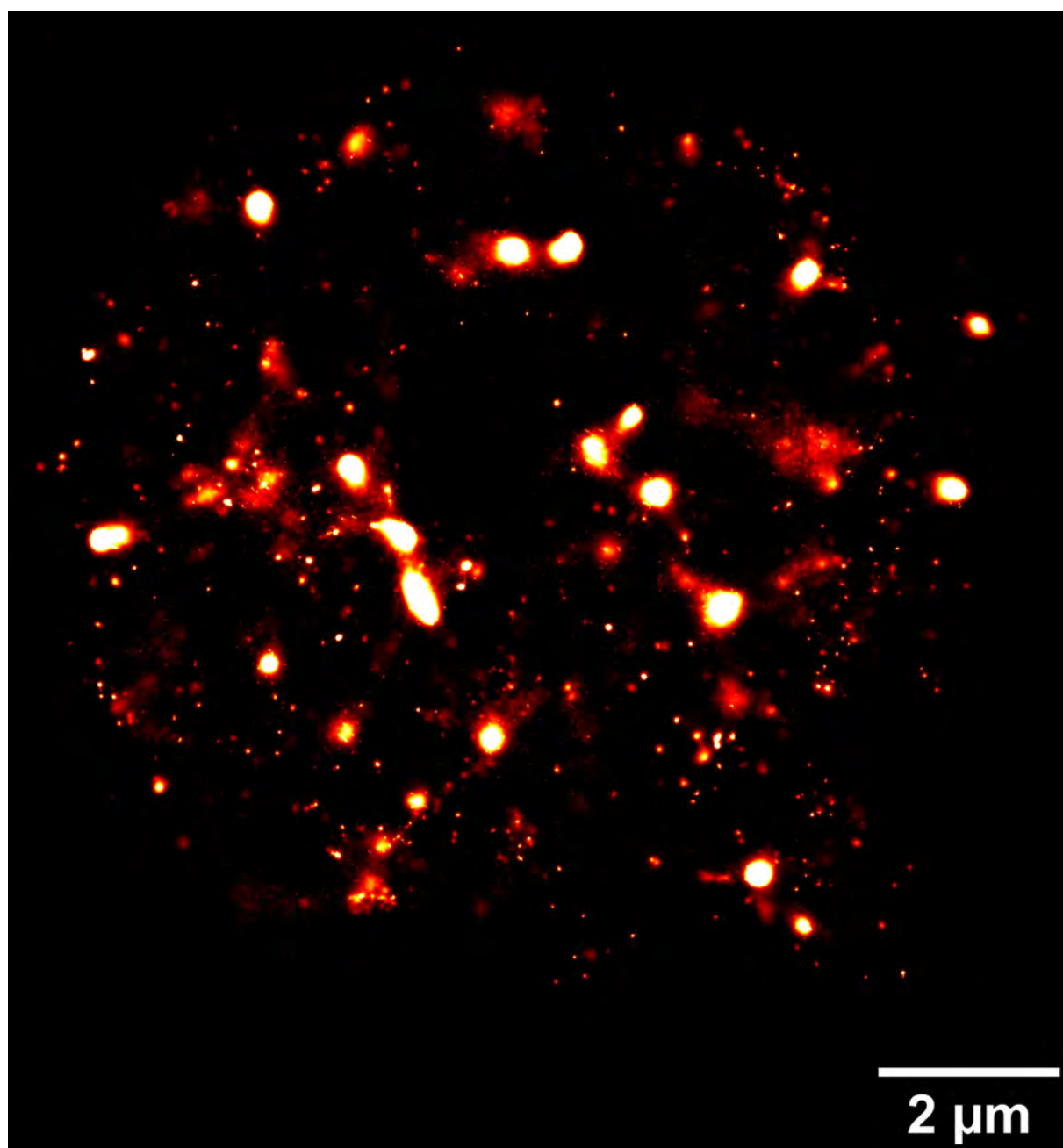
Methods: AT₁R expression was assessed in PC12 cells (an O₂ sensitive cell line used to model the CB type I cell). PC12 cells (passage number 10-30) were plated onto human placental collagen and were incubated in media equilibrated with either 1% O₂ or 20% O₂ for 4, 12 and 24 hours. At each time point, cells were fixed and stained with primary antibodies (1:500 anti-AT₁R and 1:500 anti-tyrosine hydroxylase-TH). Cells were stained with secondary F(ab) fragment antibodies conjugated with Alexa fluor 647 (1:1000) and 488 (1:1000). Cytosolic TH and membrane AT₁R expression were assessed by confocal microscopy. Membrane AT₁R clustering was visualised using direct STochastic Optical Reconstruction Microscopy (dSTORM). Data was expressed as mean $\pm$ S.E.M and significance determined with two-way repeated measures ANOVA and taken as P<0.05.

Results: Exposure of PC12 cells to hypoxia (1% O₂), caused a significant increase in cell area after 12 hours (normoxia 142.8 $\pm$ 5.8 μ m² (n=93), hypoxia 164.9 $\pm$ 6.8 μ m² (n=81), P<0.05) and 24 hours (normoxia 143.3 $\pm$ 6.2 μ m² (n=72), hypoxia 183.1 $\pm$ 8.5 μ m² (n=92), P<0.05). Confocal imaging demonstrated that cytosolic TH fluorescence levels were significantly elevated after 12 (normoxia 1333.6 $\pm$ 93.5AU, hypoxia 1903.8 $\pm$ 135.4AU, P<0.05) and 24 hours (normoxia 1175.8 $\pm$ 61.3AU, hypoxia 1765.8 $\pm$ 116.0AU, P<0.05). Furthermore, AT₁R fluorescence was also significantly increased after 12 hours (normoxia 1315.8 $\pm$ 35.1AU, hypoxia 1547.3 $\pm$ 53.4AU, P<0.05) and 24 hours (normoxia 1286.6 $\pm$ 33.5AU, hypoxia 1592.3 $\pm$ 46.6AU, P<0.05) of hypoxia. Preliminary reconstructed images using dSTORM indicated the presence of distinct AT₁R clusters on the PC12 cell surface membrane (Figure 1). The presence of clusters was consistent in all 5 cells imaged to date using dSTORM.

Discussion: This preliminary data suggests that in O₂ sensitive PC12 cells, AT₁R are present in distinct clusters or 'hotspots' in the cell membrane, raising the potential of AT₁R signalling microdomains. More experiments are required to validate this finding and quantify specific cluster parameters. In addition, this data shows that AT₁R expression is increased in response to hypoxia along with an elevation in TH and cell area. A key next step will be to assess changes in AT₁R expression and

clustering specifically in primary CB type I cells as a further move towards the generation of potential novel therapies for cardiorespiratory diseases associated with chronic hypoxia.

Figure1. Reconstructed dSTORM image showing distinct AT₁R clusters in a PC12 cell.



Reference 1 :- Aldossary, H. S., Alzahrani, A. A., Nathanael, D., Alhuthail, E. A., Ray, C. J., Batis, N., Kumar, P., Coney, A. M., & Holmes, A. P. (2020, Aug 20). G-Protein-Coupled Receptor (GPCR) Signaling in the Carotid Body: Roles in Hypoxia and Cardiovascular and Respiratory Disease. *Int J Mol Sci*, 21(17). <https://doi.org/10.3390/ijms21176012>

PC002

Cycle length dependence of electrical and mechanical alternans in guinea pig ventricular myocytes

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Cardiac alternans is thought to promote arrhythmia via mechanisms including spatial dispersion of refractoriness resulting in wavebreak, thus promoting re-entry (Tse *et al.*, 2016). The mechanisms underlying alternans remain unclear, particularly whether alternation in action potential duration (APD) or the calcium transient (CaT) is the primary disturbance (Díaz *et al.*, 2004; Kanaporis & Blatter, 2017). One hypothesis is that at high pacing frequencies, alternation in the preceding diastolic interval (DI) can drive alternans, and that this is promoted when the slope of the restitution curve of dependence of APD on preceding DI exceeds 1 (Goldhaber *et al.*, 2005). The present study investigated the cycle length (CL)-dependence of APD and mechanical (sarcomere shortening) alternans in ventricular myocytes isolated from male Dunkin-Hartley guinea pigs. Animal procedures were approved by the Animal Welfare and Ethics Review Board of the University of Bristol and performed in accordance with UK legislation. Excised hearts were mounted on a modified Langendorff apparatus and ventricular myocytes isolated as described previously (Gao *et al.*, 2005). Sarcomere shortening changes were measured as an index of beat-to-beat changes in the CaT, in order to avoid potential buffering of cytosolic $[Ca^{2+}]$ by an intracellular Ca^{2+} -sensitive fluorescent probe. Action potentials were recorded at physiological temperature (37 °C) in perforated-patch clamp measurements. Cells were paced in a steady state fashion by a train of 60 stimuli at constant CL during simultaneous recording of membrane potential and sarcomere length. The CL of the 60-stimuli train was progressively reduced to CL = 250 ms or 1:1 coupling was lost. Each train of 60-stimuli were preceded by 20 stimuli at a CL of 1,000 ms. Alternans was defined as 1:1 beat-to-beat alternation between long and short APD₉₀ or large and small changes in sarcomere length. Data are presented as mean ± standard error of the mean. APD₉₀ and mechanical alternans were seen in all cells (n=19); the incidence and duration of alternans increasing at shorter CLs (Figure 1). APD₉₀ and mechanical alternans were observed to occur in isolation, as well as simultaneously (both in- and out-of-phase). Alternans occurred despite APD₉₀ restitution slope steepness <1. In summary, the data show all cells demonstrated both APD₉₀ and mechanical alternans. While alternans occurred through the range of CLs, there was clear CL-dependence of both APD₉₀ and mechanical alternans. In conclusion, guinea pig ventricular myocytes demonstrate APD and mechanical alternans at a range of CLs, with a greater incidence at shorter CLs, which appears not to be dependent on steep APD₉₀

restitution slope dynamics. Further work is required to understand intracellular processes underlying the generation of alternans.

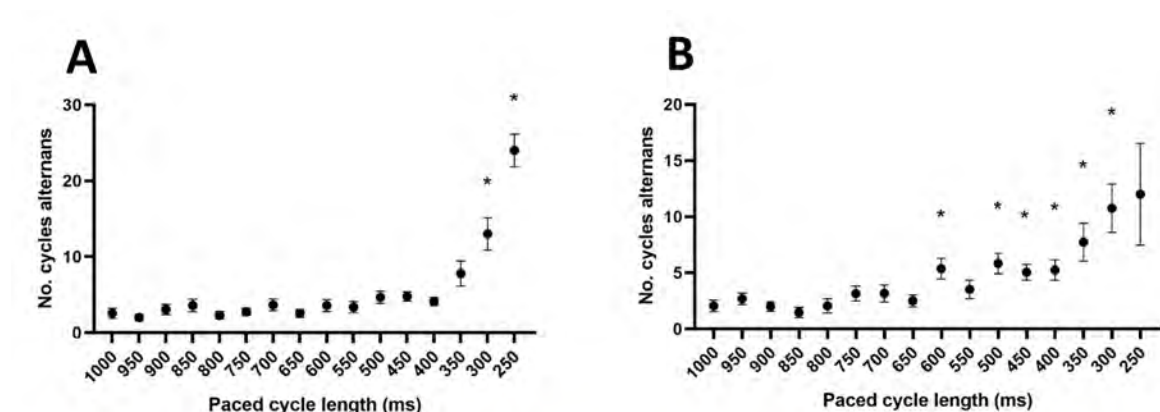


Figure 1. Rate-dependence of APD (panel A) and mechanical (panel B) alternans during steady state pacing ($n=19$; mean $\pm$ SEM). One-way ANOVA with Bonferroni post hoc tests ($* = P < 0.05$) compared number of cycles of alternans at each cycle length with that seen at 1000 ms.

Reference 1 :- Díaz ME, O'Neill SC & Eisner DA (2004). Sarcoplasmic Reticulum Calcium Content Fluctuation Is the Key to Cardiac Alternans. *Circ Res* **94**, 650–656.

Reference 2 :- Gao Z, Milnes JT, Choisy SCM, Leach MJ, Hancox JC & James AF (2005). The neuroprotective agent sipatrigine blocks multiple cardiac ion channels and causes triangulation of the ventricular action potential. *Clin Exp Pharmacol Physiol* **32**, 1088–1096.

Reference 3 :- Goldhaber JJ, Xie L-H, Duong T, Motter C, Khuu K & Weiss JN (2005). Action Potential Duration Restitution and Alternans in Rabbit Ventricular Myocytes. *Circ Res* **96**, 459–466.

Reference 4 :- Kanaporis G & Blatter LA (2017). Membrane potential determines calcium alternans through modulation of SR Ca²⁺ load and L-type Ca²⁺ current. *J Mol Cell Cardiol* **105**, 49–58.

Reference 5 :- Tse G, Mbbs H, Phd MA, Wong T, Tse V, Lee YT, Lin Y & Yeo M (2016). Cardiac dynamics: Alternans and arrhythmogenesis. ; DOI: 10.1016/j.joa.2016.02.009.

Acknowledgements :-

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PC003

Acute modification of upper-limb perfusion in vivo evokes a Prompt Adaptive Hemodynamic Response to re-establish cardiovascular homeostasis

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Introduction

Microcirculatory homeostasis depends on a highly efficient regulation involving multiple sensors and effectors, both central and peripheral. This allows compensatory response to neuroendocrine output affecting cardiorespiratory performance and systemic vascular resistance and local microcirculatory (endothelial and myogenic) activity. In previous studies a Prompt Adaptive Hemodynamic Response (PAHR) affecting systemic hemodynamics was identified in both lower limbs when acute changes of peripheral microcirculatory perfusion were evoked in only one limb.

Aims / Objectives

To investigate if the PAHR previously identified in the lower limb can be also detected in the upper limb.

Methods

Twelve healthy adults both sexes (39.3 ± 16.4 y.o.) were previous selected. Procedures respected all the principles of good clinical practice for human research. After adapting to the room's temperature humidity and light (appr. 15 minute) participants were randomly assigned to two 15 min protocols (each 3 phases of 5 min). Protocols differed only in the challenge phase (Phase 2) applied to one test limb (TL) randomly chosen while the other remained inactive (IL). In Protocol 1 (P1) (n=6) the challenge was a post-occlusive hyperaemia applied by an arterial pressure cuff in the arm; In Protocol 2 (P2) (n=6) one limb performed an active movement (TL) for 5 min. Blood perfusion (BP) and the concentration of red blood cells (CRBC) were assessed in 2nd fingertip with Laser Doppler flowmetry (LDF) and in the palm with Polarized Spectroscopy (PS). Descriptive and comparative statistics were performed and a 95% level of confidence adopted.

Results and discussion

In each individual, a perfusion asymmetry was noted at rest as expected, although not significant. Both protocols evoked similar responses of BP in the tested limb but also in the contralateral inactive limb. With P1 a clear perfusion reduction was recorded in both limbs during Phase 2, ($p=0.002$) only significant in TL - 90.2% ($p=0.028$). In the IL, values fell by an average of 23.1%. CRBC changes detected by LDF and PS were non-significant in both sides. With P2, changes were also detected during Phase 2 (during movement) also increasing perfusion IL (measurements were not possible in

TL in Phase 2). Nevertheless, perfusion modifications always occurred in the same direction. The recovery was gradually made during the final 5 min interval, with faster recovery noted in P2.

Conclusion

These exploratory results confirm that the PAHR firstly described in the lower limb can also be detected in the upper limb. This confirms the interest for the use of this potential clinical outcome for diagnostic purposes and as a reference for the disease recovery process.

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PC004

Pathological turret mutations in the cardiac sodium channel Nav1.5 induce long-range disruption to the pore geometry

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The cardiac voltage-gated sodium channel, Nav1.5, is central to cardiac action potential initiation and therefore cell membrane depolarization. The extracellular turrets of the channel extend over the central pore and play a role in ion selectivity and permeation¹. Several germ-line mutations within this region have been implicated in cardiopathologies such as Brugada Syndrome (BrS), yet it is unclear how they affect channel gating^{2,3}. From the recent cryogenic electronic microscopy structure of mammalian Nav1.5 (PDB ID:6UZ3)⁴, these mutants appear to form a complex salt bridge between an arginine residue (R878) and two acidic residues (D1430 and E1441) that connect the channel turret regions of the DII and DIII domains (Figure 1). Furthermore, the complex salt bridge is bolstered with adjacent cation- π interactions with the aromatic residues (W879, Y1426 and W1440).

We employed site-directed mutagenesis to systematically examine this region. We transiently transfected mutants into HEK293F cells to study their gating behaviour (steady-state activation, inactivation, and recovery from inactivation protocols) by whole-cell patch clamp and performed One-Way ANOVA followed by Dunnett post-hoc tests with results displayed as mean $\pm$ SEM. We also performed *in silico* Molecular Dynamic (MD) simulation modelling to investigate the structural stability of the mutants relative to the wild-type channel.

Almost all the generated mutants displayed either reduced or no detectable peak currents. Nevertheless, surface biotinylation demonstrated that all the mutants were expressed on the plasma membrane, therefore ruling out defective trafficking. Nearly 80% of our mutants that displayed no detectable gating activity were targeted to the complex salt bridge. These included all the natural BrS mutants that have been previously identified in this region. Of the mutants that retained some gating activity, 83% were targeted to the cation- π bonds. Thus, the complex salt-bridge may provide the major stabilisation to the DII-DIII turret interface, whilst the cation- π bonds contributes secondary - although still important functional reinforcement.

The Nav1.5 activator, veratridine, failed to rescue any of the null-mutants. For the mutants that retained some gating activity, only peak currents and steady-state activation parameters were perturbed relative to the wild-type channel (Table 1). Steady state inactivation parameters and recovery from inactivation kinetics were not affected. Taken together, these results suggest that the compromised gating behaviour of the mutants is not caused by large-scale protein misfolding. Rather, the mutations are more likely to introduce relatively subtle structural alterations, that selectively block the channel activation step. MD simulations revealed that the mutations destabilised the channel outer and inner vestibules through which the sodium ions move during channel activation. The mutations were also predicted to perturb the geometry of the DEKA selectivity ring that normally traps and concentrates sodium ions in the boundary between the outer and inner vestibules.

We note that this structural feature is strongly conserved in other Nav channel isoforms and pathological mutations in the same region of Nav1.1 and Nav1.7 have previously been described in clinical literature. Thus, our findings provide general insights into a class of pathological mutations occurring not only in Nav1.5 but also in other sodium channel isoforms.

	Peak I_{Na}	$V_{1/2}$ (mV)	k(mV)	n
HEK293F cells				
WT	-332.29 ± 36.29	-46.89 ± 2.43	5.22 ± 0.35	9
hW1440Y	-342.46 ± 42.15	-47.89 ± 1.47	5.27 ± 0.45	8
hW1440A	-60.85 ± 13.00****	-41.77 ± 1.51	7.85 ± 0.40***	9
hY1426F	-180.47 ± 40.13**	-44.4 ± 0.95	5.98 ± 0.28	8
hY1426W	-44.23 ± 7.54****	-41.83 ± 1.84	7.86 ± 0.47***	8
hY1426A	-68.44 ± 11.25****	-41.61 ± 1.41	7.22 ± 0.17**	7
hE1441D	-24.83 ± 2.23****	-40.57 ± 1.55	8.16 ± 0.64****	7

Table 1. Parameters from whole-cell patch-clamp steady-state activation data. Data were fit using a Boltzmann function to deduce all the parameters. The data are expressed as mean ± SEM and One-Way ANOVA statistical analysis followed by Dunnett post-hoc tests performed to determine the statistical significance between each mutant and the wild-type Nav1.5 channel. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Compared to wild-type channels, no differences were observed in $V_{1/2}$ (activation). However, the peak current (I_{Na}) and slope factor (k), of human: W1440A, Y1426W, Y1426A and E1441D were significantly different from the wild-type value. For hY1426F only the I_{Na} is significantly decreased with no change observed in k value. All the other mutants studied, namely, hW879A, hW879Y, hE1441Q, hD1430E, hR878C, hR878E/hE1441R and hR878D/hD1430R showed no current.

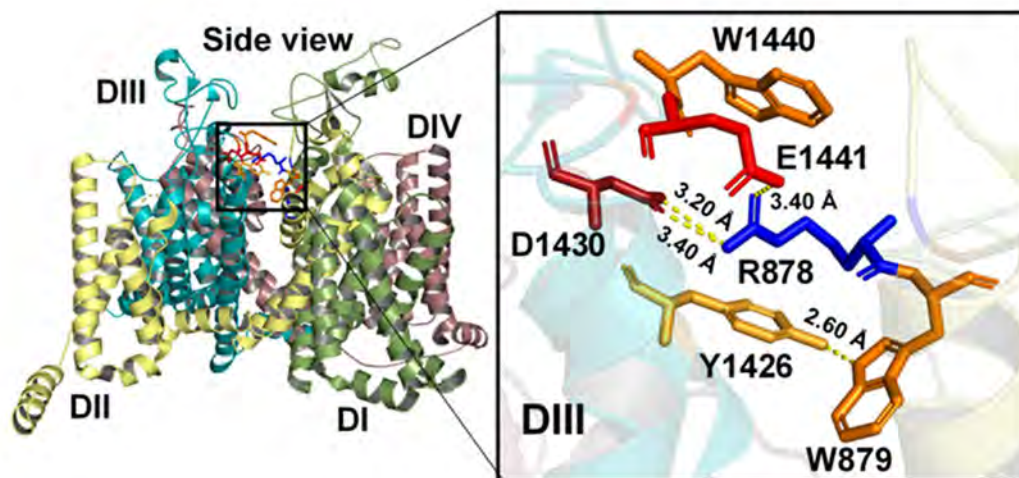


Figure 1. The rat Na_v1.5 structure (PDB ID: 6UZ3), showing the topology of the channel from the side view. Studied human equivalent residues are indicated from the DII-DIII turret interface in the enlarged image.

Reference 1 :- Chiamvimonvat N, Perez-Garcia MT, Tomaselli GF, Marban E. Control of ion flux and selectivity by negatively charged residues in the outer mouth of rat sodium channels. *J Physiol.* 1996;491

Reference 2 :- Kapplinger JD, Tester DJ, Alders M, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Hear Rhythm.* 2010;7(1):33-46. doi:10.1016/j.hrthm.2009.09.069

Reference 3 :- Maury P, Moreau A, Hidden-Lucet F, et al. Novel SCN5A mutations in two families with “Brugada-like” ST elevation in the inferior leads and conduction disturbances. *J Interv Card Electrophysiol.* 2013;37(2):131-140. doi:10.1007/s10840-013-9805-7

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PC005

Autonomic nervous system response to facial cooling increases velocity of left ventricle contraction and aortic compliance measured by pulse wave analysis.

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Introduction: Facial cooling is a simple way to stimulate trigeminal nerve receptors and evoke complex hemodynamic response that includes activation of both parasympathetic and sympathetic nervous system^[1]. We ought to investigate a very early response to facial cooling in young healthy adults using pulse wave analysis (PWA), that enables to examine hemodynamic changes in aorta during cardiac cycle and its implications affecting systolic function of the heart.

Objectives: To investigate an early hemodynamic response to facial cooling in young, healthy adults.

Method: Study was conducted according to bioethical standards and Declaration of Helsinki. 15 volunteers with no cardiovascular diseases aged 18 to 25 (males: 10) were enrolled into the study. After 5 min of rest in supine position, brachial BP was measured and pulse waveform was recorded using applanation tonometry. Then participant was exposed to 15-second facial cooling, which was followed by second pulse waveform recording and brachial BP measurement. The following parameters were obtained: heart rate (HR), cPERIOD (central pulse period) peripheral and central systolic pressure (SP) and diastolic pressure (DP), C_T1 (the time from the beginning of pulse wave to systolic peak) and C_T2 (the time from the beginning of pulse wave to the reflected wave return peak).

Results: FC caused decrease in HR and elongation of cPERIOD. There was a significant decrease in cT1, while cT2 increased. There was no significant change in peripheral and central SBP, as well as in the central DBP. However, there was a statistically significant increase in peripheral DBP. Statistical analysis was performed using R version 3.6.3 ^[2]. Due to insufficient sample size, it was impossible to accept the normal distribution as an approximation of our results' distribution. Consequently, we conducted the analysis using Wilcoxon test.

Conclusions: Despite a major rise in parasympathetic activity causing decrease in HR and elongation of cPERIOD, there was an increase in left ventricular systole velocity, manifested as shortened cT1. We hypothesized that it might be an effect of cPERIOD prolongation, consequent increase in end-diastolic volume (preload) and left ventricular systolic force and velocity. Another factor that may impact velocity of systole is decreased afterload, commonly defined as pressure in aorta. We didn't obtain any significant change in cSBP nor cDBP, however there was a notable prolongation of cT2, reflecting increase of aortic compliance. It resulted in delay of reflected wave peak return and decrease of the pressure load to be overcome by the left ventricle, without decreasing maximum

pressure in aorta. Our hypothesis is that during sympathetic activation ascending aorta acts differently than peripheral vessels.

Parameter	Before	After	p value
HR	76.8 ± 9.4	66.6 ± 9.8	0.0038
cPERIOD	793.4±96.8	917.2±129.3	0.0041
cT1	107.6 ± 15.9	100.2 ± 12.06	0.027
cT2	187 ± 28.03	198. ± 27.9	0.028
pSBP	112.4 ± 11.2	113.3 ± 9.5	0.49
pDBP	68.0 ± 5.9	70.9 ± 8.6	0.028
cSBP	94.9 ± 8.8	96.7 ± 9.7	0.26
cDBP	69.3 ± 6.2	71.5 ± 8.8	0.085

Reference 1 :- Prodel E, Barbosa TC, Mansur DE, Nóbrega AC, Vianna LC. Effects of face cooling on pulse waveform and sympathetic activity in hypertensive subjects. Clin Auton Res. 2017 Feb;27(1):45-49. doi: 10.1007/s10286-016-0391-5. Epub 2016 Dec 9. PMID: 27942951.

Reference 2 :- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Acknowledgements :-

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PC006

Lysosomal Calcium Release Modulates β -adrenergic Response and Arrhythmogenicity in Ventricular Cardiomyocytes by Increasing SERCA Activity

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Background: Dysregulation of calcium handling is a well-recognised precursor of cardiac arrhythmias. Lysosomes have recently been shown to play an important role in cardiomyocyte calcium handling through calcium release via two-pore channels (TPC2)(1). Importantly, TPC2 knock-out (TPC2-KO) was found to markedly decrease the effects of β -adrenergic stimulation on calcium transient compared to wild type (WT) cardiomyocytes. These findings accounted for a reduced tendency for

arrhythmias following acute β -adrenergic stimulation in comparison of TPC2-KO versus WT(2). The mechanisms underlying lysosomal calcium handling response to sympathetic stimulation remain unknown.

Purpose: To investigate the key mechanisms underlying the lysosomal calcium handling response to β -adrenergic stimulation and its links to arrhythmogenesis using integrated experimental and computational methodologies.

Methods: State-of-the-art computational models of mouse ventricular electrophysiology and β -adrenergic stimulation(3) were extended to incorporate lysosomal calcium handling, based on organelle localisation and action. Latin hypercube sampling was used to build an initial population of 1000 candidate models, to represent cellular variability in electrophysiology and calcium handling. Simulated action potentials and calcium transients were calibrated against experimental steady-state recordings for model acceptance in the final population. Accepted WT and TPC2-KO models were simulated in control (CTRL), under pharmacological action of the calcium-mobiliser messenger NAADP-AM, and β -adrenergic stimulation by isoproterenol (ISO). Influence of lysosomal action on calcium transients was quantified by analysis of distributions and multivariate partial correlation coefficients.

Results: Data shown as mean $\pm$ SD. The initial population of models was calibrated against experimental recordings, resulting in a population of 605 accepted models. In agreement with experimental findings (Fig 1), no differences were observed in calcium transient between WT and TPC2-KO cardiomyocyte models under control conditions, whereas WT but not TPC2-KO responded to stimulation by NAADP-AM. The normalised calcium transient response under ISO stimulation was greater in WT (simulated: 1.02 ± 0.57 ; experimental: 1.23 ± 0.24) than TPC2-KO (simulated: 0.62 ± 0.37 ; experimental: 0.57 ± 0.17). These effects were mediated by a larger sarcoplasmic reticulum (SR) release and calcium content, primarily modulated by a potentiated SR Ca^{2+} -ATPase (SERCA) reuptake due to the release of lysosomal calcium. Importantly, lysosomal calcium fluxes to the cytosolic space contributed significantly to modulation of calcium transient relaxation under β -adrenergic stimulation, surpassing the contribution of the L-type calcium current. The propensity for DAD formation as shown in the incidence of DADs in Ca^{2+} transients under hypercalcemia in β -adrenergic stimulation was increased in WT (62%) compared to TPC2-KO (48%).

Conclusion: We have extended the existing Morotti *et al.*(2) model to include lysosomal calcium handling. Our results recapitulate experimental calcium transient findings of lysosomal calcium release in WT and genetically-modified TPC2-KO cardiomyocytes(2). These data show the impact of lysosomal calcium content in modulating intracellular calcium handling by increasing cytosolic calcium, which in turn promotes SERCA uptake, increases the SR calcium content, and potentiates Ca^{2+} -induced Ca^{2+} -release via ryanodine receptors. This increase in SR calcium content also potentially leads to the observed DAD formation. Our integrated experimental and computational methodology aims to elucidate the potential role of lysosomal calcium signalling in the mechanisms that underly arrhythmia.

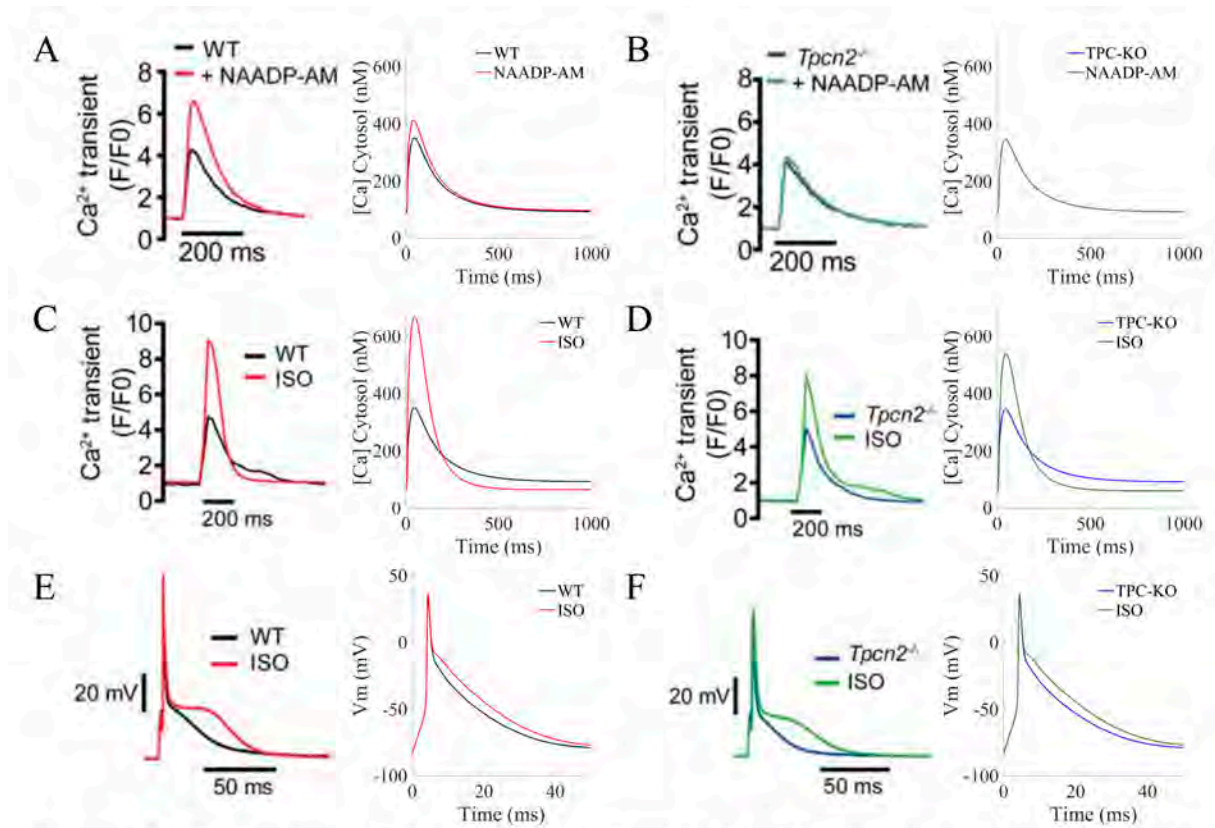


Figure 1. Calibration of the Ca^{2+} transient and action potential biomarkers in pharmacological protocols. 1A-1F show experimental data on the left panels(2) and simulation results on the right panels. 1A-1B are the Ca^{2+} transients under CTRL versus NAADP-AM comparison in WT and TPC-KO, respectively. 1C-1D are the Ca^{2+} transients under CTRL versus ISO comparison in WT and TPC-KO, respectively. 1E-1F are the action potentials under CTRL versus ISO comparison in WT and TPC-KO, respectively.

Reference 1 :- Aston, D., et al., *High resolution structural evidence suggests the Sarcoplasmic Reticulum forms microdomains with Acidic Stores (lysosomes) in the heart*. Sci Rep, 2017. 7(March 2016): 1–15.

Reference 2 :- Capel, R.A., et al., *Two-pore channels (TPC2s) and nicotinic acid adenine dinucleotide phosphate (NAADP) at lysosomal-sarcoplasmic reticular junctions contribute to acute and chronic β -adrenoceptor signaling in the heart*. J Biol Chem, 2015. 290(50): 30087–98.

Reference 3 :- Morotti, S., et al., *A novel computational model of mouse myocyte electrophysiology to assess the synergy between Na^+ loading and CaMKII*. J Physiol, 2014. 592(6): 1181–97.

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PC007

Transcriptional profiles of genes related to electrophysiological function in *Scn5a*^{+/-} murine hearts

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Introduction: The major pore-forming Na_v1.5 (α) subunit of the voltage-gated Na⁺ channel in cardiomyocytes is encoded by the *Scn5a* gene. Na_v1.5 plays a key role in action potential initiation and propagation in both atria and ventricles and hence organisms lacking *Scn5a* or carrying *Scn5a* mutations are predisposed to cardiac arrhythmogenesis. Of such pathologies, Brugada Syndrome (BrS) predisposes affected subjects to ventricular tachycardia, ventricular fibrillation, and sudden cardiac death. BrS most commonly arises due to loss-of-function mutations in the *Scn5a* gene causing Na_v1.5 haploinsufficiency. Interestingly, while patients are born with the primary ion channel dysfunction, cardiac arrhythmias tend to occur later in age suggesting later disruption to other components of the heart's electrophysiological system. The molecular mechanisms underlying this disruption remains poorly understood. A murine model with a heterozygous *Scn5a* deletion recapitulates the electrophysiological phenotypes of BrS. **Aims:** Thus, this study aims to investigate the underlying transcriptional molecular alterations which potentially contribute to atrial and ventricular arrhythmogenicity in this *Scn5a*^{+/-} murine model of BrS. **Methods:** Atrial and ventricular tissue samples were obtained from aged (11 ± 3 months) homozygous *Scn5a*^{+/-} (N = 8) and wild-type (WT). Quantitative PCR was used to examine the transcription of 60 genes underlying cardiac tissue excitability. **Results:** Of selected protein-coding genes related to cardiomyocyte electrophysiological or homeostatic function, concentrations of mRNA transcribed from 15 genes differed significantly from WT (P < 0.05). Of these, *Scn5a* expression was expectedly halved in ventricular tissue, but was contrastingly not significantly downregulated in atrial tissue suggestive of atria-specific feedback mechanisms increasing expression of the WT allele. Of the 14 remaining genes showing an altered expression, none were shared by both atria and ventricles. Notably, of the statistically significant changes in gene expression, all those in the atria were upregulations, and all those in the ventricles were downregulations. Ventricles showed downregulation of genes related to ion channels permitting surface Ca²⁺ entry (*Cacna1d*) and ion channels controlling resting membrane potential (*Abcc9*). Atria showed upregulation of genes related to ion channels permitting surface Ca²⁺ entry (*Cacna2d1*, *Cacna2d2*, *Cacna1h*, and *Cacna1c*), intracellular ion channels, transporters, and enzymes controlling Ca²⁺ homeostasis (*Ryr2*, *Atp2a2*, and *Camk2d*), Na⁺/K⁺-ATPase activity (*Atp1b1*), ion

channels initiating excitation (*HCN1*), ion channels controlling resting membrane potential (*Kcnj5*), and cardiac morphological properties (*Tbx3* and *Col1a1*). **Conclusions:** The present investigation highlights a number of important molecular alterations that may contribute to arrhythmogenesis in BrS. This demonstrates the value of future experiments exploring for and clarifying links between transcriptional control of *Scn5a* and of genes whose protein products coordinate cardiac electrophysiological activity and may potentially offer novel therapeutic pharmacological targets in the management of BrS.

PC008

The role of cardiopulmonary exercise testing (CPET) in predicting mortality and morbidity in people with congenital heart disease: a systematic review and meta-analysis.

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Background: The role of cardiopulmonary exercise testing (CPET) in predicting major adverse cardiovascular events (MACE) in people with congenital heart disease (ConHD) is unknown. **Design:** A systematic review with meta-analysis was conducted to report the associations between CPET parameters and MACE in people with ConHD. **Methods:** Electronic databases were systematically searched on the 30th of April 2020 for eligible publications. Two authors independently screened publications for inclusion, extracted study data, and performed risk of bias assessment. Primary meta-analysis pooled univariate hazard ratios (HR) across studies. **Results:** A total of 34 studies (18,335 participants; 26.2 ± 10.1 years; 54% ± 16% male) were pooled into meta-analyses. More than 20 different CPET prognostic factors were reported across 6 ConHD types. Of the 34 studies included in the meta-analyses, 10 (29%), 23 (68%), and 1 (3%) were judged as a low, medium, and high risk of bias, respectively. Primary univariate meta-analysis showed consistent evidence that improved peak and submaximal CPET measures are associated with a reduce risk of MACE (overall HR: 0.93, 95% CI: 0.91 to 0.94). This association was supported by a secondary meta-analysis of multivariate estimates and individual studies that could not be numerically pooled. **Conclusion:** Various maximal and

submaximal CPET measures are prognostic of MACE across a variety of ConHD diagnoses. Further well conducted prospective multicentre cohort studies are needed to confirm these findings.

Acknowledgements :-

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PC009

Student Evaluation: Changes to a physiology module in response to COVID-19

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Higher education providers have rapidly changed teaching, learning and assessment modes from traditional, on campus provision to online and blended modes since the COVID-19 pandemic in March 2020 (Kernohan, 2020). With blended delivery having advantages of both traditional and online teaching approaches (e.g. Sharma, 2010) introduction of course structure changes, creation of new teaching resources and assessment replacements for the 2020-21 academic year have been pressurised. The aim of this study was to determine whether delivery changes impacted on students' evaluation of an undergraduate physiology module compared to its previous 'on-campus' delivery.

Upon entry in Autumn term, year 1 BSc Medical Physiology and Therapeutics (MPT) students study a 20-credit module with content matter on haematology, alimentary physiology, nutrition and metabolism to introduce an understanding of nutritional supply, use and needs in the body. Compared to the previous 'traditional' year minimal in-class teaching was provided for 2020-21: didactic lectures were converted for asynchronous online study while practical classes and tutorials were largely delivered synchronously online via Microsoft Teams. Directed reading and activity resources supplemented material on our virtual learning platform (Moodle) to aid understanding, application and skill development which was also bolstered with online drop-ins. The online exam testing module learning outcomes was replaced by coursework.

Student Evaluation of Module (SEM) questionnaires using a 5-point Likert scale from 1 (strongly disagree) to 5 (strongly agree) and enabling free text comments, were provided electronically via Evaluate to gather our students' views. Due to COVID-19 delivery needs, the University adjusted the wording of questions for the 2020-21 cohort to maintain thematic educational comparisons between years. Scores for each question were collated and data presented as Mean $\pm$ Standard Error of the Mean (SEM).

Thirteen students from both cohorts responded to the SEMs. Comparison of the total SEM scores, and also the average scores to individual questions relating to a) activity organisation and structure, b) opportunities to explore topics, c) being challenged to achieve learning outcomes to deliver best work and d) the clarity of marking criteria, were not significantly different between the year groups despite the delivery changes made (Mann-Whitney U test, $p > 0.05$). However, significant differences in pre-COVID-19 study workload (4.31 ± 0.13 , $n = 13$) compared to the primarily online delivery (3.31 ± 0.34 , $n = 13$) suggested that the alterations had significantly increased this ($p = 0.02$). Increased workload was also corroborated by free text comments.

Overall, whilst overall student evaluation of our physiology module was not affected by the modifications for 2020-21 studies, this outcome highlights the need for increased study support for students adjusting to increased online learning.

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PC010

Perception about Teaching and Learning Methodologies Applied in Physiology, A study on Medical Students of Pakistan

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INTRODUCTION

The study of physiology is an essential part of the medical school curriculum. Medical teachers have identified the preference for a specific mode of content delivery to communicate knowledge to students in a rational, strategic, coherent, and sequential manner. In comparison to the focus on systems-based didactic lectures, more emphasis is now put on the developing critical thinking skills. Physiology is widely acknowledged as a difficult subject for medical students to grasp, incorporate, and apply in clinical sciences. Physiology is taught using a variety of teaching and learning methods, including formal interactive sessions (SIS), interactive lectures (IL), problem-based learning (PBL), and case-based lectures (CBL) Teachers are better able to keep students interested and inspired in the classroom when lectures are well planned. Physiology is interesting and preferred subject. The primary goal of medical education should be a holistic approach that improves medical professionals' problem-solving abilities through analytical and rational thought.

AIM & OBJECTIVES Until now, no research in Pakistan has evaluated whether current teaching, learning, and assessment methods are effective enough to impart information for effective learning. The aim of this study is to learn about students' perceptions of teaching, learning, and assessment approaches used in the physiology.

METHOD A quantitative cross-sectional survey was conducted online on 533 medical students from first to final year and also post graduate students in Pakistan. After the approval of the Ethical review committee of CMH Lahore Medical College, an online questionnaire made on "Google forms" was circulated to evaluate the various aspects of Physiology as a subject being taught. Cronbach's Alpha (81%) established the questionnaire's reliability which revealed excellent uniformity in the data received from responses by students. Participants answered anonymously with informed consent, and the survey was conducted for a period of two months. Data was analyzed using SPSS version 23

RESULTS

A total number of 533 students participated in this research and responded to Physiology learning and teaching. It was noteworthy to find out that the majority of students, 46% (n=246) claimed Anatomy to be the most interesting subject in 1st year MBBS which was closely followed by Physiology that 41.9% (n=224) participants selected. A similar result was also observed in another study by Rajesh K Jha in Nepal in 2015. With regards to their preferred medium of teaching, a large majority opted for traditional face to face lectures (43.7%, n=234) over online only lectures (5.2%, n=28). The likely cause for the lack of popularity of online classes can be attributed greatly to lower peer interaction, less satisfactory rapport with instructors and lower self-perceived knowledge gains. Preferred study material for most students was their college assigned textbook, "Guyton and Hall Textbook of Medical Physiology" (63.2%, n=637). It is interesting to note however, that only 9% of students claimed to rely on internet-based mediums of learning such as YouTube channels. This offers some degree of contrast to previous studies where internet resources are utilized quite often as supplementary aids. When asked whether students would wish to pursue physiology as a career option in the future, the largest response stated it was unsure (47.2%, n=252), with 32.6% (n=174) responding in the negative and only 20.2% (n=107) in the affirmative. A study in 2014 by Arun Kumar cites that a common reason for this is that most students tend to prefer the clinical subjects encountered in the later years as worthwhile career options over the basic sciences. The most important aspect of our research was to evaluate students' perception of learning and teaching methodologies used in physiology class. We asked 16 closed-ended questions assessing the general perception of students related to this topic. The result is summarized as follows in Table 1: Where as in Table 11 we have discussed the conceptual development and learning, mean rank of response of interactive sessions compared with lectures, structured Interactive sessions (SIS) and case based lectures or Problem based learning using Friedman test. P value considered significant* at <0.05

Table 1: Assessing the general perception of students related to Physiology

Question	Yes	No
Are you interested in Physiology?	88.0%	12.0%

Revision is required at the end of the lecture.	83.0%	17.
Do you like the way your physiology practical are conducted?	63.0%	37.
My teacher seems to know if something is bothering me.	47.1%	52.
In this class, my teacher accepts nothing less than our full effort.	69.5%	30.
My teacher asks questions to be sure we are following when she/he is teaching.	81.1%	18.
My teacher wants me to explain my answers -why I think what I think.	73.5%	26.
Our class stays busy and does not waste time.	57.9%	42.
If I don't understand something, my teacher explains it another way.	77.4%	22.
My teacher knows when the class understands, and when we do not.	65.6%	34.
I like the ways we learn in this class.	65.2%	34.
This class does not keep my attention -I get bored.	51.6%	48.
My teachers makes lessons interesting and innovative.	63.6%	36.
Students speak up and share their ideas about class work.	64.3%	35.
My teacher takes the time to summarize what we learn each day	71.0%	29.
The comments that I get on my work help me understand how to improve	77.0%	23.

Table 2: Use Of Teaching Methodologies (Lectures, Structured Interactive Session Sis .Case Based Lectures and PBL Sessions

Teaching Methodology	Lectures	Structured Interactive sessions(SIS)	Case Based Lectures/Problem Based	P value
Face to face Active learning	1.86	2.11	2.06	0.153
Small group discussion	1.80	2.12	2.10	<0.01*

Practical	1.70	2.22	2.18	<0.01*
Tutorials	1.76	2.07	2.24	<0.01*
Assessments and Viva	1.87	2.12	2.10	<0.01*
Assignments	1.90	2.21	1.89	<0.01*

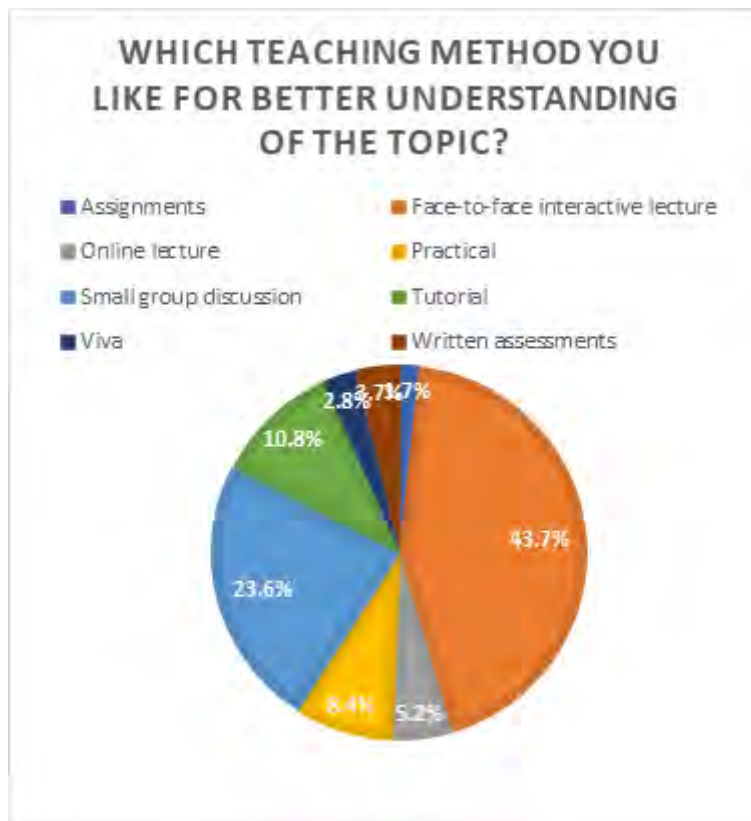


Figure 1: Which Teaching method you like for better understanding of the topic?

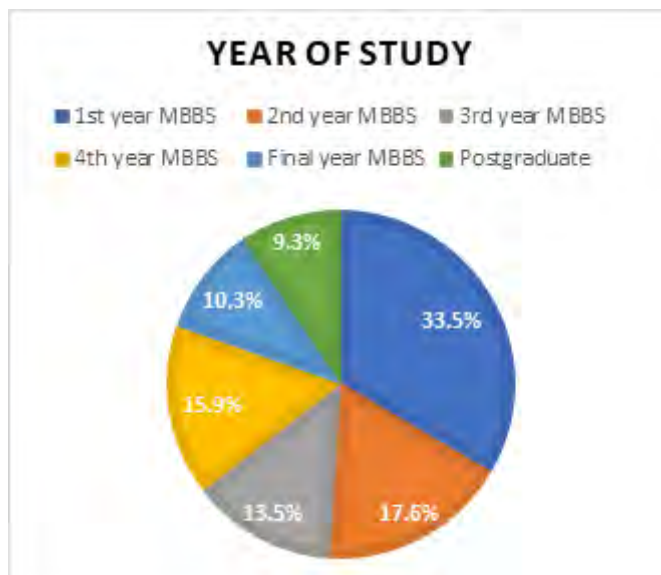


Figure 2: Year of Study

CONCLUSION

Physiology, science of life, is a discipline of biology whose goal is to comprehend the mechanisms of living things, from the ionic and molecular basis of cell activity to the integrated behaviour of the entire body and the impact of the external environment. The students overall considered their Physiology course satisfactory. Most of the participants were happy with the current teaching and learning methodologies being employed in their Physiology class but were open to their combination with multimedia aids and modern techniques. Barriers such as student teacher communication may also need to be addressed. This may allow teachers to engage their students better in the lecture and facilitate their understanding of the concepts to a greater degree.

Table 1: Assessing the general perception of students related to Physiology

Question	Yes	No
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Revision is required at the end of the lecture.	83.0%	17.0%
Do you like the way your physiology practical are conducted?	63.0%	37.0%
My teacher seems to know if something is bothering me.	47.1%	52.9%
In this class, my teacher accepts nothing less than our full effort.	69.5%	30.5%
My teacher asks questions to be sure we are following when she/he is teaching.	81.1%	18.9%
My teacher wants me to explain my answers -why I think what I think.	73.5%	26.5%
Our class stays busy and does not waste time.	57.9%	42.1%
If I don't understand something, my teacher explains it another way.	77.4%	22.6%
My teacher knows when the class understands, and when we do not.	65.6%	34.4%
I like the ways we learn in this class.	65.2%	34.8%
This class does not keep my attention -I get bored.	51.6%	48.4%
My teachers makes lessons interesting and innovative.	63.6%	36.4%
Students speak up and share their ideas about class work.	64.3%	35.7%
My teacher takes the time to summarize what we learn each day	71.0%	29.0%
The comments that I get on my work help me understand how to improve	77.0%	23.0%

Table 2: Use Of Teaching Methodologies (Lectures, Structured Interactive Session Sis .Case Based Lectures and PBL Sessions

Teaching Methodology	Lectures	Structured Interactive sessions(SIS)	Case Based Lectures/Problem Based	P value
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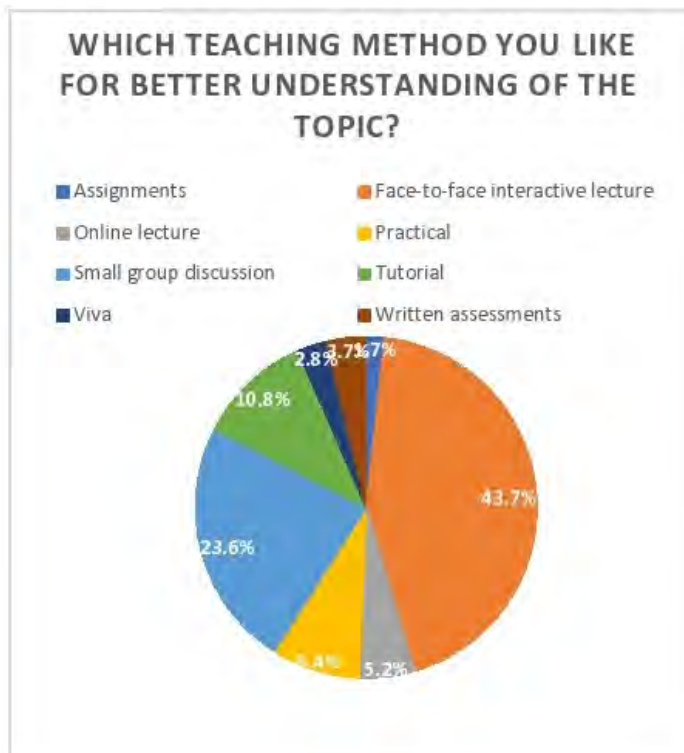


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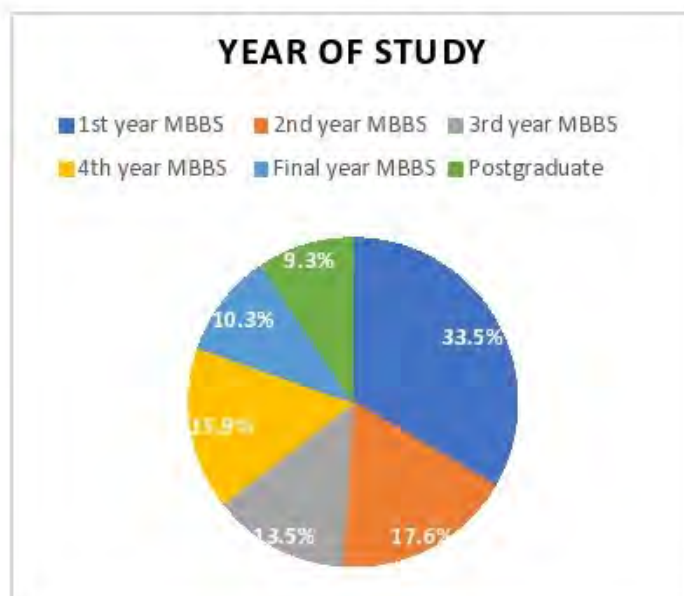


Figure 2: Year of Study

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Acknowledgements :- My Institution CMH Lahore Medical College and IOD

PC011

Physiology Education in Nigeria: Faculty Opinions on Challenges and Suggested Solutions

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Introduction:

Physiology is one of the core subjects in undergraduate medical education and has practical implications for medical practice. Though Physiology is widely studied in Nigeria, it is faced with series of challenges that demand periodic assessments to proffer solutions. (1)

Objective:

This qualitative study explores faculty opinions regarding the current challenges facing Physiology education in Nigeria and subsequently proposes possible solutions.

Methods:

An online descriptive, cross-sectional study was conducted between March and April 2021. Existing faculty members in Physiology departments across several Nigerian Universities who are engaged in Physiology teaching and research constituted the study population. Using Google forms, a pre-tested and validated web-based questionnaire was administered to participants via WhatsApp messenger and emails. The questionnaire was developed using self-designed and validated questions from published studies, and used to obtain participant's sociodemographic data, opinions regarding the problems facing Physiology education, and recommended solutions.

Results:

Overall, 50 participants responded (response rate = 62.5%). Of the 48 valid responses, 40 (83.3%) were males, and the majority (n=23, 47.9%) were in the age bracket of 21-30 years. Of the 48 faculty members who responded, 23 (47.9%) work in federal universities, 16 (33.3%) in state universities, while 9 (18.8%) work in private universities. Findings from our study revealed the opinions of Nigerian faculty members regarding the challenges facing Physiology education. More than half (n=30, 62.5%) of the participants stated the lack of professional (clinical/industrial) experiences, 16 (33.3%) opined a poor job/career prospects, 12 (25%) and 13 (27.1%) participants stated poor funding and inadequate learning/teaching/research facilities respectively. More so, 12 (25%) respondents opined a poor and outdated curriculum, while 8 (16.7%) and 7 (14.5%) participants stated lack of awareness and recognition of Physiology, and a poor regulatory body/framework respectively amongst others. Furthermore, data obtained from the recommended solutions for improving Physiology education as opined by faculty members are; incorporation of professional (clinical/industrial) experiences (n=26, 54.2%), adequate funding for teaching and research (n=24, 50%), curricular review and integration (n=18, 18.8%), promoting public awareness and recognition of Physiology (n=10, 20.8%), and the establishment of a regulatory body (n=8, 16.7%).

Conclusion:

Challenges in Physiology education in Nigeria are numerous and on multiple fronts. The faculty's recommended solutions if implemented diligently will go a long way in enhancing the discipline. This will further provide students with rewarding educational experiences and consequently impact positively on their career upon graduation.

Keywords: Physiology education; Nigeria; Challenges; Solutions

Reference 1 :- Sofola S. (2014). Challenges of teaching and researching physiology in Sub-Saharan Africa. *Physiology*; 29: 150-152.

PC012

Assessment of Repeatability using Structured Light Plethysmography Technique Compared to Spirometry

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Introduction:

Structured Light Plethysmography (SLP) is a non-invasive, contactless, self-calibrating, easily portable technique that utilises a projected grid of white light and cameras to track, measure, capture Thoraco-Abdominal (TA) displacement and record quiet tidal breathing. Repeatability is the assessment of measurements from two devices for the same test subject when recorded simultaneously. Spirometry is considered the gold standard for measuring lung function but it requires the application of forceful manoeuvres and full cooperation and understanding that might not be attainable by patients. Thus, SLP could provide an opportunity to evaluate these subjects in addition to spirometry data.

Aim:

To determine the agreement and repeatability between SLP recordings and simultaneous spirometry measurements of tidal breathing.

Methods:

Quiet breathing in 14 healthy volunteers (18-35 years) was simultaneously recorded via SLP (Thora3Di, Pneumacare Ltd) and by spirometry (Power lab 4/20, AD Instruments Ltd). Statistical analysis using a paired t-test to evaluate the correlation and agreement of these techniques was assessed under three different breathing conditions: Normal, Deep and Shallow for a total of 3 minutes and a minute for each pattern. Respiratory rate (RR), inspired, expired and total breathing times were analysed (Ti, Te, Ttot), and duty cycle (Ti/Ttot) were calculated under the three different breathing conditions. All the work carried in this study was under the ethical review licence (ERN_19-0016) approved by the Science, Technology, Engineering and Mathematics Ethical Review Committee at the University of Birmingham with a screening questionnaire and a written, signed consent form completed by participants.

Results:

There was a significant difference in shallow breathing measurements found in inspiratory time and duty cycle (Ti $P=0.0128$, Ti/Ttot $P=0.0346$) with no significant changes during normal and deep breathing (see Figure 1. a-b).

Conclusions:

SLP shows a good correlation with spirometry for all timing indices under normal and deep breathing, indicating that it is a reliable technique under these conditions. However, a significant difference in shallow breathing was observed, suggesting either that body movement during rapid breathing disturbed SLP detection of breathing as a result of the high frequency or that this pattern of breathing affected spirometry through an increased dead space ventilation and resistance. It is safe

to say that SLP can be a useful method in the detection of breathing and useful addition to other techniques with attention to changes in breathing patterns.

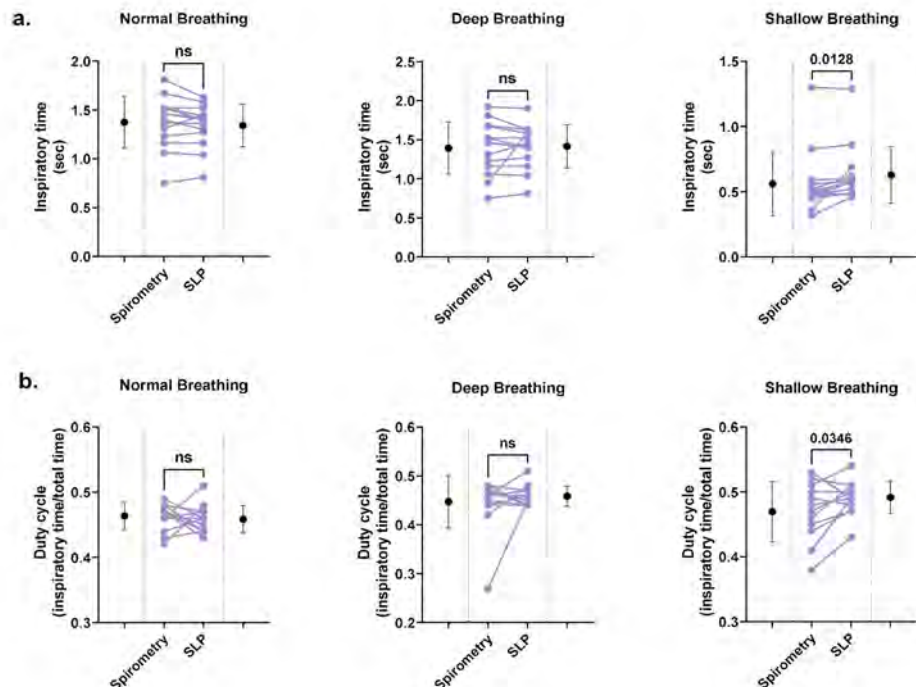


Figure 1. A comparison of the (a. Inspiratory time) and a calculated inspiratory time to total time ratio (b. Duty cycle) recorded in (n=14) healthy participants using spirometry and SLP simultaneously during three different breathing patterns (Normal, Deep, and Shallow). Compared using a paired t test presenting Mean $\pm$ SD with $p < 0.05$ considered significant.

Acknowledgements :- This research was funded by a Scholarship from King Saud bin Abdulaziz University for Health Sciences (KSAU-HS) (Riyadh, Saudi Arabia) provided through the Saudi Arabian Cultural Bureau (SACB) in London.

PC013

Influence of posture on the foot perfusion in the upright position

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Introduction

The upright position depends on postural stability which can be quantified by displacement parameters, including the length of the center of pressure (CoP) and oscillation, path, and average CoP speed). The contact area is also an important parameter to study the distribution of plantar pressure, indicating the duration of the load applied to a specific area. We might expect that both gravity and muscle tensions impact and influence the vascular dynamics of the foot, even in the maintenance of an orthostatic position.

Aims/objectives

To look deeper into the potential relationships between posture and perfusion variables affecting foot vascular dynamics in the upright standing position.

Methods

Eight healthy participants (25.1 ± 5.2 years) of both sexes (4 women and 4 men), with $ABI = 1.0 \pm 0.1$, were selected. Procedures respected all the principles of good clinical practice for human studies research. Perfusion was measured on both feet, participants in the orthostatic position at rest for 5 min using Laser Doppler flowmetry (LDF, Perimed 5000). Sensors were applied on the dorsum of both feet between the 3rd and 4th toes. The dorsal region perfusion was also assessed by polarized spectroscopy (PS, TiVi, WheelBridge AB). Postural data was obtained with the Foot Scan® RsScan International® Balance pressure plate. Plantar pressure of the foot was assessed in three functional segments - the forefoot, the midfoot, and the backfoot. Statistical analysis was performed with GraphPad Prism software. Parametric and non-parametric statistics were applied and a 95% confidence level adopted.

Results and Discussion

LDF showed different baseline perfusion values between both feet with slightly higher values in the left foot also showing a statistically significant higher concentration of red blood cells (CRBC) ($p = 0.03$). The PS system detected a higher perfusion index in the right foot, ($p = 0.007$) in the same area. These observations are not contradictory, as the light-tissue interaction differs between these two systems, with LDF penetrating to a much greater depth than the superficial plexus measurements provided by PS. Regarding posture, the hindfoot region has shown higher values compared with the forefoot and midfoot. Changes in displacement of the CoP and speed over the five minutes recorded had no statistical significance, as expected, as the applied protocol is a quasi-static activity. The CoP offset is correlated to the CoP velocity during the protocol and to the plantar pressure in the three selected areas. Correlations between pressure variables and LDF perfusion variables were noted. In the right limb, a negative correlation ($p < 0.01$) was observed between the CoP displacement and CoP velocity, while in the left limb, a negative correlation ($p < 0.05$) with CoP displacement and velocity in the midfoot.

Conclusion

Permanent interaction between perfusion and CoP and plantar pressure seems to exist and to mutually influence each other even in the orthostatic position, however, although these variables are poorly understood.

Acknowledgements :- Fundação de Ciência e Tecnologia (FCT) UIDB/04567P/2020 and UIDP/04567B/2020.

PC014

Evaluating the Efficacy of Primary Dysmenorrhoea Management Methods and their Accessibility under Universal Health Coverage

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Introduction

Primary dysmenorrhoea (PD) describes a recurrent, cyclical, cramp-like pain that occurs around the onset of menstruation in the absence of any discernible underlying cause and with an unclear pathophysiology. Estimates of prevalence are broad, with anywhere between 16% and 91% of adolescent girls and young women affected. A further 7-15% are estimated to suffer from PD severe enough to affect work, school and socialisation.

The typical first-line treatment for PD is prescription of the non-steroidal anti-inflammatory drug (NSAID) mefenamic acid, with oral contraceptives then used if the patient is unresponsive or derives insufficient pain relief. However, accessibility to these pharmaceuticals may be limited in countries with low levels of Universal Health Coverage (UHC), a measure utilised by the World Health Organisation (WHO) to quantify countries' provision of affordable essential healthcare services.

Aims & Objectives

This poster seeks to establish the efficacy of pharmaceutical and non-pharmaceutical management methods for PD. This may allow improvement of the WHO Model List of Essential Medicines (a tenet of UHC) to promote access and reduce the burden of this prevalent disease. Understanding of non-pharmaceutical management methods may also prove critical to supplementing conventional pharmaceutical therapies, particularly where they are inaccessible or unaffordable.

Methods

PubMed, EMBASE and the Cochrane Central Register of Controlled Trials were searched for English-language full-text primary research articles published between October 2015 and October 2020 investigating PD management methods. Inclusion criteria included use of pharmaceuticals, supplements, exercise or education on adolescent girls or women with a diagnosis of primary dysmenorrhoea. The primary exclusion criteria were diagnosis of secondary dysmenorrhoea or any other pelvic pathology, as well as investigation of alternative and complementary medicine, which was defined as any therapy not administered by a healthcare professional or approved by the Medicines and Healthcare Products Regulatory Agency. The PRISMA guidelines (Moher et al, 2009) and CASP checklists (Critical Appraisal Skills Programme, 2019) were used for screening and appraisal, respectively.

Results

Of the 1570 primary research articles identified, 23 were included, containing 3155 participants. Management categories included NSAIDs (n=822), contraceptives (n=915), exercise (n=56) and supplements (n=1262). NSAIDs, contraceptives and exercise were largely effective at alleviating PD symptoms, with contraceptives most frequently totally eradicating symptoms. Efficacy of supplements varied, with calcium, Vitamin E and omega-3 presenting the most promising management methods, subject to further rigorous RCTs.

Discussion

Expansion of the WHO Model List of Essential Medicines to include more NSAIDs effective for PD will improve access to pharmacological treatments, thus reducing the social and economic burden of PD. Targets for further research include elucidating the mechanism of action of supplements and focusing research on adolescents, as they are a sizeable subset of the PD population.

Reference 1 :- Critical Appraisal Skills Programme (2019). CASP Randomised Controlled Trial Checklist. [online] Available at: <https://casp-uk.net/wp-content/uploads/2018/01/CASP-Randomised-Controlled-Trial-Checklist-2018.pdf>. Accessed: 18/04/21

Reference 2 :- Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G. and Prisma Group, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS medicine*, 6(7), p.e1000097. Moore, R.A., Derry, S. and Wiffen, P.J., 2013. Challenges in design and interpretation of chronic pain trials. *British journal of anaesthesia*, 111(1), pp.38-45.

Acknowledgements :- School of Life Sciences, College of Medicine, Veterinary Medicine and Life Sciences, University of Glasgow

Microbial-metabolite pathways associated with cervicovaginal infection and spontaneous preterm birth: a systematic review

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Introduction: Delivery before 37 completed weeks of gestation (preterm birth, PTB) is still a major global health challenge, with 15 million babies born premature annually [1]. Majority (~75%) of PTBs occur spontaneously with still unresolved pathogenesis due to the multiple aetiologies. Intrauterine infection commonly ascending from an altered (dysbiotic) vaginal microbiota accounts for ~50% of spontaneous PTB (sPTB) [2]. Choriodecidual bacterial colonisation induces feto-maternal immunological responses that lead to production of proinflammatory mediators, uterotonins and matrix degrading enzymes that activate the common pathway to spontaneous labour [3, 4]. The cervicovaginal (CV) space is metabolically active and the metabolome is critical in understanding eubiosis, dysbiosis, infection and infection-associated sPTB [5]. Studies identifying CV microbial-metabolic pathways and developing predictive models based on metabolites obtained from pregnant women are limited.

Aim/objective: We conducted a systematic review of the literature to identify studies that employed cervicovaginal fluid (CVF) microbial-metabolites and metabolic pathways to investigate the pathophysiology of and/or predict sPTB.

Method: The review was conducted according to the PRISMA statement. Medline/PubMed and Web of Sciences databases were searched for relevant articles using specific search terms. Original studies that included CVF metabolites and metabolic-pathways to determine mechanisms and/or risk of PTB were eligible. The eligibility and exclusion criteria are listed in Table 1. The search results were reviewed by two authors (EA and MC) independently, first by title, followed by abstract and finally by full text. Cases of discordant views on a particular article were deliberated until a consensus was reached, otherwise a third author's (NK's) review of such article was adopted as the decider.

Results: Of 115 studies identified, only 8 met the inclusion criteria. Seven of these studies reported a distinct CV metabolic profile in women destined to deliver preterm, while one study did not identify any significant difference between the groups. Only 2/8 of the studies reported microbial changes associated with the distinct metabolite profiles observed. The metabolites fit into common human-microbial metabolic pathways including the glycolytic, tricarboxylic acid, hydroxyglutarate and methylaspartate pathways; aspartate-arginosuccinate shunt and urea cycle; and ammonia assimilation cycle. Women with PTB showed increased/upregulated acetate, acetate:glutamate, mannitol/sorbitol, methyl phosphate ($p<0.05$) especially when they present with symptoms of preterm labour. Asymptomatic women that eventually deliver preterm also showed elevated xanthine, NADH, glucose, glucose-6-phosphate, N-acetylneuraminate, palmitoleate, 10-heptadecanoate, 1-palmitoyl glycerol, nicotinamide and 2-pyrrolidinone ($p<0.05$) at mid to late second trimester. At other instances, both symptomatic and asymptomatic women with PTB showed elevated ethanol, ethylene glycol, glycolate, methanol, isopropanol and formate ($p<0.05$). They also

had elevated acetone and trimethylamine N-oxide ($p<0.001$), whereas lactate, branched chain amino acids, phenylalanine, tyrosine, alanine, aspartate and glycine are reduced compared to women without PTB ($p<0.05$).

Conclusions: The CVF metabolite profile differs between term and preterm women, and could also reveal new pathways associated with sPTB. This could be a potential source of predictive biomarkers of sPTB. The metabolites and pathways could be exploited in developing predictive algorithms that incorporate other omic data for stratification of women at risk of sPTB and guide bespoke preventive and therapeutic interventions.

Table 1. Eligibility and exclusion criteria.

Eligibility	Pregnant women Cervicovaginal fluid Metabolites and/or metabolic pathways Mechanisms and/or prediction of PTL and PTB Original articles Written in English Published in the last 30 years
Exclusion	Non-pregnant women Non-CVF samples e.g. placenta, amniotic fluids, oral, gut, breast milk etc. Non-human samples e.g. animal models <i>In vitro</i> studies Genomic, transcriptomic and proteomic studies Fetal metabolome and microbiome Review articles

CVF, cervicovaginal fluid; *PTB*, preterm birth; *PTL*, preterm labour.

Reference 2 :- Witkin SS (2015). *Bjog* 122(2), 213-218.

Reference 3 :- Parris KM et al. *J Clin Path* 74, 10–18.

Reference 4 :- Romero R et al. (1992). *Am J Obstet Gynecol* 167(4 Pt 1), 1086-91.

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PC016

Glucagon-like peptide 1 (GLP-1) secretion from gut endocrine cells is pH dependent

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Glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted from enteroendocrine cells of the gut, that are called L cells. GLP-1 is released in response to the arrival of digested food in the gut, with glucose being one of the most potent stimulants of GLP-1 release. The mechanism of glucose-induced GLP-1 secretion has been well established. First glucose enters the L cells via a sodium-glucose co-transporter (SGLT1), the influx of sodium depolarises the cell membrane, which in turn opens the voltage-gated calcium channels and the subsequent rise in intracellular calcium levels triggers GLP-1 exocytosis.

Previous reports showed that secretion of insulin from pancreatic β -cells, as well as glucagon from α -cells is pH sensitive. We therefore decided to investigate the GLP-1 secretion at different extracellular pH. Here, we report for the first time that secretion of GLP-1 from L-cells is also pH dependent.

We tested the effect of lowering the extracellular pH on GLP-1 secretion in two murine model L cells – GLUTag cells and primary intestinal cell cultures from wild-type mice. **We discovered that lowering extracellular pH below neutral 7.0 decreases glucose-induced secretion of active GLP-1 by almost 50% at pH 6.3, while secretion remains unaltered at pH ranging 7.0-7.6.** We show that the effect of low extracellular pH on GLP-1 secretion can be mimicked by the inhibition of intracellular V-ATPase proton pumps by bafilomycin A1. This result strongly suggests that the pH dependence of GLP-1 secretion is mediated via proton influx and accumulation in the cytoplasm, rather than an external protonation effect. We further speculated that the point of entry of protons might involve ion-coupled transport. We therefore replaced sodium in the secretion buffer with non-permeable cation, NMDG. As predicted, glucose-induced GLP-1 secretion was impaired by the absence of sodium and no pH dependence of GLP-1 secretion was observed. This would suggest the pH effect on GLP-1 secretion is Na⁺dependent. However, further depolarising the membrane with 30mM KCl, both in the presence or absence of sodium, still show a preserved pH dependence of GLP1 secretion, which would suggest that the pH

effect is depolarisation independent. Further study is required to investigate how the protons are imported into the cell as well as to elucidate the exact mechanism by which increased levels of intracellular protons decrease GLP-1 secretion. In a future study we will specifically address two pH sensitive steps involved in GLP-1 secretion: cleavage of proglucagon into active peptide by prohormone convertase 1 that would impact availability of the active GLP-1 for secretion and also docking/fusion of mature secretory vesicle into plasma membrane. GLP-1 analogues and inhibitors of its degradation are currently under investigation as a new treatment of type 2 diabetes. Understanding how pH affects GLP-1 secretion could be of potential clinical relevance since intraluminal pH of the gut is very variable throughout and also changes with the food passage, health status of the patients and microflora content of the gut.

PC017

Transcriptome and methylome of the term human placenta in gestational diabetes mellitus: A systematic review and meta-analysis.

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Background: Gestational diabetes mellitus (GDM) is defined as the development of glucose intolerance during pregnancy. It is one of the most common complications of pregnancy, is linked to fetal overgrowth, and predisposes the mother and her child to develop metabolic disease like type-2 diabetes in later life. The placenta is a multifunctional organ that develops during pregnancy and may be central to the pathogenesis and sequelae of GDM, but the metabolic pathways and epigenetic mechanisms possibly involved are not fully understood. This study aimed to perform a systematic review and meta-analysis using existing transcriptome and methylome datasets of the placenta to identify particular types/classes of genes and epigenetic events that may be of significance for understanding the role of placenta in the molecular pathophysiology of GDM.

Methods: We retrieved published transcriptome-wide and methylome-wide studies performed on term human placenta by performing searches on PubMed, Google Scholar, Gene Expression Omnibus and Europe PMC. For inclusion, studies needed to show a list of differentially expressed or methylated genes using high-throughput sequencing technologies or microarray, comparing control and GDM samples. Studies which included participants with other health or pregnancy complications (e.g. pre-existing diabetes and preeclampsia) were excluded. Different R packages such as upsetR, among other Bioconductor packages were used to compare the results between the studies.

Results: We identified 14 studies for assessment. Ten contained a list of differentially expressed (DEG) RNAs between GDM and control placenta and provided pathway analyses. Of the DEGs identified, two were non-coding RNAs. Five studies showed differentially methylated genes. The sample size and number of DEGs between GDM and control placentas varied from 41 to 3 samples per group and from 300 to 20 genes. Genes including CXCL10, CXCL11, GBP1, FABP5, LPL, CFH, LUM, and several encoding HLAs (e.g. HLA-C and HLA-DQA2) were shown to be dysregulated in GDM in more than 3 transcriptome datasets surveyed while MIR30B was consistently altered in the GDM placenta of 2 transcriptome datasets. Biosynthesis, cytokine activity, immune response regulation and oxidative phosphorylation were pathways commonly enriched in the transcriptome studies. In addition, methylation of the TPO, VIPR1, BCAR1, CAPN1 and SEPT9 genes were shown to be aberrant in at least 2 of the surveyed methylome studies. Meta-analysis of the transcriptome and methylome studies identified different intersection sizes between specific studies. Work is underway to correlate transcriptome and methylome datasets.

Conclusion: Overall, the number of studies performed on placenta in GDM concerning RNA, and specifically DNA methylation changes by sequencing is restricted. The overlap between studies is limited, and likely due to variations in participant characteristics including ethnicity, BMI, GDM treatment and diagnosis, as well as offspring sex. However, the overlap of DEGs and differentially methylated genes identified in the current study may inform on the pathogenesis and impact of GDM on maternal and offspring metabolic health. Correlating transcriptome and methylome datasets will provide information of the mechanisms regulating gene expression in the GDM placenta. Future work could also be improved by including maternal and cord blood sequencing data.

PC018

Carotid body modulation decreases weight gain and reverts dysmetabolism by ameliorating adipose tissue function and restoring sympathetic integration

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Introduction: Therapeutic options to treat obesity are scarce⁽¹⁾. We previously demonstrated that carotid body (CB) is involved in the genesis of metabolic disturbances by showing that the abolishment of its activity, through resection of carotid sinus nerve (CSN) prevents and reverses metabolic dysfunction^(2,3). Furthermore, CSN resection decreased weight gain and body fat mass in an obese animal model⁽⁴⁾. Herein, we investigated if these effects are related with a recovery of white (WAT) and brown (BAT) adipose tissues metabolism and a restauration of sympathetic integration.

Methods: Experiments were performed in male Wistar rats and C75BL/6 J mice submitted to a high-fat diet (60% lipids) for 10 weeks or 12 weeks, respectively. HF animals were compared with aged-matched controls submitted to standard diet (CTL). After diet period, groups were randomly divided and half submitted to CSN resection or to a sham procedure. Animals were kept under the respective diets for 9 and 3 weeks, respectively. Insulin sensitivity, glucose tolerance, caloric intake and body weight were monitored. At a terminal experiment WAT and BAT depots were collected for: 1) analysis of proteins involved in adipose tissue metabolism (UCP1, PPAR γ and PGC1 α) by immunohistochemistry and western blot; 2) tissue oxygen consumption rate (OCR) by seahorse in basal conditions and in response to Norepinephrine [15mM] or Dopamine [100nM]; 3) catecholamine levels by HPLC and; 4) sympathetic innervation by light-sheet microscopy. Laboratory care was in accordance with the European Union Directive 2010/63/EU and approved by NOVA Medical School Ethics Committee.

Results: HF diet decreased, both in WAT and BAT: 1) the mitochondrial density, by 30% and 21%, respectively and 2) UCP1 expression by 34% and 21%, respectively. It also decreased PGC1 α expression by 27%, in WAT, with no alterations observed on PPAR γ expression. CSN resection increased, both in WAT and BAT: 1) mitochondrial density by 61 and 50%, respectively and 2) UCP1 expression by 63 and 45%, respectively; It also increased WAT PGC1 α and PPAR γ expression by 51% and 73% . Moreover, HF diet decreased WAT basal activity by 51% while did not impact BAT basal OCR. CSN resection promoted an increase of 77% above control levels. HF diet also decreased WAT activation promoted by NE and dopamine by 57 and 44%, respectively, while in BAT, HF prompted a decrease of 20% in response to NE with no alterations in OCR after Dopamine injection. CSN resection totally restored the effect of HF diet on NE and dopamine activation of adipose tissue depots. Catecholamine levels, in WAT, were decrease by 47%, with HF diet, while CSN resection increased it by 29%. These results are coincident with the results obtained with light-sheet microscopy where we observed a decrease on sympathetic innervation after HF diet whereas CSN promotes a recovery of innervation.

Conclusion: Herein we demonstrated for the 1st time that CB controls adipose tissue metabolism, both WAT and BAT and that the beneficial effects of CSN resection on weight gain and dysmetabolism are related with an improvement of adipose tissue metabolism/thermogenesis accompanied by a restauration of sympathetic integration.

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Reference 3 :- Sacramento, J. F., et al. *Diabetologia* 2017, 60 (1), 158–168.

Reference 4 :- Melo, B.F., et al. *Diabetologia*, vol. 60, n. Suppl 1:S275, 2017.

Acknowledgements :-

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PC019

Identification of Novel Human Gut Microbiota Metabolites from Cyanidin- and Delphinidin-Type Anthocyanins

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Background: Anthocyanins are a class of flavonoids responsible for the red-purple pigmentation of foods such as strawberries, bilberries and black rice. Numerous studies report beneficial physiological effects linked to anthocyanin consumption. But, due to their rapid degradation following consumption, bioavailability of anthocyanins is low, and any protective effects are likely to be attributed to their metabolites [1]. However, much remains to be understood regarding the plethora of metabolites generated and the role of the gut microbiota in this.

Objective: To determine the metabolites produced from cyanidin- and delphinidin-type anthocyanins, highlighting inter-individual variation in metabolite profiles, differences in the metabolites from these two anthocyanins, and their relationship with the gut microbiota.

Approach and Study Design: In a placebo-controlled randomised crossover trial (performed in compliance with the guidelines laid down in the Declaration of Helsinki), 52 volunteers consumed capsules of bilberry (mainly delphinidin-type) or black rice (mainly cyanidin-type) anthocyanins for 28 days. Urine samples were collected pre- and post-intervention, subjected to solid-phase extraction, and analysed using UHPLC-MS/MS. For a subset of participants (n = 24) faecal samples were preserved as glycerol stocks and used to establish the real-time anthocyanin metabolising capacity of the microbiota using an in-house method (Shehata, Day-Walsh & Kroon, unpublished).

Results: For the first time, 5-hydroxyferulic acid was identified as a bilberry anthocyanin metabolite. Additionally, several other phenolic metabolites were identified that were not reported in a study of human metabolism of penta[13C]-cyanidin-3-glucoside [2]. We observed catechol and its phase 2 conjugates to be major metabolites of both cyanidin- and delphinidin-type anthocyanins. Furthermore, we have provided evidence for the role of the gut microbiota in the production of key *in vivo* metabolites, and have shown clear correlations between *in vitro* fermentation and *in vivo* metabolism.

Conclusion and Future Work: We observe specific differences in the metabolite profiles derived from cyanidin- and delphinidin-type anthocyanins, along with considerable inter-individual variation in metabolism. For the first time we are also able to compare *in vivo* urinary metabolites with those produced in an *in vitro* gut fermentation model, highlighting the critical role of the gut microbiota in anthocyanin metabolism, and consequently the physiological effects of an anthocyanin rich diet. An important point of future work will be to elucidate any physiological bioactivity of these microbial metabolites.

Reference 1 :- [1] Kay CD, Kroon PA, Cassidy A (2009). Mol Nutr Food Res 53, 92-101.

Reference 2 :- [2] De Ferrars RM, Czank C, Zhang Q, Botting NP, Kroon PA, Cassidy A, Kay CD (2014). Br J Pharmacol 171, 3268-3282.

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PC020

Effect of BMI on the Motor Nerve Conduction Study among Medical Student of AL-Neelain University

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Introduction:

Body mass index (BMI) is an important parameter regarding adiposity and obesity. Many studies have shown higher incidence of cardiovascular diseases especially coronary artery diseases in population with BMI greater than 25. (1) Studies also reveal median and ulnar nerve compression associated with an increasing incidence of higher BMI. (2) This study is an attempt to establish a relationship between median nerve conduction parameters and BMI in a healthy population group from Alneelain university faculty of medicine. .

Methodology:

In the present study –a descriptive institutional based pilot study- the effect of Body Mass Index (BMI) on Median nerve motor conduction latency, amplitude and velocity was analysed in 84 healthy students from Alneelain university faculty of medicine in the age group of 18-25 years.13 were underweight,49 had normal BMI and 20 were above normal.

The data was collected using a self-administered questionnaire and the nerve conduction study was measured by power lab serial number G607.

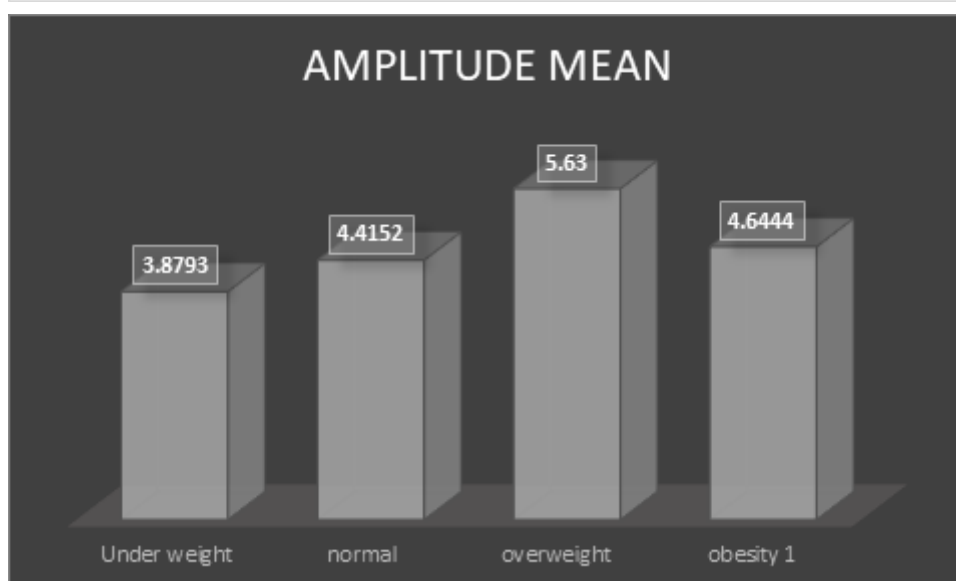
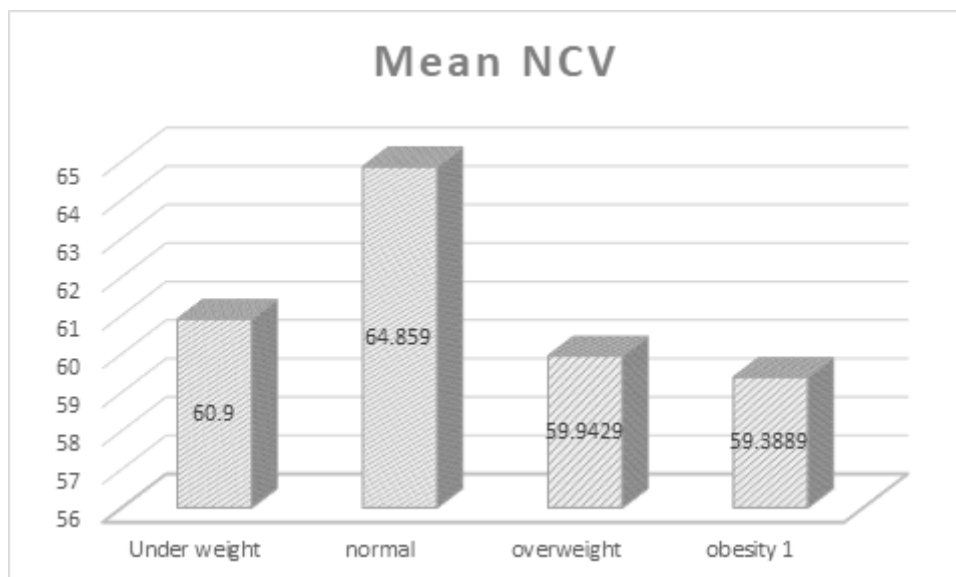
The median nerve was stimulated supra-maximally at two points along its course at the wrist and antecubital fossa. Nerve conduction velocity, amplitude, proximal latency and distal latencies were measured

ethical approval was obtained from alneelain university ethical committee.

The data was analyzed using Statistical Package for the Social Sciences (SPSS) Version 23 (SPSS) .

Results:in this study there was a tendency for amplitude to increase with increasing BMI however this does not reach the level of significance P value 0.266

No statistical significant difference in the nerve conduction velocity and latencies between BMI categories with p values 0.157 for the velocity ,P-value .617 for proximal latencies and p-value .581 for distal latencies



Relationship between NCS parameters and BMI

NCS parameters	BMI	N	Mean	Std. Deviation	Sig.
motor nerve conduction velocity	Under weight	12	60.9000	9.32767	.157
	normal	39	64.8590	7.56511	
	overweight	7	59.9429	10.13687	
	obesity 1	9	59.3889	8.50815	
	Total	67	62.9015	8.43631	
Amplitude	Under weight	12	3.8793	1.69063	.266
	normal	39	4.4152	1.69906	
	overweight	7	5.6300	2.32577	
	obesity 1	9	4.6444	2.34280	
	Total	67	4.4769	1.87284	
Proxy	Under weight	7	.00324286	.000763451	.617
	normal	29	.00351034	.001285453	
	overweight	4	.00360000	.000541603	
	obesity 1	5	.00282000	.000875785	
	Total	45	.00340000	.001128958	
Distal	Under weight	7	.00770000	.000611010	.581
	normal	28	.00739643	.001516937	

	overweight	4	.00782500	.000865544	
	obesity 1	6	.00670000	.002103331	
	Total	45	.00738889	.001454026	

Conclusion: Our study reveal that there is no effect of BMI on nerve conduction study amplitude,velocity and latencies

Recommendation: larger Studies involving higher numbers of both genders and a variety of ages and with measuring the sensory component of the median nerves and other nerves is recommended.

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Reference 2 :- Dipti Thakker, N J Shah, R.S. Trivedi, TO STUDY THE EFFECT OF BMI ON NERVE CONDUCTION VELOCITY, Int J Basic Appl Physiol., 8(1), 2019

PC021

Psychoneuroimmunology of Meditation

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Objective: Meditation, a major component of a yoga-based lifestyle, has been implicated in relaxation and is known to reduce stress and anxiety. A plausible mechanism of such relaxing effect is psychoneuroimmunology (PNI), based on a relation and interaction between mind, physical health, and self-healing; that conceptualizes that stress and the emotional state of an individual may play a significant role in increasing vulnerability to diseases. Research to date suggests that meditation may play a vital role in resetting the imbalance between psycho-physical health by modulating the psychoneuroimmunological effects of stress. However, to date, this multi-faceted psychoneuroimmune aspects of meditation has not been completely elucidated.

Methodology: On this concept, evidence-based mechanism has been framed for the first time from India to explain the psychoneuroimmunological aspects associated with regular and long-term meditation practice using an innovative 18FDG-PET methodological approach.

Conclusion: The innovative methodology provides the backbone to frame the psychoneuroimmunological mechanism of meditation. Therefore, the present mechanism confirms prefrontal cortex (PFC) acts as a 'Connector Hub Region' where all the components of meditation that include attention control, emotional regulation, and altered self-awareness function

simultaneously to exert the positive benefits in the regulation of cognitive and emotional behavior. Also, this mechanism will help us to understand how a particular pathway fosters brain plasticity to overcome various neuropsychiatric illnesses.

Acknowledgements :- NA

PC022

Chronic intermittent hypoxia *in utero* depresses basal carotid body activity and hypoxic sensitivity in adult offspring

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Introduction:

Chronic intermittent hypoxia (CIH) is a common feature of obstructive sleep apnoea (OSA) and is prevalent in pregnancy, thus exposing the fetus to CIH [1]. In adults exposed to CIH, neurogenic hypertension develops as a consequence of carotid body (CB) hyperactivity. Evidence in animal models suggests that CIH *in utero* (CIHU) may cause subsequent hypertension in the adult [2]. Any role of the CBs in promoting hypertension development in adult offspring following CIHU programming is undefined.

Aim:

We aimed to identify whether CIHU alters basal CB activity and the hypoxic sensitivity in adult offspring, and if this is associated with alterations in cardiovascular and respiratory function.

Methods:

All animal procedures were approved by the Home Office (UK) (PPL number PF4C074AD) and University of Birmingham. 12 female Wistar rats (Charles River, UK) arrived on day 6 of pregnancy and were housed in individually ventilated cages with free access to food and water (n= 1 animal per

cage). On day 10 of pregnancy, animals were randomly assigned to 3 different groups: normoxia (N; n= 4), mild maternal CIH (8 cycles/hour, 8 hours per day; CIHU 8; n=4), and severe maternal CIH (15 cycles/hour, 8 hours per day, CIHU 15; n=4). Following birth, male offspring were matured to adults (10-20W) without further exposure to hypoxia. The *in vitro* CB chemoafferent activity was assessed on freshly isolated CBs, in normoxic conditions (superfusate PO₂ ca 300mmHg) and during hypoxia (PO₂ca 100mmHg). Respiratory measurements were collected on awake animals using whole body plethysmography and cardiovascular reflex responses to hypoxia were performed under terminal anaesthesia (alfaxan i.v 17 – 20 mg kg⁻¹ h⁻¹), as described [3].

Results:

Basal chemoafferent activity was significantly decreased in adult offspring exposed to severe but not mild CIHU (N 1.±0.2 vs. CIHU 15 0.2±0.04 Hz, P<0.05, Fig.1a). Similarly, the CB response to hypoxia was depressed in the animals exposed to severe CIHU, when measured at fixed, defined superfusate PO_{2s}. E.g. At a superfusate PO₂ of 100mmHg: N 4±1 vs. CIHU 15 0.5±0.15 Hz, P<0.0005 (Fig. 1c). Absolute peak chemoafferent responses were consistent between groups but occurred at lower PO_{2s} in the CIHU animals, consistent with a reduced CB O₂sensitivity. Interestingly, both mild and severe CIHU did not alter the mean arterial blood pressure in normoxia or hypoxia. Furthermore, baseline ventilation and the hypoxic ventilatory response were unaltered by CIHU.

Conclusion:

These results suggest that prenatal CIH leads to attenuation of CB function in the adult without completely abolishing it. However, this change did not alter the basal cardiovascular and respiratory measurements or attenuate reflex responses to hypoxia. The inconsistency of the current findings with the evidence that showed CIH in utero causes hypertension may be due to the lower CIH frequency and the duration used in this study compared to other studies. Further adaptations in the chemoreflex may also act to counter this reduction in the CB activity, preventing systemic cardiorespiratory dysfunction- future experiments are needed to explore this further.

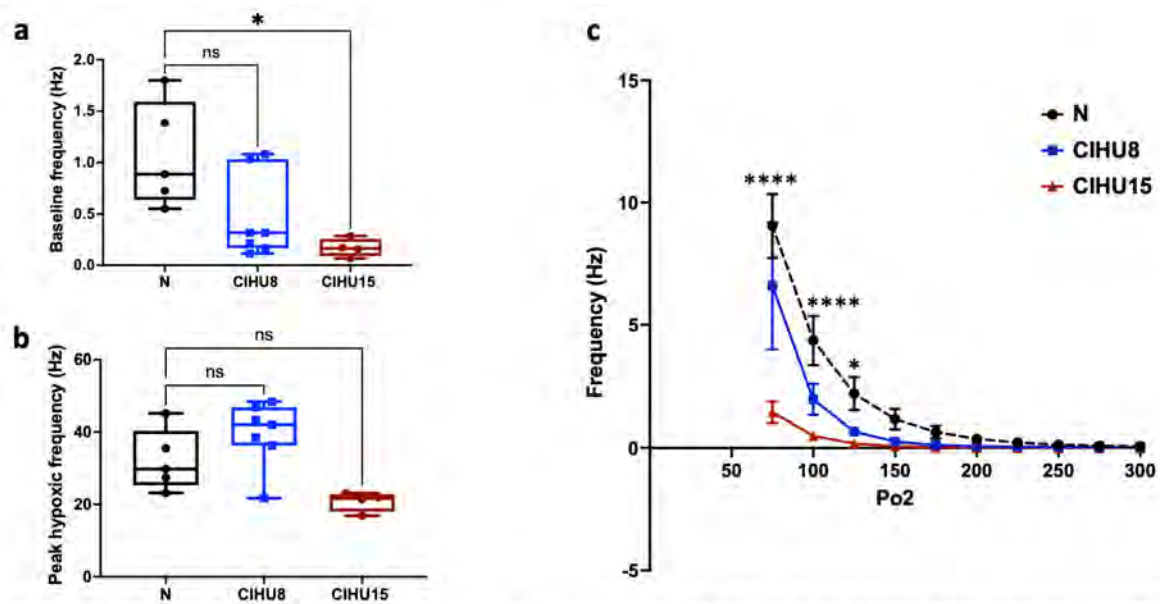


Figure 1. Chronic intermittent hypoxia in utero depresses basal carotid body activity and hypoxic sensitivity. **a** Mean baseline single fibre CB frequency for N (n= 5), CIHU 8 (n= 7), and CIHU 15 (n= 4). **b** Peak hypoxic frequency evoked during hypoxia. **c** Mean hypoxic response curves. **** denotes $p < 0.05$; $p < 0.0001$ N vs CIHU 15; one-way ANOVA followed by Bonferroni multiple comparisons test.

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Acknowledgements :- This work was supported by a scholarship from Umm A-Qura University (Makkah, Saudi Arabia) Provided to Abdulaziz Alzahrani through the Saudi Arabian Cultural Bureau in London.

PC023

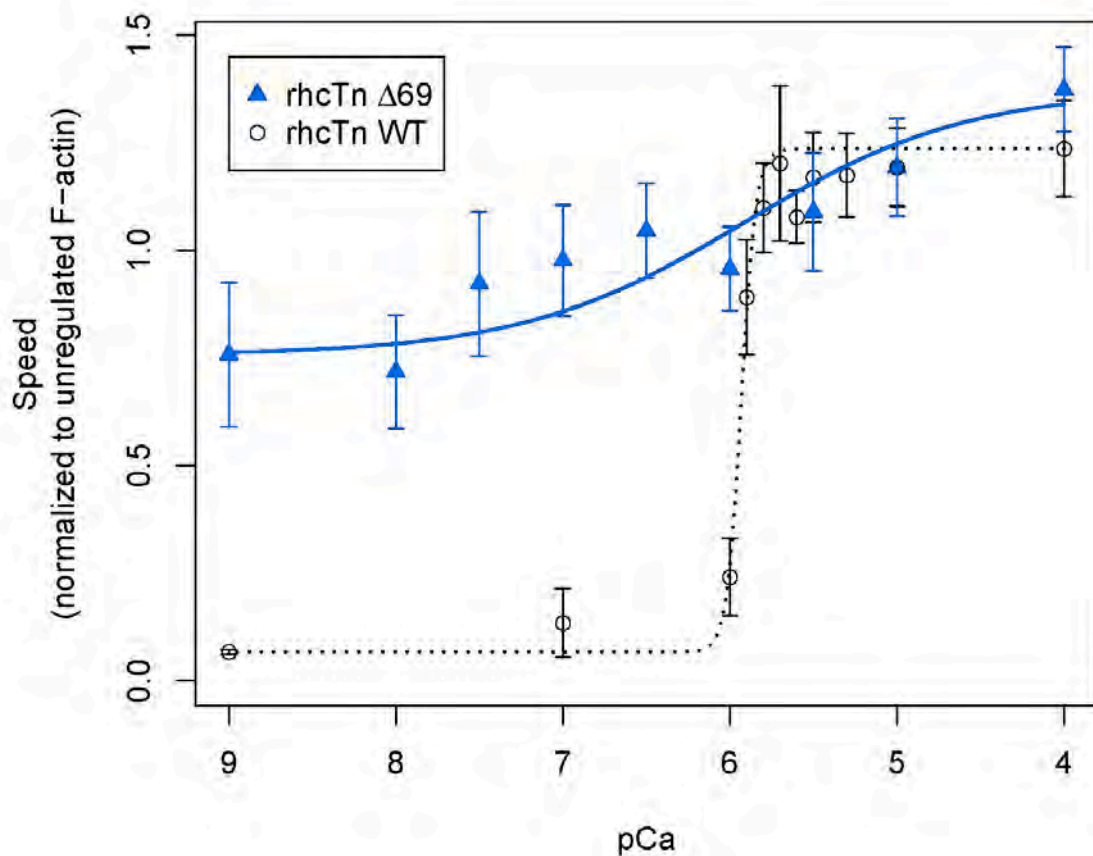
Cardiac troponin T amino terminus is required for inhibition of Ca^{2+} -dependent sliding of thin filaments

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Final steps of excitation-contraction coupling in vertebrate striated muscles involve Ca^{2+} -binding to troponin (Tn), which alters thin filament structure to allow actomyosin cross-bridge formation and sarcomere contraction in response to myocyte depolarization that transiently elevates cytoplasmic Ca^{2+} . Published cryo-EM structures of the cardiac thin filament regulatory unit provide valuable information about Ca^{2+} -induced structural changes in Tn and associated movement of tropomyosin (Tm) on actin, but not all portions of troponin are resolved (Yamada *et al.*, 2020; Risi *et al.*, 2021). We examined the functional role(s) of one region that is not fully resolved, the N-terminal “tail” of the cardiac troponin T (TnT) subunit, where T refers to Tm-binding. The tail of skeletal muscle TnT (sTnT) on its own inhibits actomyosin (Tobacman *et al.*, 2002). Cardiac thin filament regulatory unit cryo-EM structures show, in diastolic conditions, the cTnT tail extends in one direction from the cTn core domain along actin-Tm, while the C-terminus of the troponin I (TnI) subunit (consisting of the inhibitory peptide, switch region and mobile domain) extends in the opposite direction (Yamada *et al.*, 2020; Risi *et al.*, 2021). Those cryo-EM structures are consistent with a model in which the cTn core binds one Tm strand while the cTnT “tail” domain reaches across the thin filament to the other Tm strand. We therefore hypothesized that the N-terminal tail of cTnT is required for full inhibition of thin filament sliding by myosin in diastolic (low Ca^{2+}) conditions, and that apparent cooperativity of Ca^{2+} -activation would be altered when the cTnT tail is missing.

To examine the role of cTnT’s N-terminal tail, we co-expressed all three subunits of human cardiac Tn in *E. coli* and purified the assembled Tn complex as described (Schoffstall *et al.*, 2011). We demonstrated through RP-HPLC purification and N-terminal peptide sequencing that the N-terminal 69 amino acids of cTnT can be removed by thrombin proteolysis at a single consensus cleavage site without affecting the other subunits. The resulting product (rhcTn Δ 69) was compared in functional assays with intact cTn (rhcTn WT). Interestingly, rhcTn Δ 69, unlike rhcTn WT (Schoffstall *et al.*, 2011), does not enhance myosin (HMM) ATPase in solution in the absence of actin and Tm. Co-sedimentation demonstrates that both rhcTn Δ 69 and rhcTn WT bind F-actin-Tm. In vitro motility assays of thin filament sliding were conducted as described (Schoffstall *et al.*, 2011; Meyer & Chase, 2016). Thin filaments containing cTn missing the first 69 amino acids of cTnT only partially inhibited at diastolic Ca^{2+} , unlike thin filaments containing rhcTn WT (left side of figure). The slope of the speed-pCa relationship was less steep (Hill coefficient < 1) for rhcTn Δ 69 compared to rhcTn WT. In contrast to solution ATPase assays, maximum sliding speed at high Ca^{2+} levels was not reduced with rhcTn Δ 69 (right side of figure). We conclude that both extensions from the core domain of cTn—the N-terminal tail of cTnT (this communication) and the C-terminus of cTnI (Meyer & Chase, 2016)—are required for full inhibition of actomyosin interactions at diastolic Ca^{2+} and for normal cooperative activation of actomyosin interactions.



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Regular walking significantly improves foot perfusion independently of age

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Introduction

Ageing is regarded as a natural process capable of triggering or accentuating the progression of many pathologies. This is true of peripheral vascular disease and microcirculatory impairment specially in the lower limb, associated or not with other ongoing diseases (metabolic syndrome, diabetes). Dynamic movement, rather than exercise, is likely one of the most useful, broadly applicable activities to prevent or delay the negative impact of ageing on lower limb microcirculation.

Aims / Objective

To understand the effect of the common dynamic movement of casual walking on foot perfusion in subjects with different ages.

Methodology

This work involved 49 healthy volunteers, both sexes, healthy with confirmed no signs of peripheral vascular disease (ankle-brachial index = 1.07 ± 0.07). Participant data was grouped into Young (< 30 years) and Older Adult (> 30 years old), and BMI categories (Normal weight BMI <25; Excess weight BMI >25). All applied procedures respected the principles of good clinical practice for human studies research. Perfusion was measured by laser Doppler flowmetry (LDF) in both feet 5 minutes before and 5 minutes after a casual walking period in the lab through a predetermined track. After walking, perfusion was measured for another 5 minutes in the recovery period. Age, blood pressure (systolic and diastolic) and Body Mass Index (BMI) were measured. Statistical analysis was performed with a SPSS version 22.0. Parametric or non-parametric tests were performed to assess differences between variables. All tests adopted a 5% significance level.

Results and Discussion

Within each group, participants were of similar age (Mann-Whitney; $p=0.421$) but men had a higher BMI (t-test, $p<0.0001$). Higher perfusions at baseline were also found in men (t-test; $p=0.026$) but after walking, those differences were not significant. Younger individuals had lower perfusions than older, both at baseline and post-walking, but the differences were non-significant (Mann-Whitney $p=0.371$ and $p=0.121$). Perfusion difference at baseline and post-walking per BMI groups were also non-significant (Mann-Whitney $p=0.133$ and $p=0.277$). Overall, 38,8% of individuals showed an increase in perfusion after walking. No single categorical variable was associated with the increase in

perfusion, although individuals with BMI >25 had a tendency (adjusted for age and sex) to show an increased perfusion post-walking, which might signify increased microcirculatory stress.

Conclusions

Our results suggest that gait consistently increases foot perfusion, though the impact of walking was not the same for all subjects. Intraindividual resting differences between feet were only significant in men and were reduced, in some cases to the point of disappearance, with gait. Although increased perfusion was independent of sex, variations in the recovery period apparently related to BMI (> 25) were noted.

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PC025

Angiotensin II mediated pericyte contractility in the spinal cord drives pain development and can be alleviated through local administration of Losartan

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Vascular degeneration is a key factor in the development of many neurological diseases. Accumulating evidence implies altered blood brain barrier (BBB) integrity affecting cerebral perfusion in patients of Alzheimer's, Huntington's and Amyotrophic lateral sclerosis disease. The increase acknowledgment of the vascular component within the central nervous system (CNS) highlights the importance when considering disease onset and progression. More recently, models of disease within the CNS spinal cord (SC) microvasculature have suggested reduced blood perfusion greatly influencing pain perception. Pericytes, part of the BBB abluminally positioned on small capillaries, demonstrate contractile abilities within cerebral tissue to modulate blood perfusion of nervous tissues. Our preliminary work supports angiotensin II type 1 (AT1) receptor positive pericytes in the SC as a fundamental modulatory component of vasoconstriction. Therefore, this study aims to determine whether Angiotensin II (ANGII), via pericyte AT1 receptor, induces reduced SC blood perfusion through pericyte constriction and whether this impacts upon pain behavioural phenotypes. *In vivo* studies implied that intrathecal (i.t.) ANGII (100nM, N=6) injected mice develop pain following mechanical and thermal behavioural withdrawal response tests (pre+post i.t.) upto 5hours post-dose compared to vehicle treated group (PBS, N=6) (*P<0.05). To support pericyte activation within the dorsal horn, fluorescently stained paraformaldehyde-fixed SC indicated an increased proportion of constricted CD31-vessels associated with NG2-pericytes 30-minute post i.t. ANGII injection versus vehicle (*P<0.39). Systemic Losartan (AT1 antagonist; 20mg/kg intraperitoneal

injection) inhibited ANGII-induced nociceptive behavioural hypersensitivity compared to ANGII (*P<0.0037) 5 hours post-dose, while returning to basal level after 24 hours, however no change was recorded from mechanical behavioural withdrawal response. After repeating this study with local administration of Losartan (10µM i.t.), mechanical pain alleviation occurred 1 hour (N=3; *P<0.022) and 5 hours (N=3; **P<0.009) post-dose versus ANGII i.t. dose alone. These studies highlight an ANGII dependent modulation of pericyte vasocontractility in the SC and pain perception. Furthermore, ANGII induced nociceptive behavioural hypersensitivity can be mechanically and thermally alleviated through local administration of Losartan.

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PC026

Does a reduction in Na_v1.5 protein expression within the right atria increase an elderly patient's susceptibility to atrial fibrillation?

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Background: Na_v1.5 is the predominantly expressed voltage-gated sodium channel isoform in the heart, responsible for the upstroke of the action potential. Changes in sodium currents may lead to an increased frequency of arrhythmogenic episodes.¹

Aim: To investigate the protein expression of Na_v1.5 in the right atrial appendage of tissue from patients in sinus rhythm compared with those diagnosed with atrial fibrillation (AF).

Methods: Tissue from the right atrial appendage was obtained from patients undergoing routine cardiac surgery; coronary artery bypass or valve repair (NHS-REC approval 197493). The two patient groups of 'sinus rhythm' and 'AF' were matched on age, operation-type, and their co-morbidities. Expression of Na_v1.5 protein was quantified by specific protein-directed primary antibody (Alomone, Israel) coupled with a secondary antibody conjugated to HRP for western blot (Abcam, UK), or Alexa Fluor 488 (ThermoFisher, UK) for immunohistochemistry with confocal microscopy (LSM 710 Zeiss, UK).² Densities were quantified using Image J software.

Results: Arranged in chronological age order (43 - 87 years of age) we demonstrated a negative correlation between increasing age and Na_v1.5 protein expression (n = 26, Pearson correlation coefficient of -0.25): The gradient demonstrates a decline of 9 % Na_v1.5 protein per decade of ageing. Comparison of 'matched patients' (age range 63 - 80 years; mean 72 years per group) revealed that those diagnosed with AF expressed 50% less Na_v1.5 protein when compared with patients in normal sinus rhythm (n=7, t-test P<0.001). This novel finding using the technique of western blot was reflected by immunocytochemistry, as elderly patient tissue showed a lower density of labelled Na_v1.5 protein with a florescent tag than their younger counterparts where label was strongly visualised at the t-tubules and intercalated disks.

Conclusion: With increasing chronological age of the patient from which the right atrial tissue was derived we determined reduced expression of Na_v1.5 protein, and furthermore Na_v1.5 protein expression was reduced in AF patient tissue when compared with age-matched tissue from patients in sinus rhythm: Thus, demonstrating a reduction in Na_v1.5 protein expression contributes to susceptibility of arrhythmias in the elderly heart.

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Reference 2 :- Jones SA, Lancaster MK, Boyett MR. Ageing-related changes of connexins and conduction within the sinoatrial node. *J Physiol*. 2004;560:429-37.

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PC027

Reliable identification of cardiac liability in drug discovery using automated patch clamp:
Considerations and best practices for high throughput recordings of Na_v1.5

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For reliable identification of cardiac safety risk, compounds should be screened for effects on cardiac ion channels in addition to hERG, including Na_v1.5 and Ca_v1.2. Automated patch clamp (APC) instruments are increasingly adopted for cardiac safety measurements but cross-site and cross-platform comparisons of IC₅₀ values has identified the need for standardized protocols for reliable pharmacology. In this study, we identified different parameters that might affect IC₅₀s of compounds

on $\text{Na}_v1.5$ peak and late currents recorded using APC. For example, we found that using frozen cells or running cultures did not influence IC_{50} values of compounds tested on $\text{Na}_v1.5$ peak, but that voltage protocol, holding potential, temperature and incubation time could all influence IC_{50} s. Temperature affected the V_{half} of inactivation, shifting the V_{half} of inactivation by approximately 7 mV to more negative potentials at 37°C vs room temperature, and also affected the IC_{50} of mexiletine which was $82.1 \pm 19.4 \mu\text{M}$ ($n = 14$) at 37°C compared with $181.5 \pm 29.0 \mu\text{M}$ ($n = 10$) at room temperature. The IC_{50} s of compounds were compared on peak current elicited using different voltage protocols and using different holding potentials. For example, ranolazine and tamoxifen, two blockers known to block $\text{Na}_v1.5$ in the open state showed more potent IC_{50} s at a holding potential of -80 mV compared with -95 mV (ranolazine $\text{IC}_{50} = 145.64 \mu\text{M}$ at holding potential -95 mV and $99.75 \mu\text{M}$ at holding potential -80 mV; tamoxifen $\text{IC}_{50} = 21.43 \mu\text{M}$ at holding potential -95 mV and $2.90 \mu\text{M}$ at holding potential -80 mV). Compound incubation time also influenced IC_{50} s and whereas compounds such as lidocaine reached steady state within 3 mins, some compounds, e.g. flecainide and bepridil required 5 mins or more to reach steady state.

We suggest outlines for best practices for recording $\text{Na}_v1.5$ peak and late current using APC which include the use of the CiPA step-ramp protocol at physiological temperature, a minimum compound incubation time of 5 min, a replicate number of 4 and the use of positive and negative controls to ensure reliable IC_{50} s.

PC028

Carbon Nanotube-Based Scaffolds for Cardiac Tissue Engineering – Systematic Review and Narrative Synthesis

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Introduction: Cardiovascular disease is currently the top global cause of death, yet research into new therapies is in decline. Tissue engineering is a solution to this crisis enabling the development of biomaterials which facilitate more dynamic and complex *in vitro* models. A material that has drawn attention are carbon nanotubes (CNTs). CNTs' electrical conductivity and dimensional similarity to cardiac extracellular proteins provides a unique opportunity to deliver scaffolds with stimuli that mimic the native cardiac microenvironment *in vitro* more effectively. This systematic review aims to evaluate the use and efficacy of CNTs for cardiac tissue scaffolds and was conducted according to the PRISMA guidelines. **Methods:** Three databases were searched: PubMed, Scopus and Web of Science. Papers resulting from these searches were then subjected to analysis against pre-determined exclusion, inclusion and quality appraisal criteria. From 249 results, 27 manuscripts met the criteria and were included in this review. **Results:** Neonatal rat cardiomyocytes were most commonly used in experiments (74%), with multi-walled CNTs being most common in tissue scaffolds (74%). Immunofluorescence was the experimental technique most frequently used (78%), employed for staining of cardiac-specific proteins relating to contractile and electrophysiological function. Few

[papers considered using electrophysiological techniques \(22%\), such as whole cell patch clamping, indicating a gap in the research landscape for the development of CNT-based scaffolds for cardiac tissue engineering. **Conclusions:** CNTs displayed versatility as a biomaterial for cardiac tissue engineering through applications in a diverse range of scaffold designs and formulations. Limited toxic effects were observed for all papers retrieved in this review and the revolutionary effects brought about solely by the CNTs warrant continued effort in seeking their optimal use. CNT scaffolds could most successfully be applied in reliable models for cardiac pathologies and testing novel pharmaceuticals – thus reducing dependency on animal models and clinical trials, but also reinvigorating research in this struggling but enormously important field.](#)

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PC029

Noradrenaline stimulates human atrial L-type Ca^{2+} current by activating β_1 -adrenoceptors, and to a smaller degree α_1 , with a mixed and minor contribution from β_2 and α_2 .

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Introduction: Atrial fibrillation resulting from elevated adrenergic activity may involve an increase in atrial L-type Ca^{2+} current (I_{CaL}) by noradrenaline (NA). However, the contribution of the individual adrenoceptor (AR) sub-types to such I_{CaL} -increase is poorly understood, particularly in human atrium. **Aim:** To investigate effects, on NA-stimulated I_{CaL} , of various broad-action and sub-type-specific α - and β -AR antagonists, alone or in combination, in human atrial myocytes. **Methods:** I_{CaL} was recorded by whole-cell-patch clamp at 35-37°C in myocytes isolated enzymatically from right atrial tissue samples obtained from consenting patients undergoing elective cardiac surgery. **Results: 1)** Noradrenaline substantially increased peak (at 0 mV) I_{CaL} , in a concentration-dependent manner, to a maximally effective value (E_{max}) of 7.29 pA/pF (from E_{min} 2.82 pA/pF); and with a half maximally-effective concentration (EC_{50}) of 156 nM; EC_{75} 310 nM ($n=8-31$ cells, 4-8 patients). **2)** Broad-action β - and α -AR antagonism, of I_{CaL} -responses to NA at EC_{75} , were studied using propranolol ($\beta_1+\beta_2$ -AR antagonist; 0.2 μM) and phentolamine ($\alpha_1+\alpha_2$ -AR antagonist; 1 μM). In a group of 9 cells (3 patients), in which NA increased I_{CaL} by $188\pm 22\%$ (mean $\pm$ SE; $P<0.001$ vs control, 1-way-ANOVA), propranolol substantially decreased I_{CaL} , by $60\pm 4\%$ ($P<0.001$ vs NA). Subsequently applied phentolamine decreased the remaining (i.e. α -stimulated) I_{CaL} , by $37\pm 4\%$ ($P<0.001$ vs NA+propranolol). The degree of I_{CaL} -reduction from phentolamine was significantly smaller ($P=0.007$) than that from propranolol. **3)** β -AR-subtype-specific antagonism of I_{CaL} -responses to NA (again at EC_{75}) was investigated with CGP20712A (β_1 -antagonist; 0.3 μM : CGP) and ICI118551 (β_2 -antagonist; 0.1 μM : ICI). In each of 6 cells (2 patients) studied, CGP substantially decreased NA-stimulated I_{CaL} , on average by $71\pm 4\%$ ($P=0.032$ vs NA). By contrast, ICI, in the continued presence of NA+CGP (studied in 5 of these cells), produced a mixed I_{CaL} -response: a marked and reversible (upon washing out ICI) decrease in 2 cells (by 72 and 78%), and a reversible increase in the other 3 cells (by 12, 35 & 37%); with no significant effect on average ($P=0.949$ vs NA+CGP). **4)** α -AR-subtype-specific antagonism of NA-stimulated I_{CaL} was studied with prazosin (α_1 -antagonist; 0.5 μM) and yohimbine (α_2 -antagonist; 10 μM). In each of 6 cells (3

patients) studied, prazosin decreased NA (EC₇₅)-stimulated I_{CaL}, on average by 37±8% ($P=0.017$ vs NA). By contrast, yohimbine (still in the presence of NA+prazosin), produced a mixed I_{CaL}-response: a moderate decrease in 4 cells (of 19, 36, 43 and 49%; reversible in 3 of these), a marked and reversible increase (by 78%) in one cell, and no effect in the other cell. There was no significant effect on average ($P=0.940$ vs NA+prazosin). The degree of reduction in NA-stimulated I_{CaL} by prazosin was significantly smaller ($P=0.002$, un-paired t-test) than that by CGP. **Conclusion:** Stimulation of I_{CaL} by NA in human atrial myocytes is mediated, based on adrenoceptor sub-type-antagonist responses, predominantly by β_1 -AR activation, with a smaller (though still substantial) contribution from α_1 -activation, and either a contributing or attenuating (and, on average, negligible) action of β_2 - or α_2 -activation.

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PC030

Assessment of hair cortisol in euthyroid, hypothyroid, and subclinical hypothyroid subjects

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Introduction: Hypothyroidism is associated with an increase in serum cortisol level while the long-term activity of hypothalamic-pituitary-adrenal (HPA) axis in hypothyroid, and subclinical hypothyroid (SCH) subjects has not been studied.

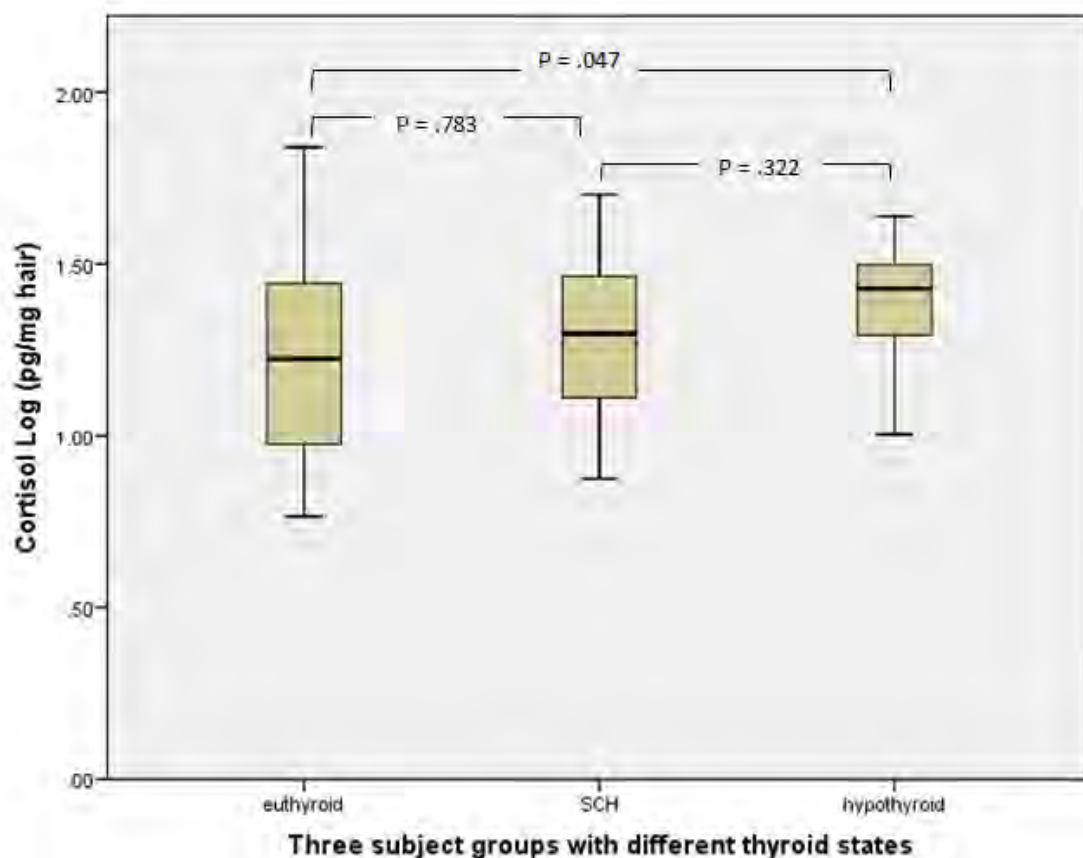
Objectives: This study aimed to assess the hair cortisol levels as a long-term activity of HPA axis in hypothyroid, SCH, and a group of healthy adult subjects. Also, it aimed to examine the correlation of hair cortisol levels with hypothalamic-pituitary-thyroid (HPT) axis and anthropometric measures.

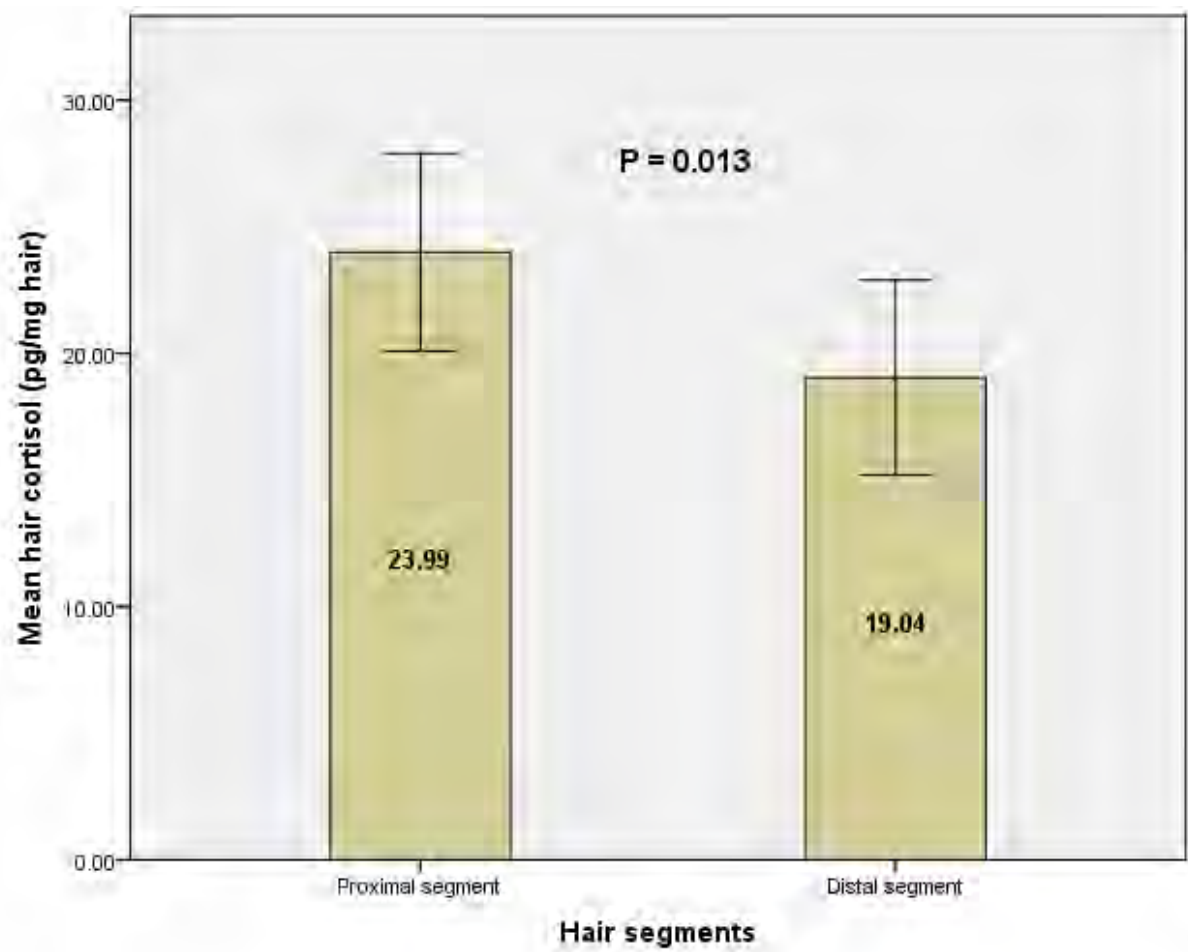
Methods: We prospectively evaluated a group of normal (n=65), SCH (n=26), and hypothyroid subjects (n=27). Serum TSH, FT4, and FT3 were measured as a component of the HPT axis. Hair samples were collected, prepared, followed by extraction of hair cortisol and measurement in pg/mg of hair. Hair cortisol levels were compared in normal, SCH, and hypothyroid groups and correlated with HPT axis and anthropometric data. The study was approved by the ethics committee of the College of Medicine-University of Sulaimani under meeting number 62, and all procedures in the study have been performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Informed consent Written informed consent was obtained from all individual participants

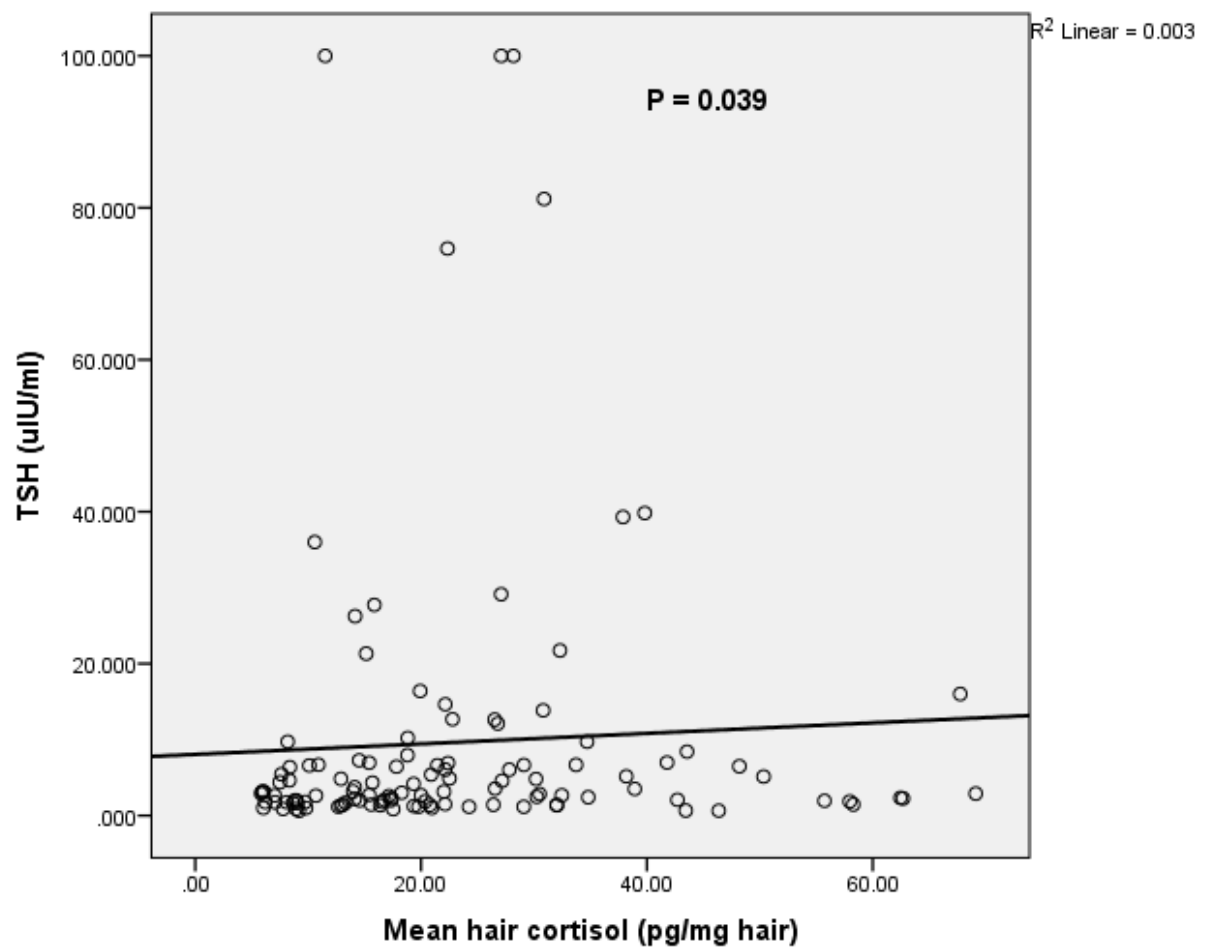
included in the study. The hair cortisol log transformed to normalize the distribution. After log transformation, the skewness was corrected, mean of hair cortisol log transformed (hair cortisol log) was used for the comparison and correlations. The Kruskal–Wallis H test and Mann–Whitney U test was used to compare the median hair cortisol and TSH levels between the groups. The mean of each data was compared between euthyroid, SCH, and hypothyroid subjects using ANCOVA, body weight, BMI, and waist circumference used as a covariate. Mean hair cortisol levels were compared between subjects with different BMI, using independent sample t-test, and mean of hair cortisol level between proximal and distal segment were compared using Paired samples T test. Correlation between hair cortisol levels with other variables was evaluated using Pearson's correlation with p-value of ≤ 0.05 regarded as significant.

Results: A total of 118 subjects were analyzed, and the mean hair cortisol level in healthy volunteers was reported to be 17.38 pg/mg of hair. Hair cortisol level was slightly higher in the SCH subjects, 18.19 pg/mg of hair; however, the difference was not significant. Compared to the euthyroid subject, a significantly higher hair cortisol level was recorded in the hypothyroid subjects, 24.17 pg/mg hair, $p < .05$. Hair cortisol was significantly and positively associated with each of the serum TSH, age, weight, and BMI ($p < .05$).

Conclusions: Overt hypothyroidism but not SCH is significantly associated with higher hair cortisol levels compared to normal subjects, and significant relation between hair cortisol with HPT axis was found. Also, weight and BMI were positively correlated with hair cortisol level.







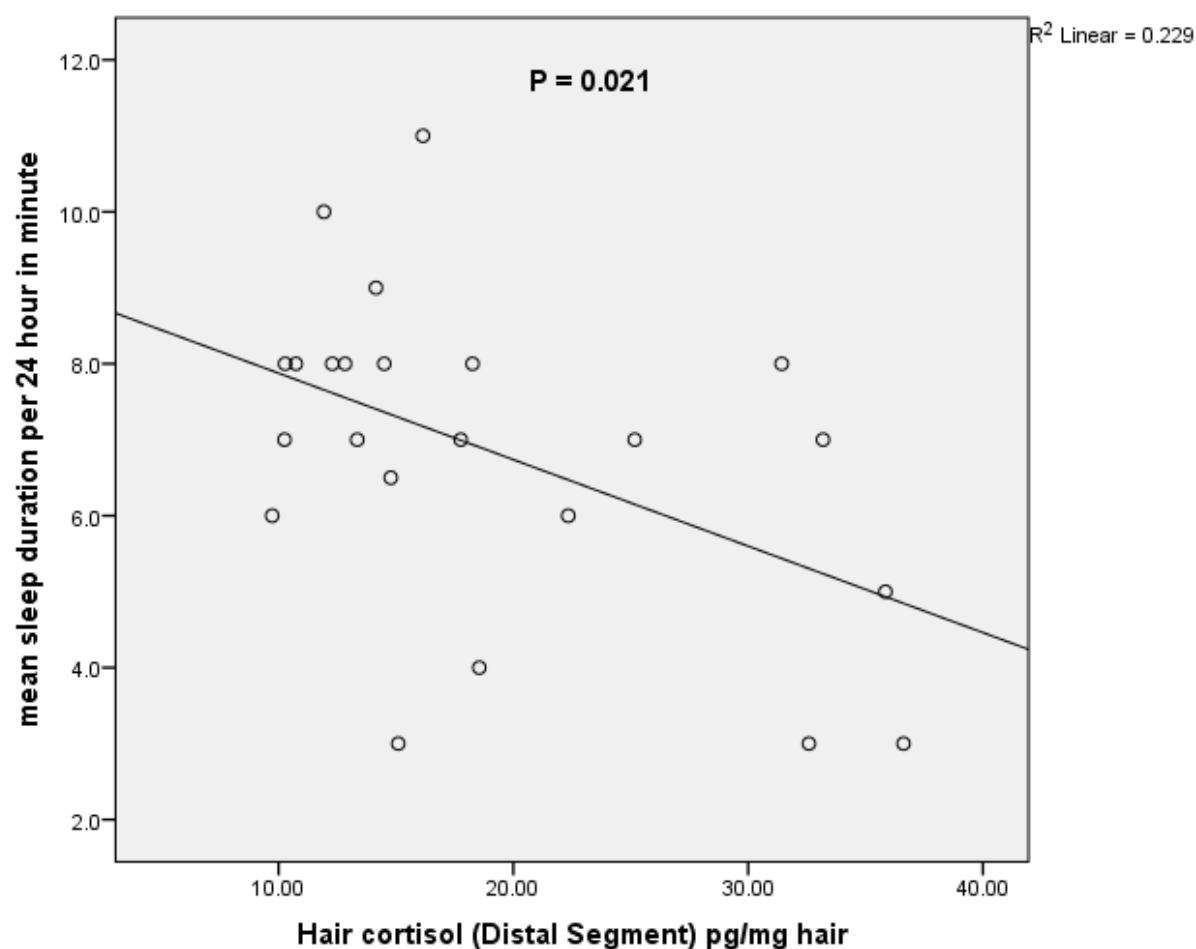


Table 3 Correlations between hair cortisol and other variables among main study sample

Parameters	Age	Weight	BMI	Waist circumference	TSH(uIU/ml) ^a	FT4(ng/dl)	FT3	TPO titer
Hair cortisol								
Pearson correlation	.215*	.203*	.206*	.106	.192*	-.129	-.182	.092
Sig. (2-tailed)	.021	.031	.029	.322	.039	.171	.171	.357

Pearson correlation was used for correlation between hair cortisol log and all normally distributed variables

^aSpearman's correlation is used for correlation between original hair cortisol and non-parametric variable, TSH

*p value < 0.05

Table 2 Comparison between euthyroid, SCH and hypothyroid subjects in demographic and biochemical data

Variables	Euthyroid (n = 65)	<i>p</i> ^a	SCH (n = 26)	<i>p</i> ^b	Hypothyroid (n = 27)	<i>p</i> ^c
Age (years)	33.12(10.38)	.329	36.85(13.44)	.948	37.97(10.91)	.134
Weight (Kg)	64.51(13.94)	.073	70.47(9.33)	.928	72.49(11.71)	.027
BMI (Kg/m ²)	26.36(5.74)	.198	28.25(4.36)	.888	29.32(4.53)	.062
Waist circumference (cm)	82.24(12.62)	.048	89.9(15.24)	.917	92.82(11.03)	.009
TSH (uIU/ml) ^d	1.95 [.82]	<0.001	6.41 [.65]	<0.001	16.4 [98.64]	<0.001
FT4 (ng/dl)	1.25(.169)	.219	1.17(.15)	<0.001	0.829(.34)	<0.001
FT3 pg/ml	3.29(.42)	.210	3.49(.46)	<0.001	2.56(.73)	<0.001
TPO titer (IU/ml)	23.59(73.86)	.124	74.0(137.22)	<0.001	228.27(192.26)	<0.001
Cortisol (pg/mg hair)						
Median ^d	16.74 [63.3]	.417	19.86 [42.84]	.115	26.79 [57.65]	.010
Cortisol Log	1.236(.299)	.783	1.277(.249)	.322	1.383(.193)	.047
Mean (antilog)	17.22		18.92		24.17	
Range	5.75-69.18		7.41-50.12		10-67.61	

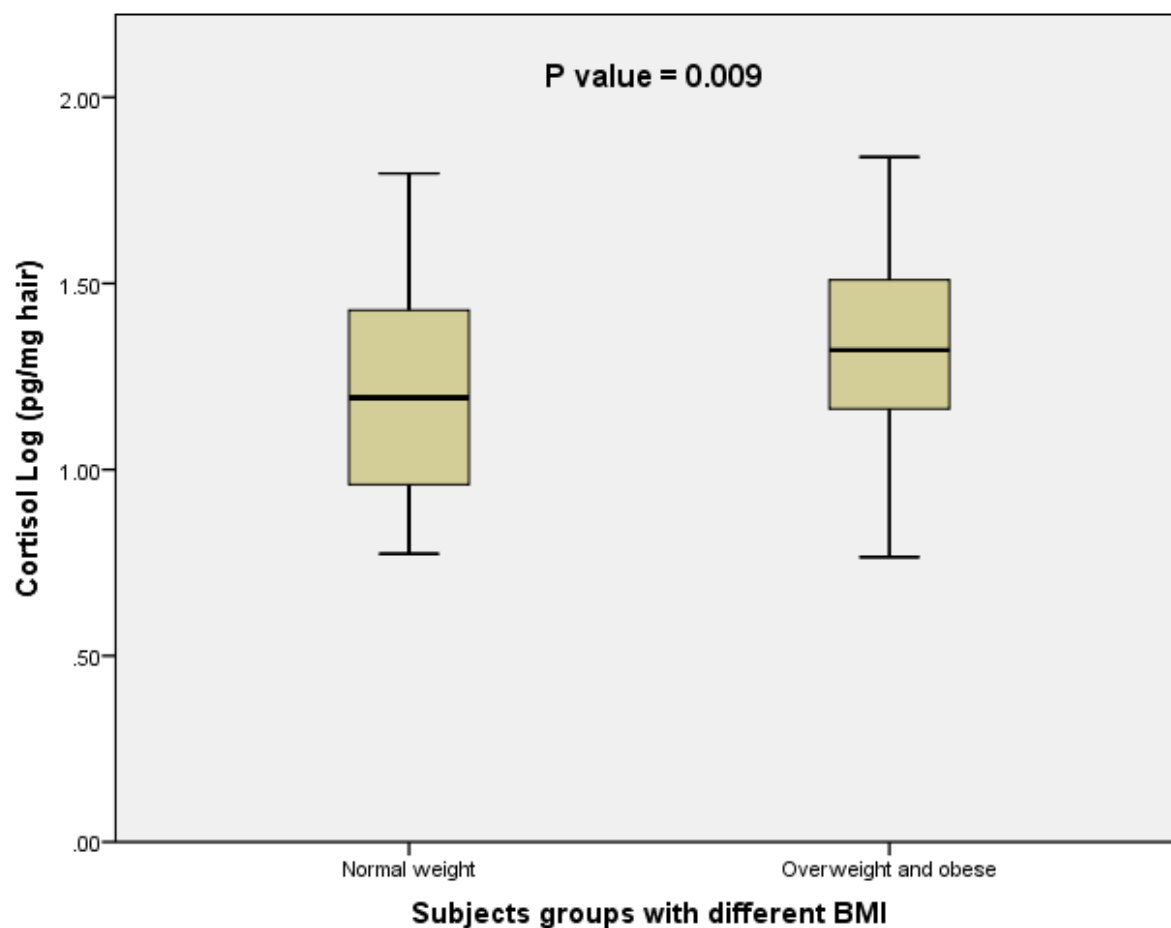
All variance were compared between the groups using ANCOVA. *p*-value < .05 was regarded as significant (Bold)

^a*p* value between euthyroid and SCH groups

^b*p* value between SCH and hypothyroid groups

^c*p* value between hypothyroid and euthyroid groups

^dMedian of non-parametric data between the groups were compared using Kruskal–Wallis H test and Mann–Whitney U test



PC031

Modulation of L-type voltage-gated calcium channels interferes with intracellular calcium mobilisation in mouse white fat adipocytes

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Dihydropyridines such as BAY K8644 and the benzoylpyrrole derivative FPL 64176 are pharmacological agents well known for their agonist action to activate L-type VGCCs. Studies exploring the modulation of GPCR mobilization of intracellular calcium often involve the use of agonists and antagonists of L-type voltage-gated calcium channels (L-type VGCCs) to distinguish between increases in intracellular calcium due to extracellular influx from those associated with intracellular calcium release due to activation of inositol triphosphate receptors (IP3R) on intracellular calcium stores. However, direct interference on calcium mobilisation due to L-type calcium channels drugs is an unreported observation; one which could confound interpretation of calcium measurement data.

White fat adipocytes (WFA) were isolated from epididymal fat of CD-1 mice by standard methods. Intracellular calcium ($[Ca^{2+}]_i$) measurements were made by epifluorescent microscopy at 28°C.

We found that the L-type VGCC agonists 10 μ M Bay K 8644 (n=15) and 1 μ M FPL 64176 (n=20) abolished the mobilisation of intracellular calcium in WFA evoked by 100 nM oxytocin ($p < 0.05$, Kruskal Wallis). In contrast, 20 nM growth hormone which biochemically upregulates L-type VGCCs in these cells was without effect on the oxytocin response. The inhibition of intracellular calcium mobilisation by BAY K8644 and FPL 64174 was neither time-dependent, nor vehicle-mediated; as oxytocin was able to elicit a characteristic transient increase in intracellular calcium after a prolonged period of 25 minutes (n=20) in the absence of both agents. In addition, dimethyl sulfoxide (n=18), the vehicle for both L-type VGCC agonists had no inhibitory effect on oxytocin action.

Our data suggest that the actual drug themselves: BAY K8644 and FPL 64174 directly interfere with calcium mobilisation by oxytocin, and not by an increase in L-type calcium channel activity per se. Whether this effect is unique to WFA or/and oxytocin remains to be investigated.

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PC032

Vitamin D receptor activation exerts anti-secretory actions in colonic epithelial cells

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Background: The vitamin D receptor (VDR) is a nuclear receptor that is expressed in many tissues throughout the body, but is particularly abundant within the colon. VDR is activated by the active form of vitamin D, calcitriol, but is also known as a receptor for the secondary bile acid, lithocholic acid. VDR has previously been shown to be anti-inflammatory and barrier promoting in the colon. However, the role VDR plays in regulating colonic epithelial transport function is less known. Interestingly, previous studies from our laboratory have shown the bile acid receptor, farnesoid X receptor (FXR), to exert anti-secretory actions in colonic epithelial cells. The current study aims to investigate a role for VDR in regulating colonic Cl⁻ secretion and whether it may be a target for the treatment of diarrhoeal diseases.

Aim: To investigate the effects of VDR activation by calcitriol on colonic epithelial Cl⁻ secretion.

Methods: Polarised monolayers of T₈₄ cells were used as a model of the colonic epithelium. Protein expression of VDR was measured by western blotting. Calcitriol (1 - 20 nM) activated VDR and the VDR target protein, CYP24A1, verified activation of the receptor by qRT-PCR. Expression of the FXR target protein, FGF19, and FXR activation measured by luciferase activity verified the specificity of calcitriol to VDR. The effects of VDR activation by calcitriol (1 - 100 nM) on chloride secretion was determined using the Ussing chamber technique. Changes in short-circuit current (I_{sc}) induced by the cAMP-dependent agonist, forskolin (FSK; 10 µM) or the Ca²⁺-dependent agonist, carbachol (CCh; 100 µM) were assessed.

Results: VDR was expressed in T₈₄ cell monolayers as determined by western blotting. Treatment of T₈₄ cells with calcitriol (1 - 20 nM) significantly (n = 3, *p<0.05) increased expression of CYP24A1, indicating activation of VDR. Calcitriol did not alter the activity of FXR, either in an FXR/luciferase reporter cell line or in T₈₄ cells, indicating specificity of the agonist for VDR. LCA (10 µM) induced activation of both VDR and FXR. In Ussing chambers, the FXR agonist, GW4064, inhibited Cl⁻ secretory responses to both FSK and CCh, as previously reported (n = 7, ***p< 0.001). Calcitriol (10 - 100 nM) was also found to significantly inhibit FSK-stimulated Cl⁻ secretory responses by 37.9 ± 4.7 (p< 0.01) and 32.6 ± 7.2 (p< 0.05), respectively (n = 7), while 100 nM calcitriol significantly reduced CCh-induced responses by 31.4 ± 6.5 (n = 7, *p≤ 0.05). Analysis of the time course over which calcitriol exerts its effects revealed its antisecretory actions to be apparent after 24 - 48hrs (n = 5, p≤0.05).

Conclusion: VDR is expressed in colonic epithelial cells where its activation by its natural ligand, calcitriol, inhibits agonist-induced Cl⁻ secretory responses. Future studies will aim to determine a possible role for VDR in mediating anti-secretory actions of luminal bile acids, such as LCA. Since Cl⁻ secretion is the primary driving force for fluid secretion in the intestine, our data suggest that VDR may serve as a target for the development of new anti-diarrhoeal agents.

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Vaginal chemokine delivery system for pelvic floor muscular dysfunction and urinary incontinence prophylaxis and therapy

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Gestational diabetes mellitus (GDM) associates with high risk factor of gestational hyperglycemic myopathy (GHM) leading to pelvic floor muscular dysfunction (PFMD) and urinary incontinence (UI) at two years postpartum (PP) [1,2]. There are new approaches for the treatment of PFMD and UI based on regenerative medicine and mesenchymal stem cell therapy (MSCs) [3]. The MSCs therapy has limitations and challenges such as homing and retention of the injected MSCs. Thus, the sustained release of the chemotactic chemokine like CCL7 provides a great prospect for endogenous stem cell homing and improvement of the response to MSCs therapy in the prophylaxis in women at high risk of developing PP and UI and treatment of PFMD and UI. Our goal was developing an CCL7 sustained-release vaginal delivery device in the preclinical phase (experimental model in female rats), which can be used later translationally in humans. The *in vivo* study was approved by Botucatu Medical School ethics committee (protocol number 1234/2017-CEUA). Aliquots of 2 mL natural rubber latex (NRL) were mixed with a solution containing 0.2 µg/µL CCL7. During polymerization the devices were molded according to the rat vaginal anatomy and dried at 37°C during 48 h. *In vitro* release kinetics were evaluated by mimicking the vaginal environment. Elisa was used to evaluate sustained release of CCL7 over 10 days. Scanning electron microscopy analyzes were performed to analyze the structure of the vaginal devices. The *in vivo* release kinetics were evaluated by insertion of the devices into the animals vagina during 10 days. Virgin and young female rats (45) were used in the experiment in three different groups (n = 5): with device + CCL (CL+), with device without CCL7 (CL-) and control without the device and without CCL7 (CC). The groups were subdivided by 3 collection period (1, 7 and 10 days). The plasma, vagina, urethra, bladder and caudal portion of rectus abdominis muscle (RAM) were collected in these periods after the device insertion. The *In vitro* release kinetics results shows sustained CCL7 release over ~9 days without burst 2,500 ± 100 S.D pg/mL. CCL7 level in the vagina, urethra, bladder and RAM increased exponentially up to 10 days after the use of the device in the group CL+. The CCL7 level observed in the 10 days period using the vaginal device was 193± 83 S.D pg/mL (ANOVA and Tukey's post hoc test) in bladder, 45 ± 10 S.D pg/mL in vagina/urethra, and 16 ± 6 S.D pg/mL RAM. However, CCL7 concentration was lower in the RAM when compared with the bladder (15 ± 2,5 fold p<0.01) and with the vagina/urethra (2.81± 1,5 fold p<0.01). There was no systemic release since no CCL7 was detected in the plasma. In

the group CL- and CC the CCL7 was not detected in the tissues. The results show that the implanted vaginal device allows delivering CCL7 through the vagina with a sustained and continuous release increasing the CCL7 bioavailability in all regions of interest for prophylaxis and treatment.

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Acknowledgements :- São Paulo Research Foundation (FAPESP) Scholarships grant number: 2017/21783-4, 2018/25410-0, 2019/02405-4 and Thematic project grant number 2016/01743-5; National Council for Scientific and Technological, CNPq (for support Universal project grant number: 409902/2018-7) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, grant number 88882.432902/2019-01), (CAPES, grant number 88882.432900/2019-01) (Brazil). FONDECYT 1190316, and International Sabbatical (UMCG, University of Groningen, The Netherlands) from the Vicerectorate of Academic Affairs, Academic Development Office of the Pontificia Universidad Católica de Chile (Chile).

PC034

Carotid body chemosensitivity at rest and during exercise in patients with hypertension

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Hypertension is the single risk factor leading to the greatest number of directly attributable deaths worldwide (Murray *et al.*, 2020). The carotid body (CB), a highly vascular gland situated bilaterally at

the bifurcation of the common carotid arteries, is the body's primary oxygen sensor. It also modulates sympathetic tone and has been shown to be hyperactive at rest in autonomic diseases such as hypertension (McBryde *et al.*, 2013). The CB plays a role in the control of ventilation during exercise in healthy humans (Wasserman *et al.*, 1975), although the exact mechanisms of exercise hyperpnea are incompletely understood. We aimed to assess CB chemosensitivity in human hypertension during submaximal exercise & gain insights into its role in exercise hyperpnea.

All study protocols were approved by Frenchay South West National Research Ethics Service Committee (15/SW/0030) and conformed to the Declaration of Helsinki.

We compared 12 hypertensive (HTN) patients (7 men; mean $\pm$ SD age 65.3 $\pm$ 7.6 years; 10 on anti-hypertensive therapy) to 8 healthy, normotensive (NTN) controls (6 men; 61 $\pm$ 7.9 years). We assessed CB chemosensitivity as Hypoxic Ventilatory Response (HVR) in both groups at rest then during submaximal exercise ($\approx$ 45% $\text{VO}_{2\text{peak}}$, assessed previously using a standard protocol on an upright cycle ergometer). A poikilocapnic, hypoxic technique was used, targeting oxygen saturation (SpO_2) levels of 87% for 2-3mins by the addition of nitrogen into inspired air. HVR was calculated using the method described by (Goldberg *et al.*, 2017), such that the change in ventilation is divided by the change in SpO_2 . The same method was used to calculate Hypoxic Respiratory Rate (RR) and Tidal Volume (V_T) Responses.

Responses to hypoxia were analysed using 2-way ANOVA's, with Sidak's multiple comparison's test. Peak exercise data were compared using unpaired T tests. Alpha was set at $P < 0.05$. Data are reported as mean $\pm$ SD.

$\text{VO}_{2\text{Peak}}$ test variables are seen in Table 1. While $\text{VO}_{2\text{Peak}}$ was similar between groups, ventilatory efficiency was lower and peak RR higher in HTN vs NTN. The respiratory responses to hypoxia are seen in Figure 1. HVR was increased to the same extent from rest during exercise in both NTN and HTN ($P < 0.0001$; Figure 1A). Hypoxic RR response was also increased overall by exercise ($P = 0.0015$) but with post hoc analysis showing only an increase in HTN (Figure 1B). There was no change in Hypoxic V_T Response with exercise in either group ($P = 0.8633$; Figure 1C). These results indicate that there is no difference in HVR at rest and during exercise between HTN and NTN. HVR did increase from rest to exercise in both groups showing that exercise sensitizes the hypoxic chemoreflex control of ventilation. Interestingly, during exercise, the increase in HVR appears to be driven by RR (rather than V_T) in HTN but not in NTN. The HTN group had a higher RR at maximal exercise versus controls. Potentially, inappropriate increases in RR during exercise are driven by the CB and may drive some ventilatory inefficiency in patients with hypertension. More work is needed to investigate this.

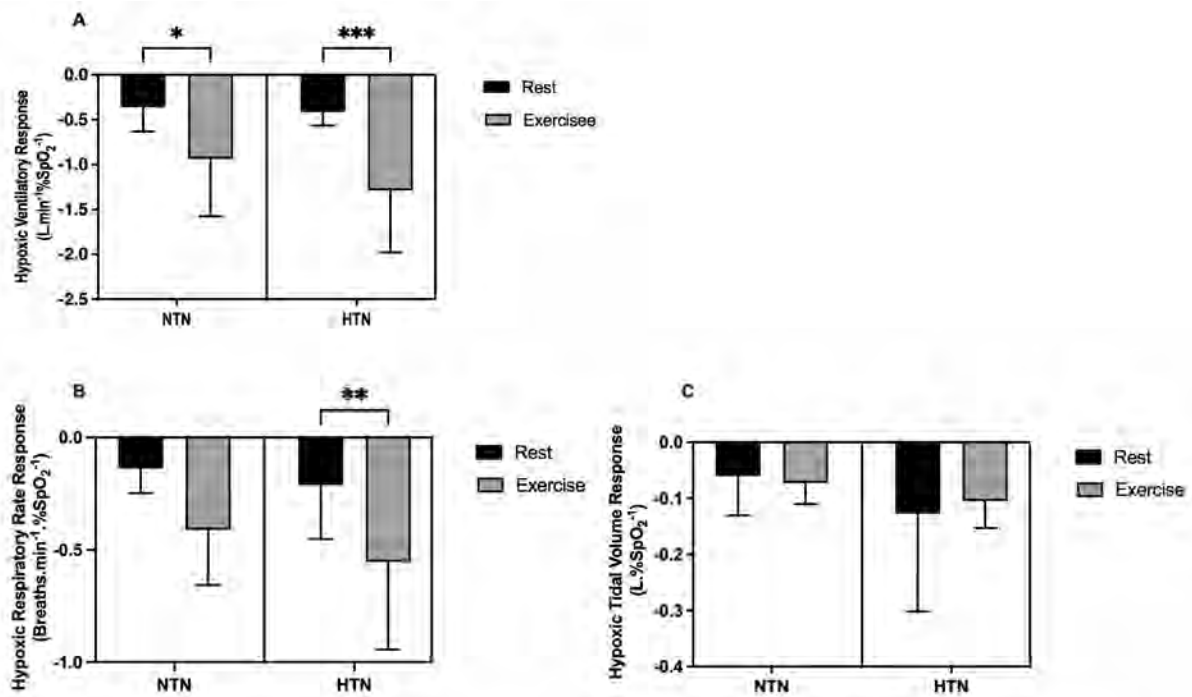


Figure 1. Respiratory responses to hypoxia. A: Hypoxic Ventilatory Response B: Hypoxic Respiratory Rate Response C: Hypoxic Tidal Volume Response

Table 1. VO₂Peak Test Variables

Exercise variable	NTN	HTN	P value
VO ₂ Peak (ml/kg/min)	26.9 ± 10.7	26.3 ± 7.2	0.8897
% of predicted VO ₂ Peak reached	100 ± 40.2	97 ± 22.4	0.8546
V _E /VCO ₂ during VO ₂ Peak	27.5 ± 2.1	32.1 ± 5.9*	0.0497
Respiratory variables at peak exercise			
RER	1.2 ± 0.1	1.2 ± 0.1	0.2652
V _E (l/min)	78.4 ± 34.1	82.3 ± 25.8	0.8016
RR (breaths/min)	27.6 ± 5.1	33.2 ± 4*	0.0165
V _T (l)	2.8 ± 1.1	2.4 ± 0.7	0.3613
P _{ET} CO ₂ (kPa)	5.3 ± 0.3	5.2 ± 0.6	0.4536

Values are mean ± SD; NTN = normotensives; HTN = hypertensives; VO₂ peak = peak oxygen consumption during maximal exercise testing on upright cycle ergometer; V_E/VCO₂ = ventilatory efficiency; RER = Respiratory Exchange Ratio; P_{ET}CO₂ = Partial Pressure of End Tidal Carbon Dioxide; *P<0.05 HTN vs NTN.

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PC035

Observations on the interaction between perfusion and postural stability in both feet during movement

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Introduction

Body positioning and perfusion changes have been studied to better understand cardiovascular adaptive mechanisms when moving from our bipedal condition to seated or supine. Biomechanics are thought to challenge hemodynamics and trigger regulation mechanisms, although these relationships are still poorly described. Center of pressure (CoP) location and displacement and plantar pressure are common measures to assess postural stability.

Aims/objectives

To explore how the differences in plantar pressure and location and displacement of the CoP might relate to the perfusion variations *in vivo* during upright standing and functional movement.

Methods

Eight participants (25.1 ± 5.2 y.o) of both sexes (4 female and 4 male) were selected following specific inclusion criteria. All participants were healthy and without peripheral vascular disease ($ABI=1.0\pm0.1$), and procedures respected the principles of good clinical practice adopted for human research. The experimental design involved the application of two protocols with three phases - five minutes standing at rest (Phase 1), two minutes performing a challenge movement - squat (protocol I) or single leg squat (protocol II) (Phase 2) and five minutes recovery in the standing position (Phase 3). Perfusion was assessed on both feet with laser Doppler flowmetry (LDF) sensors applied to the dorsum of the foot between the 3rd and 4th toes. The dorsal region perfusion of both feet was also measured by contactless polarized spectroscopy (PS). To assess postural data, a Foot Scan® RsScan International® Balance pressure plate was used to access center of pressure displacement and velocity, along with pressure for each foot within specific foot regions. Statistical analysis was performed with GraphPad Prism. Parametric tests were performed to assess variables by paired t-test. A 95% level of confidence was adopted.

Results and Discussion

A consistent increase in perfusion was recorded with both protocols (Phase II). With the squat (protocol I), a significant difference relative to rest (Phase I) was noted using LDF in Phase II and III ($p = 0.0001$). In protocol II, only the foot of the supporting limb for the one-legged squat shows significant differences in recovery (Phase III) with both LDF and TiVi. During the orthostatic posture, the distribution ratio of plantar pressure between the forefoot and the hindfoot was 4:6 when the feet were parallel to each other (standing position). The maximal elliptical displacement exhibited the expected significant differences in the center of mass between resting Phase I and recovery Phase III as compared to the challenge Phase II. In Protocol 1, Correlations between pressure and perfusion variables were noted. In protocol 1, Phase II, LDF correlates negatively ($p<0.05$) with forefoot pressure and positively ($p<0.05$) with midfoot pressure. In Phase III, LDF correlates negatively ($p<0.05$) with forefoot pressure, CoP displacement correlates positively ($p<0.05$) with forefoot pressure and negatively ($p<0.05$) with midfoot pressure, while CoP velocity correlates positively ($p<0.05$) with forefoot pressure. CoP displacement and velocity correlate negatively ($p<0.05$) with LDF in Phase III.

Conclusions

Results suggest that body position plays a fundamental role in the magnitude and duration of hemodynamic responses from rest to active movement.

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Continuous compared to accumulated multicomponent-training on physical function and health-related quality of life in sedentary elderly

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Background: Increasing evidence suggests that prolonged sedentary bouts [1] and/or few breaks in sedentary time [2] may damage metabolic health, independently of total sedentary time and moderate–vigorous physical activity. Accumulative exercise (distributing exercise training in the morning and in the afternoon) could be a good strategy to break and reduce sedentary time in older adults, especially in the less active time-slots. However, scarce studies have investigated whether accumulative proposals convey some advantage compared to similar doses of continuous exercise regarding physical function in sedentary older people. In addition, it remains unknown whether accumulated training enhances, more than does continuous training, the benefits in functional outcomes or health-related quality of life.

Objective: The present study aimed to analyze the impact of a Multicomponent Training (MCT) program in a group of sedentary older adults, comparing two different dose-distributions.

Method: In this quasi-experimental and longitudinal study, we recruited twenty-four sedentary older adults (71.75 ± 4.51 years) who were assigned to two groups of the EFAM-UV© program (a MCT program based on the gait-retraining and improving postural control with enriched environments that combining strength and cardiovascular proposals under the dual-tasking methodology) [3]. The continuous group (CMCT) trained for 60 min/session in the morning, while the accumulated group (AMCT) performed the same duration and intensity of exercise, but it was distributed twice a day (30 min in the morning and 30 more in the afternoon). All the participants attended voluntarily the program and gave their written consent for this study approved by the ethics committee of the University of Valencia (H1484058781638).

Results: After 15 weeks of intervention (2 days/week), Bonferroni post-hoc comparisons revealed significant ($p < 0.001$) and similar large improvements in both groups in lower limb strength. In addition, large gains were found in preferred walking speed and instrumental daily life activity, which were higher for CMCT and AMCT respectively; improvements in cardiorespiratory fitness, which were moderate for CMCT and large for AMCT; and medium and similar improvements in agility in both groups. Health-related quality of life showed a trend to significance only when the whole sample was considered. None of the training protocols had impact on the executive function.

Discussion: Our results confirm the importance of strength and cardiovascular training in order to maintain autonomy with aging and gives a hint to health fitness professionals and personal trainers about different functional changes in accumulated or continuous MCT programs [3]. As walking

programs [4], starting a multicomponent training in sedentary older adults improves physical function regardless of the type of dose-distribution performed. However, the slightly positive differences of accumulated strategies may also be related with benefits in other variables (as lipid profile or body composition) since helping to break afternoon sedentary behaviors.

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PC037

The Effect of Gum Arabic Supplementation on Cathelicidin expression in Monocyte Derived Macrophages in Mice

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Background:

Antimicrobial peptides (AMPs) are important effectors of the innate defense system. Cathelicidins, (CRAMP in mouse/rat, LL-37 in human) is one of the two major classes of AMPs in humans. The upregulation of LL-37 synthesis is a novel non-antibiotic approach to prevent or treat infectious diseases. Butyrate was found to induce Cathelicidin expression. Gum Arabic (GA), an exudate from *Acacia senegal* tree, is known for its prebiotic effects. Fermentation of GA by colonic bacteria increases serum butyrate concentrations. This study was conducted to investigate if GA supplementation can increase Cathelicidin expression in macrophages. **Methods:** The study was an in-vivo experiment in mice. Thirty mice were randomly divided into three groups, of ten mice per group. The two intervention groups received GA dissolved in drinking water in two different concentrations (15% and 30%) for 28 days. The third group served as a control. Blood was collected on Day 29 to isolate peripheral blood mononuclear cells (PBMC) which were cultured to obtain monocyte derived macrophages (MDMs). The transcription level of CRAMP was determined in MDMs by qPCR.

Results: We detected a significant increase in CRAMP expression in MDMs following 28 days of 15% but not 30% GA supplementation.

Conclusion: GA supplementation can induce Cathelicidin expression in MDMs and the effect is dose dependent

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PC038

Influence of Season on the Modulating Role of Ascorbic Acid and L-Carnitine on Respiratory Rate of African Giant Rats (*Cricetomys gambianus*, Waterhouse - 1840)

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1. Nutritional supplements may impact vital physiological parameters differently, depending on season. Seasonal change in respiratory rate of homeotherms may be a physiological mechanism for heat dissipation/conservation (Furtado *et al.*, 2008; Dzenda *et al.*, 2015; Kumar *et al.*, 2018).
2. The aim of the present study was to determine the influence of season on the modulating role of ascorbic acid and L-carnitine, administered singly (100 mg/kg) or combined (50 mg/kg each), to captive African Giant rats (*Cricetomys gambianus*, Waterhouse) of either sex. The trap, capture, handling and management of the rats conformed to ethical guidelines (Gannon and Sikes, 2007; Cooper, 2008). The supplements were administered in drinking water, 3 hours before commencement of the experiment for each day. The respiratory rate of the rats (n = 10 per group per season) was counted in the morning, afternoon and evening for 3 days (1 week apart), each during the cold-dry (Dec/Jan), hot-dry (Mar/Apr) and early-rainy (May/Jun) seasons in Zaria, Nigeria. Data were subjected to one-way analysis of variance and Tukey's *post-hoc* test.
3. The mean $\pm$ SEM value of respiratory rate in control rats (administered only water) was highest ($P < 0.01$) during hot-dry (120.4 ± 2.97 breaths/min), followed by cold-dry (113.0 ± 3.57 breaths/min), and the least ($P < 0.01$) was recorded in early-rainy (79.29 ± 3.13 breaths/min) seasons. Overall, the respiratory rate was significantly ($P < 0.01$) lower in rats

administered with ascorbic acid (95.43 ± 1.64 breaths/min) than in those given only water (103.2 ± 1.74 breaths/min) or L-carnitine (107.2 ± 1.84 breaths/min). Relative to control values, the respiratory rate was significantly ($P < 0.01$) lower and higher during the dry and early-rainy seasons, respectively in rats administered ascorbic acid only. The respiratory rate was significantly ($P < 0.01$) higher during both hot-dry and early-rainy seasons in L-carnitine-administered than in control rats. Co-administration of ascorbic acid and L-carnitine to the rats during hot-dry season did not significantly ($P > 0.05$) alter the respiratory rate count.

4. It was concluded that oral administration of ascorbic acid and L-carnitine modulated the respiratory rate in African Giant rats in a season-dependent manner. Ascorbic acid modulated the respiratory rate by decreasing and increasing the count to ameliorate its seasonal peak and nadir, associated with the hot-dry and early-rainy seasons, respectively. L-carnitine, on the other hand, raised the respiratory rate even during the hot-dry season, when the count was at its seasonal zenith. The administration of ascorbic acid may be more beneficial than that of L-carnitine in modulating respiratory rate across the seasons in the rats.

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PC039

Physiology of body composition – how do vegetarians and omnivores differ?

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Introduction: Dietary factors play a significant role in the accumulation of body fat. Plant-based diets are characterized by a reduction or elimination of animal product consumption and are believed to exert beneficial effects concerning the incidence and clinical course of different chronic diseases, especially those related to overweight and obesity. Despite the consistency by which plant-based diets are associated with reduced body composition, the clinical reality and the mechanisms by which this occurs have not yet been well-defined.

Aims/ Objectives: To examine the total body composition differences among vegetarians-vegans and omnivorous individuals.

Methods: The present study was a pilot and cross-sectional analysis of a university student sample consisting of 12 healthy individuals, 6 vegetarian-vegan, and 6 omnivores. The mean age of the study population was 27 years old, and 83% were women. Body composition was assessed using a dual-energy x-ray absorptiometry (DXA Lunar Prodigy Advance - General Electric Healthcare®), and dietary habits were collected using a Food Frequency Questionnaire and a 3-day dietary recall. Other general and descriptive variables were also collected by trained dietitians, including weight, height, abdominal circumference, smoking status, sleeping hours, intestinal and urinary rhythm, physical activity practice, among others. All individuals gave their informed and written consent to participate in the study prior to data collection. All procedures respected the principles of good clinical practice adopted for human research studies. Statistical analysis (descriptive and regression analysis) was performed using SPSS software. All statistical tests were two-tailed and the significance level was set at $p < 0.05$.

Results and Discussion: Our results have shown that the vegetarian-vegan group had a lower volume of any type of mass (total bone mass, fat mass, lean mass, tissue mass, and fat-free mass) evaluated ($p\text{-value} > 0.05$). However, these vegetarian-vegan participants also presented consistently higher values ($p\text{-value} > 0.05$) of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (281.00 and 1025.00 cm^3 , respectively) compared to those omnivores (219.17 and 831.00 cm^3 , respectively). SAT and VAT are two structurally and functionally distinct fat depositories playing major mechanical, protective and regulatory roles. VAT is associated with a higher risk of cardiovascular and metabolic diseases, while SAT seems to prevent and improve type 2 diabetes. No other differences were observed for weight, height, BMI, smoking status, physical activity, or other general characteristics that could influence the observed results.

Conclusions: Higher levels of visceral and subcutaneous adipose tissue were found in this vegetarian-vegan group. Other studies are needed to better understand the significance of this finding.

PC040

Anti-inflammatory and anti-oxidative properties of aqueous extract of *Triticum aestivum* (common wheat) on acetic acid-induced colitis in Wistar rats

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Introduction: Colitis falls under a group of disorders called inflammatory bowel disease (IBD) and marked with the presence of reactive oxygen species (ROS). The therapeutic target is to reduce inflammation and ROS.

Aim: This study investigated the anti-inflammatory and anti-oxidative properties of *Triticum aestivum* on acetic acid-induced colitis in Wistar rats.

Materials and Methods: Male Wistar rats (n = 12, 150-200g) were randomly divided into 4 groups as follow; group I(negative control) distilled water, group II- acetic-acid (6%) induced colitis (positive control), group III- acetic-acid induced colitis + mesalamine (400mg/kg) orally and group IV-aqueous extract of common wheat (200mg/kg) orally for three weeks. Ulcerative colitis was induced on day 1 of the study using acetic acid via anal injection, and colitis was confirmed on day 3 by the presence of bloodstains in the stool counts. The rats were euthanatized (ketamine) on days 7 and 21. The colon tissue was harvested and homogenized. Activities of superoxide dismutase (SOD), malondialdehyde(MDA), glutathione(GSH), and expression of interleukin-6 (IL-6) was evaluated.

Results: Data were expressed as mean $\pm$ SEM, analyzed using one-way ANOVA, with $P < 0.05$. *Triticum aestivum* significantly decreases the MDA level in group IV on days 3 (8.9180 ± 0.574) and 7 (9.531 ± 0.133) when compared with group III on days 3 (12.950 ± 0.457) and 7 (12.360 ± 1.414) and group II on days 3 (13.380 ± 0.501) and 7 (9.876 ± 0.633). It also reduces the SOD levels and significantly increases the GHS level. A Significant decrease in interleukin-6 expression was observed in group IV on days 3(7884 ± 77.2) and 7(5425 ± 384.3) when compared with group II on days 3(10600 ± 240.3) and 7 (8584 ± 495.4).

In conclusion, aqueous extract of *Triticum aestivum* possesses anti-inflammatory and anti-oxidative properties in Wistar rats induced with colitis.

Keyword: Colitis, Anti-inflammatory markers, *Triticum aestivum*, anti-oxidative

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PC041

Evaluating the leucine trigger hypothesis to explain the postprandial regulation of muscle protein synthesis in young and older adults: A systematic review

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Background: The 'leucine trigger' hypothesis was originally conceived to explain the postprandial regulation of muscle protein synthesis (MPS). This hypothesis implicates the magnitude (amplitude and rate) of postprandial increase in blood leucine concentrations for regulation of the magnitude of MPS response to an ingested protein source. Recent evidence from experimental studies has challenged this theory, with reports of a disconnect between blood leucine kinetics and postprandial rates of MPS in response to protein ingestion.

Aim: The primary aim of this systematic review was to qualitatively evaluate the leucine trigger hypothesis to explain the postprandial regulation of MPS at rest and post-exercise in young and older adults. We hypothesized that experimental support for the leucine trigger hypothesis will depend on age, exercise status (rest vs. post-exercise), and protein source (i.e. isolated proteins vs. protein-rich whole food sources).

Methods: This qualitative systematic review extracted data from studies that combined measurements of postprandial blood leucine kinetics and rates of MPS following ingested protein at rest and following exercise in young and older adults. Data relating to blood leucine kinetics and postprandial MPS rates were extracted from all studies and reported as providing sufficient or insufficient evidence for the leucine trigger hypothesis.

Results: Overall, 16 of the 28 eligible studies provided sufficient evidence to support the leucine trigger hypothesis for explaining divergent postprandial rates of MPS in response to different ingested protein sources. Of these 16 studies, 13 were conducted in older adults (eight of which conducted measurements post-exercise) and 14 studies included the administration of isolated intact protein.

Conclusion: This systematic review underscores the merits of the leucine trigger hypothesis for the explanation of the regulation of MPS. However, our data indicate that the leucine trigger hypothesis confers most application in regulating the postprandial response of MPS to ingested proteins in older adults. Consistent with our hypothesis, we provide data to support the idea that the leucine trigger hypothesis is more relevant within the context of ingesting isolated protein sources rather than protein-rich whole foods. Future mechanistic studies are warranted to understand the complex series of modulatory factors beyond blood leucine kinetics within a food matrix that regulate postprandial rates of MPS.

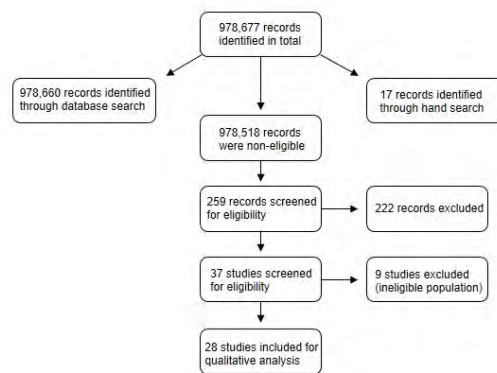


Figure 1. A flow diagram of the screening process for selecting eligible studies

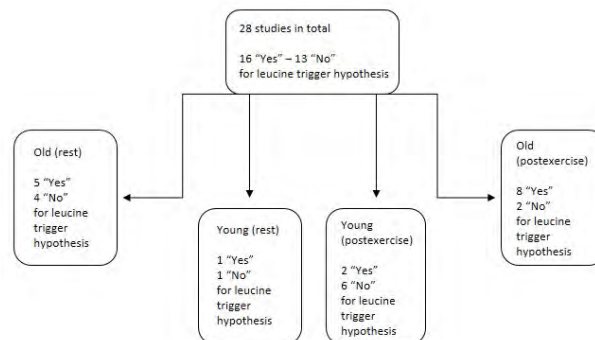


Figure 2. Synthesis of findings from 28 studies that support or refute the leucine trigger hypothesis to explain the postprandial regulation of muscle protein synthesis in young and older adults at rest and following exercise, using 29 study arms overall

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PC042

Mapping the daily rhythmic transcriptome in the diabetic retina

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Purpose: The eye is a rhythmic organ specifically evolved to function around the light cycle via functional circadian clocks. Diseases such as diabetes have been reported to disrupt circadian rhythms and circadian disruption emerges as an important factor in the prognosis of disease outcomes and treatment success. Herein we mapped the rhythmic transcriptome in the mouse retina to understand the extent of circadian disruption due to diabetes.

Methods: Healthy control and Ins2Akita/J diabetic mice were kept under a physiological 12h:12h light-dark cycle until 4 months of age. Retinas were collected from 4-5 mice every 4 hrs around the day/night cycle. Deep mRNA sequencing was conducted and transcripts were identified. Computational approaches were used for detection of rhythmicity (empirical JTK_Cycle, emp $p < 0.05$, FC > 1.2), acrophase prediction (Harmonic Regression with a set period of 24 hrs and normalization set to false), differential rhythmic patterns (DORD analysis BH corrected $p < 0.05$), phase set enrichment analysis (PSEA, BH corrected p -value < 0.01) and upstream regulator predictions (IPA, $p < 0.05$). Animal studies were carried out at the institutional animal care facilities at the Indiana University School of Medicine in accordance with institutional and national guidelines for the care and use of laboratory animals (IACUC #10604 and #11167)

Results: Almost 10% of the retinal transcriptome was identified as rhythmic with a clear 12hr axis of transcriptional activity, peaking at midday and midnight. Although the 12-hour transcriptional axis is retained in the diabetic retina, it was phase advanced by approximately 1-3 hours. Interestingly, only mild changes in the circadian rhythms were observed, as those were entrained by the light cycle. However, oxidative phosphorylation and HIF1A and NUPR1 were identified as the major upstream regulators for the phase shifts. An altered peak in daily glucose levels in diabetic mice may drive the phase shifts in the retina.

Conclusions: To our knowledge this is the first study mapping the effects of diabetes in the rhythmic output in the retina. Importantly, we identified that many of the daily rhythmicity in the retina is altered and the source of this shift is more related to abnormal metabolic adaptation rather than circadian disruption.

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PC043

In Silico Investigation on Possible Involvement of Neuroendocrine Modulations in Diabetic Peripheral Neuropathy

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AIM: Peripheral neuropathy is one of the most common diabetic complications in a chronic process in the absence of glycemic control, and the pathogenesis of diabetic peripheral neuropathy (DPN) is not fully understood yet. Clinical experiments are on-going and also recent evidences indicates hope from *in silico* analysis. The aim of this study was to examine possible involvement of neuropeptides in DPN by using bioinformatics tools, by examining the expression levels of genes, known to have neuroendocrine functions through genome analyzes performed in sciatic nerve tissues obtained from diabetic rats.

METHODS: GSE147732¹ dataset obtained from GEO (Gene Expression Omnibus) database was re-examined for this research. In the dataset, sciatic nerve tissue samples (n=6) derived from normal and streptozotocin-induced diabetic Sprague-Dawley rats are recruited. After the gene expression levels in the dataset were re-analysed in the R program, gene set enrichment analyses were performed in Gene Ontology (GO) and ENRICH tools.

APPROACH FOR STATISTICAL ANALYSIS: Expression levels of genes, commonly implicated to play a role in neuroendocrine modulations, in GSE147732 dataset are re-analysed in R program. Based on Benjamini-Hochberg correction, adjusted p-values <0.05 were accepted as significant.

RESULTS: Gene expression levels on the dataset indicated that neuropeptide Y (NPY), neuropeptide receptor 1Y and 2Y (NPY1R, NPY2R), proopiomelanocortin (POMC), pancreatic polypeptide receptor 1 (PPYR1), calcitonin receptor-like peptide (CALCRL), endothelin 1 (EDN1), vasoactive intestinal peptide (VIP), angiotensinogen (AGT), neurotensin receptor 1(NTSR1), parathyroid hormone 1 receptor (PTH1R), neuropeptide W (NPW), thyrotropin releasing hormone receptor (TRHR), chromogranin A (CHGA), adiponectin (ADIPOQ), resistin (RETN), neuromedin B (NMB), cerebellin 2 precursor (CBLN2), period homolog 1 and 2 (PER1 and PER2), clock homolog gene (CLOCK), chemokine (C-C motif) ligand 21, (C-X-C motif) ligand 12 and (C-X3-C motif) ligand 1 (CCL21, CXCL12, CX3CL1), galectin 1,5,8,9 (LGALS1, LGALS5, LGALS8, LGALS9) genes were down-regulated (p<0.05); and galanin (GAL), somatostatin receptor 3 (SSTR3), neuropeptide FF-amide peptide precursor (NPFF), adrenomedullin (ADM), endothelin 3 (EDN3), secretin (SCT), gastric inhibitory polypeptide (GIP), corticotropin releasing hormone receptor 2 (CRHR2), urocortin 2 (UCN2), tachykinin 1 (TAC1), neuromedin U (NMU), nerve growth factor receptor (NGFR) genes were up-regulated (p<0.05) in the diabetic group, compared with normal group.

CONCLUSION: Results from this *in silico* analysis indicate imbalances in the expression levels (down- and up-regulation) of genes encoding neuropeptides known to be involved in many neuroendocrine process, implicating involvement of impaired neuroendocrine signalling in the pathogenesis of DPN.

Keywords: Diabetic neuropathy, sciatic nerve, neuropeptides, gene expression, bioinformatics.

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PC044

Biochemical signalling pathways are disrupted in the myocardium of mdx 5cv mice and not rescued by $\Delta R4-R23/\Delta CT$ micro-dystrophin

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Background: Cardiac failure is a primary cause of mortality in patients with Duchenne (DMD) or Becker (BMD) muscular dystrophy. DMD and BMD are caused by mutations in the X-linked *DMD* gene that affect the expression and/or function of the dystrophin protein in striated muscles. Dystrophin plays an important structural function in protecting the muscle cell membrane from the repeated mechanical stresses of contraction. However, dystrophins with deletions that do not disrupt its structural functions, delay but do not prevent cardiac disease. This suggests the existence of additional non-structural functions of dystrophin that remain to be elucidated in order to develop effective treatments for heart failure in DMD and BMD.

Aims: To investigate signalling changes associated with disease in the dystrophin deficient heart that are not corrected by an internally deleted dystrophin with preserved structural function ($\Delta R4-R23/\Delta CT$ micro-dystrophin ([μDys](#))).

Methods: Kinase arrays were performed on whole heart tissue lysates from wild-type mice, dystrophin deficient mdx 5cv mice and mdx 5cv mice expressing μDys. Signalling pathways disrupted in mdx 5cv mice compared to wildtype that were not normalised by micro-dystrophin were further investigated by Western blotting and immunofluorescence assays to validate the array results. Additionally, we also assessed proteins known to be disrupted in dystrophin deficient hearts and to play a role in maintaining the structural integrity of cardiomyocytes or in cardiac conduction. To

quantify Western blots densitometric analyses were performed and data expressed as mean fold change (FC) $\pm$ standard deviation versus wildtype. All groups were N=5 and statistical assessment was performed using one-way ANOVA with Bonferroni post hoc test for multiple comparisons. All animal procedures were performed in accordance with the Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (UK Home Office).

Results: Our group have recently shown that expression of μ Dys fully prevents cardiomyocyte hypertrophy and collagen fibrosis developing in the heart, despite perturbation to ERK 1/2 signalling (Wang et al., 2021). Here, we show that upstream of ERK 1/2, MEK 1/2 was hypo-phosphorylated in mdx 5cv (0.15 ± 0.08 FC $P < 0.01$) and not corrected by uDys (0.21 ± 0.31 FC $P < 0.01$). Akt was also hypo-phosphorylated in mdx 5cv (0.49 ± 0.21 FC $P < 0.05$ and not corrected by uDys (0.45 ± 0.26 FC $P < 0.05$). Additionally, sarcomeric protein α -actinin increased in abundance in mdx 5cv (2.90 ± 0.48 FC $P < 0.01$) but was normalised by uDys (1.52 ± 1.25 FC N.S.). Finally, connexin 43, a key protein of intercalated disks that mediates cardiac conduction was mislocalised and over-abundant in mdx 5cv (3.19 ± 1.05 FC $P < 0.01$) and not normalised by uDys (2.27 ± 0.57 FC $P < 0.05$).

Conclusions: In line with current understanding, our results confirm the ability of Δ R4-R23/ Δ CT micro-dystrophin to restore the structural integrity of the dystrophin-deficient cardiomyocyte. However, key biochemical signalling pathways appear to remain perturbed and efforts to address such issues are required for fully effective therapies towards DMD and BMD.

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PC045

Palmitoylation and regulation of the “funny” current HCN4 channel

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The sinoatrial node (SAN) and subsidiary pacemakers in the cardiac conduction system can generate spontaneous electrical activity. The hyperpolarisation-activated cyclic nucleotide-gated channel HCN4 is responsible for genesis of the pacemaker “funny” current (I_f) during diastolic depolarisation

(Choudhary et al, 2015). HCN4 channels localise to lipid rafts and disorganisation of rafts results in redistribution of the channels, altering their kinetic properties (Barbuti et al, 2004). S-palmitoylation regulates key cardiac Na^+ and Ca^{2+} handling proteins, influencing their membrane microdomain localisation and function (Howie et al, 2018; Reilly et al, 2015) and acting as a mechanism of targeting transmembrane proteins and transporters into lipid rafts (Gök et al, 2020). This *in vitro* study was conducted to investigate HCN4 palmitoylation and its functional consequences. Resin assisted capture (acyl-RAC) of acylated proteins was used to assess palmitoylation of full length HCN4 and its intracellular amino and carboxyl termini fused to YFP in human embryonic kidney (HEK) cells as well as endogenously expressed HCN4 in neonatal rat ventricular myocytes. Site-directed mutagenesis using In-Fusion Cloning was used to construct alanine mutations of the palmitoylation sites. Whole-cell patch-clamp recordings from stable wild-type (WT) and mutant C93A/C179A HCN4-expressing HEK 293 cells employed an internal solution comprised of (in mmol/L): 130 KCl, 1 MgCl_2 , 5 EGTA, 5 MgATP, and 10 HEPES (titrated to pH 7.2 with KOH) and external solution comprised of (in mmol/L): 140 NaCl, 4 KCl, 2.5 CaCl_2 , 1 MgCl_2 , 10 glucose, and 5 HEPES (titrated to pH 7.4 with NaOH). Phylogenetic analysis was used to explore the evolutionary emergence of HCN4 palmitoylation within the pre-metazoan and metazoan lineage. Acyl-RAC ($n = 5$) established that palmitoylation of full length HCN4 occurs. Mutagenesis of intracellular cysteines at the N-terminus revealed that C93 and C179 are both sites of HCN4 palmitoylation ($n = 3$). A double cysteine-to-alanine mutation C93A/C179A of full length HCN4 resulted in an $\sim 77\%$ reduction (0.52 ± 0.05 for WT vs 0.12 ± 0.06 for C93A/C179A; mean $\pm$ SEM, $n = 3$; unpaired t test; $P < 0.01$) in palmitoylation in comparison to wild type HCN4. Under whole-cell patch clamp, removal of the two N-terminal palmitoylation sites did not significantly alter the half maximal activation voltage ($V_{0.5}$: -82.4 ± 5.20 mV for WT vs -82.8 ± 1.15 mV for C93A/C179A; mean $\pm$ SEM, $n = 3$ and 9 respectively; unpaired t test; $P = 0.91$) but altered the activation slope factor (k : 6.21 ± 0.17 mV for WT vs 10.54 ± 0.64 for C93A/C179A; $n = 3$ and 9; unpaired t test $P < 0.05$). Phylogenetic analysis revealed that although cysteine 93 is widely conserved across all classes of HCN4 vertebrate orthologs, conservation of cysteine 179 is restricted to placental mammals. A polybasic cassette potentially driving the palmitoylation of cysteine 93 in human HCN4 was conserved widely across all classes of HCN4 vertebrate orthologs. Collectively, the work in this study provides clear evidence for N terminal palmitoylation of the HCN4 channel.

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Action potential clamp characterization of a short QT syndrome “hotspot” hERG mutation

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Short QT syndrome (SQTS) is a rare but potentially fatal cardiac channelopathy (Hancox et al, 2018; Harrell et al, 2015). The T618I mutation in the hERG channel pore has been termed a “hotspot” mutation as it is the mutation observed most frequently in successfully genotyped cases (Hu et al, 2017; Sun et al, 2011). A distinct U wave has been observed in the electrocardiogram of ~70% of T618I carriers and these patients are vulnerable to ventricular fibrillation; however, atrial fibrillation has not been reported (Hu et al, 2017). This study was undertaken to compare the effect of the T618I mutation on hERG current (I_{hERG}) elicited by action potential (AP) waveforms from different cardiac regions. HEK293 cells were transiently transfected with wild-type (WT) or T618I hERG constructs and whole-cell patch-clamp measurements of I_{hERG} were made at 37 °C, using ventricular, Purkinje fibre (PF) and atrial AP waveforms as previously described (McPate et al, 2009). Data are presented as mean $\pm$ SEM. Under ventricular AP clamp, WT I_{hERG} attained a maximal amplitude of 125.4 ± 34.5 pA/pF during AP repolarization, peaking at -23.5 ± 1.8 mV (n=5). During the same waveform, T618I I_{hERG} attained a maximal amplitude of 565.9 ± 155.8 pA/pF (n=6; $p < 0.05$ vs WT, unpaired t test), whilst peaking earlier during the AP (at $+19.0 \pm 2.6$ mV; $p < 0.001$ vs WT). For WT I_{hERG} , application of PF and atrial AP commands to the same cells to which ventricular APs had been applied elicited peak repolarizing currents of 101.0 ± 25.0 pA/pF and 45.2 ± 9.1 pA/pF respectively (n=5; PF $p > 0.05$ vs ventricular; atrial $p < 0.01$ vs ventricular, repeated measures 1-way ANOVA, with Tukey post-test). Normalizing peak repolarizing current to that elicited by the ventricular AP command showed maximal repolarizing WT I_{hERG} current during the PF waveform to be $84 \pm 6\%$ of maximal ventricular repolarizing current and for the atrial AP command the comparable value was $41 \pm 6\%$ (n=5). For T618I, maximal I_{hERG} during the PF and atrial APs was 282.0 ± 94.6 pA/pF and 103.9 ± 33.3 pA/pF respectively (n=6; $p < 0.05$ and $p < 0.01$ vs ventricular AP respectively). When normalised similarly to WT data, peak repolarizing current during the PF and atrial APs were respectively $49 \pm 4\%$ ($p < 0.001$ vs WT; unpaired t test) and $18.8 \pm 2\%$ ($p < 0.01$ vs WT) of peak ventricular AP repolarizing current. Collectively, these results indicate that the T618I mutation increases repolarizing ventricular I_{hERG} , whilst it also augments differences in I_{hERG} between PF and ventricular APs. This in turn may contribute to heterogeneity of ventricular-PF repolarization and consequently to the U waves seen in T618I carriers. The comparatively brief duration and lack of pronounced plateau of the atrial AP is likely to account for the disparity in T618I effect between atrial and ventricular APs.

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PC047

LQTS-Associated Calmodulin Mutations Disrupt Interactions with the Kv7.1 Voltage-Gated Potassium Channel

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Long-QT syndrome (LQTS) is a life-threatening cardiac arrhythmia whereby ventricular recovery time is extended, prolonging the QT interval as seen on an electrocardiogram. Affecting 1 in 2000 people, LQTS predisposes sufferers to an increased risk of suffering catastrophic cardiac events, typically resulting in sudden cardiac death (SCD). The most common cause of LQTS are congenital mutations in the Kv7.1 voltage-gated potassium channel which facilitates one of the main ventricular repolarising currents (I_{Ks}). Calmodulin (CaM) is a ubiquitous calcium-sensing protein which regulates Kv7.1 activity. Several mutations in CaM have been identified in human patients displaying LQTS phenotypes, suggesting a key role in the molecular aetiology of LQTS. However, the molecular mechanisms of CaM-associated LQTS remain unclear.

Here, we present novel data regarding the biophysical properties of four LQTS-associated mutations located at calcium co-ordinating residues within the C-lobe of CaM, and their interactions with a CaM-binding domain (Helix B) at the C-terminus of the alpha subunit (KCNQ1) of the Kv7.1 channel. Using circular dichroism, we showed that single amino acid substitutions confer significant changes to the thermostability and secondary structure of CaM variants. Additionally, using equilibrium calcium binding titrations, we showed that the affinity of CaM C-lobe for calcium can be reduced up to 2-fold. Isothermal titration calorimetry experiments provided evidence of both calcium-dependent and independent binding of CaM to Helix B (Kv7.1). In its calcium-free state, CaM binds Helix B through hydrophobic interactions with moderate affinity ($K_d = 2.2 \pm 0.2 \mu\text{M}$). Ca^{2+} dependent binding to Helix B is considerably higher affinity, with over a 2000-fold reduction in dissociation constant (K_d). LQTS-associated CaM variants were found to reduce affinity for Helix B with the largest reductions found in fourth EF hand mutants. These mutants also adopted a highly consistent, alternative complex with Helix B compared to WT CaM as determined through HSQC ^1H - ^{15}N NMR. Using whole cell configuration voltage-clamp electrophysiology, we demonstrated that CaM modulates the Kv7.1

channel to produce larger currents without altering channel activation kinetics. The I_{Ks} current was found to be Ca^{2+} sensitive, in response to increases in cytosolic Ca^{2+} , larger I_{Ks} currents are generated (from 47 ± 6 to 150 ± 31 pA pF⁻¹ at +60 mV, $n = 22$ and 9 respectively). Interestingly, LQTS-associated CaM mutants impair this Ca^{2+} sensitivity by reducing current density.

Here we outline mechanistic insights as to how LQTS-associated CaM variants contribute to electrical disease of the heart, mutations in the highly conserved structure of CaM appear to perturb protein structure, its ability to bind calcium, and disrupt complex formation with the KCNQ1 channel. This results in reduced generation of the I_{Ks} current, ultimately decreasing the repolarising capacity of cells and therefore extending the QT interval.

Acknowledgements :- Supported by the Institute of Translational Medicine University of Liverpool PhD studentship (to LFM), the Wellcome Trust PhD Studentship (to NG), the British Heart Foundation Intermediate Basic Science Research Fellowship FS/17/56/32925 (to NH) and the British Heart Foundation Project Grant PG/14/55/30973 (to CD).

PC048

Computational study of SARS-CoV-2 infection inhibitor Hydroxychloroquine on Cardiac toxicity

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Objectives: The outbreak of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/2019-nCoV) poses a serious threat to global public health and local economies. With the heightened interest in the potential use of hydroxychloroquine (HCQ) to treat patients with SARS-CoV2, it may be prudent to reflect on the risks of therapy, particularly their cardiac toxicity. This quantitative study investigates the propensity of HCQ in relation to cardiac safety.

Methods: The sinoatrial node (SAN) cell is presented as an equivalent electrical circuit comprising nine ion channels. All ionic currents are described by the ordinary differential equations [2 and 3]. The SAN action potential (AP) and respective ionic currents are constructed in silico. A HCQ drug model for the hyperpolarizing-activated current or funny current (I_f) is simulated after mining data from the experimental studies [1].

Results: The resting membrane potential is set at -80 mV. The HCQ blocked I_f with IC_{50} concentration. The steady-state value of the activation parameter of the I_f is shifted to the positive side after applying HQN of 1μ M. The action potential (AP) timing (shown in Figure 1) is altered when we incorporated the biophysically modified I_f after the application of HCQ. The results show that the modified I_f plays an important role in reducing the frequency of the spontaneous AP at the SA node.

Conclusions: We simulated the effects of HCQ drug upon funny current and action potential. Our quantitative investigation identifies hydroxychloroquine, a well-established drug used against COVID-19 reduces the frequency rate of the spontaneous action potential firing by inhibiting the funny current. Therefore, we should prevent it as a potential drug for COVID-19.

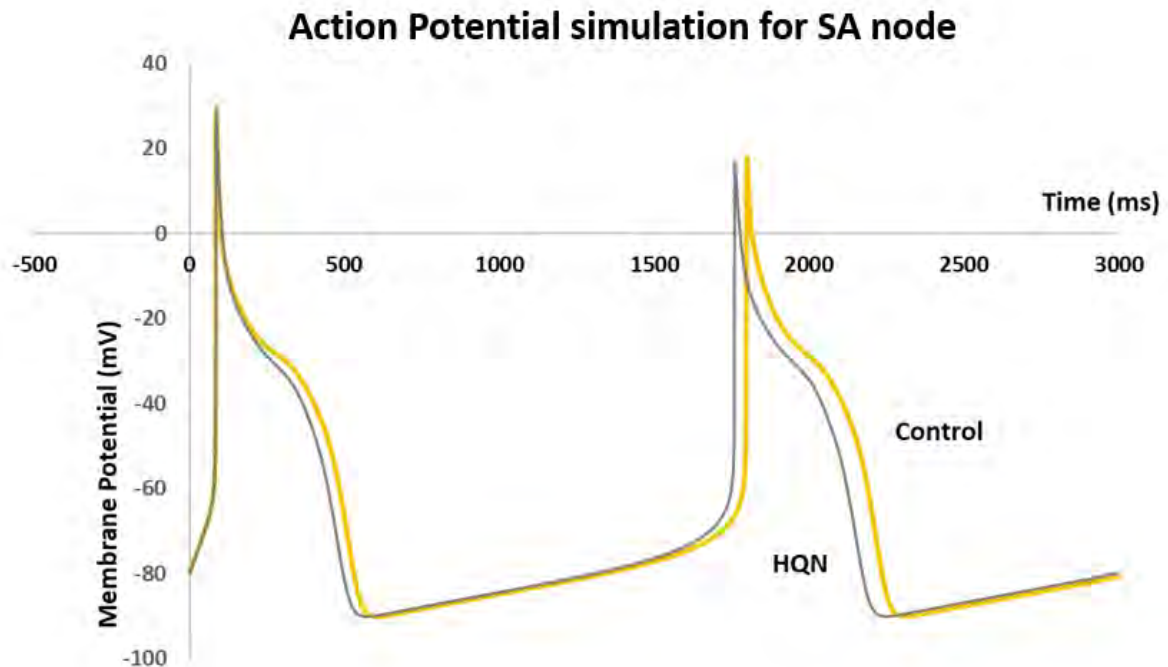


Figure 1. SA node AP simulation in both control and applied drug

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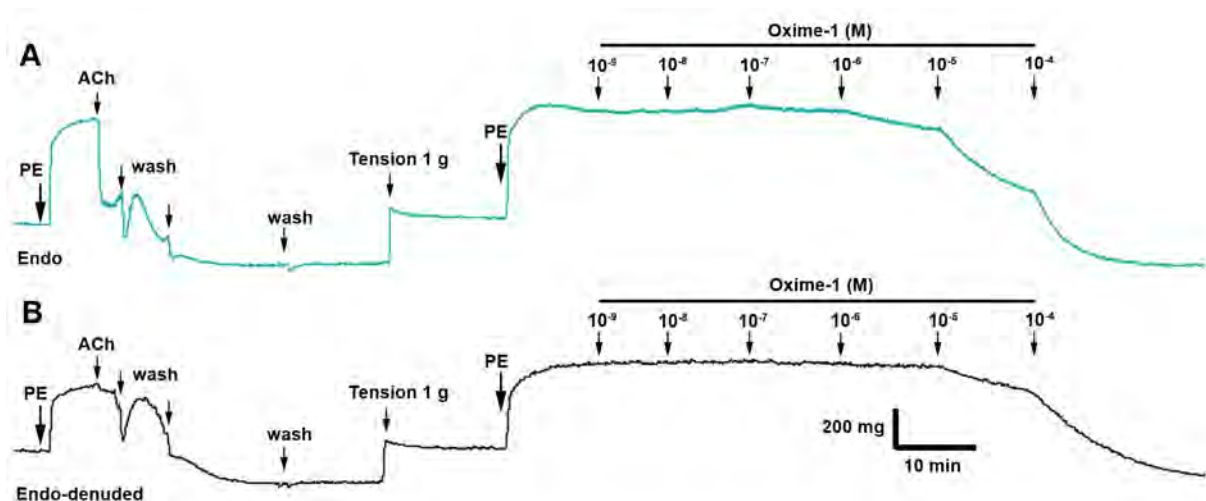
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Vasorelaxation Effect of Oximes Synthesized from Hydroxyacetophenone and Hydroxytremetone derivatives Isolated of Endemic Medicinal Plants of North Chile in Rat Aorta

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Introduction: The search for bioactive molecules extracted from plants used by American indigenous ethnicities is gaining much consideration as source of new compounds for treating hypertension. **Objective:** To evaluate the vascular effect of bioactive molecules isolated and chemically modified from medicinal plants used for the treatment of mountain sickness. **Method:** We isolated two compounds from *Senecio nutans* Sch. Bip., such as 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (metabolite-1) and 6-Hydroxytremetone (metabolite-2). In addition, we carried out chemical modification of both metabolites-1 and -2, which produced oxime-1 and oxime-2, respectively. Vascular reactivity experiments with two metabolites isolated and their oximes in rat aorta were performed. The results obtained from these experiments were expressed as mean $\pm$ standard error of mean. Statistical analysis of the data was performed using analysis of variance (two-way ANOVA) where applicable followed by Bonferroni post-hoc test. In addition, the determination of the sensitivity (EC_{50}) was performed using nonlinear regression (sigmoidal) via Graph Pad Prism software, version 5.0. Statistical significance is set at $p < 0.05$. The trials were approved by the Ethics Committee of Universidad Antofagasta (CEC-275/20). **Results:** All compounds caused vascular relaxation in intact rat aorta pre-contracted with phenylephrine (PE; 10^{-6} M): 41 ± 6 % metabolite-1, 73 ± 2 % oxime-1, 58 ± 2 % metabolite-2, and 48 ± 2 % oxime-2 (10^{-5} M). The denudation of endothelium in aortic rings provoked a drastic decreased of relaxation induced by the compounds tested in this study. The pre-incubation of the intact aortic rings with 10^{-4} M L-NAME significantly decreased relaxation in response to all molecules (10^{-5} M). Moreover, contractile vascular response to PE (EC_{50} 34.04 ± 8.49 nM) was reduced when oxime-1 (EC_{50} 67.28 ± 8.67 nM; $p < 0.05$) or metabolite-2 (EC_{50} 77.06 ± 7.69 nM; $p < 0.01$) were present. **Conclusion:** Since metabolite-2 and oxime-1 showed the highest vascular relaxation effect, they could be potential bioactive molecules for regulating blood pressure.



Oxime-1 causes relaxation in rat aorta via the endothelium-dependent pathway. Aortic rings were pre-contracted with 10^{-6} M PE, and cumulative concentrations of oxime-1 (10^{-9} to 10^{-4} M) were added in bath.

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Acknowledgements :- Financial support was provided by FONDECYT 1200610 to JP

PC050

The role of angiotensin II-mediated pericyte contractility in sensory neurodegeneration in diabetic neuropathic pain

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Diabetic neuropathic pain is a common symptom of diabetic neuropathy, which is a complication that affects many people living with diabetes and is caused by damage to nerves. To date it is acknowledged that neurodegeneration of the somatosensory nervous system underlies the development of this neuropathology however the mechanisms in which this arises are not yet certain. The sensory neurons in the dorsal horn of the spinal cord are typically hyperactive in diabetic individuals and there is now evidence reporting microvessel damage as a putative cause. Pericytes are cells found along microvessel walls and have been found to constrict blood vessels in other diabetes-linked complications such as retinopathy as well as other neurodegenerative conditions (e.g. Alzheimers), reducing blood flow to vital systems. In this study, the mechanism of pericyte contractility via angiotensin II, an effector molecule upregulated during hyperglycaemia, was explored as a direct link to diabetic neuropathy. Fluo-4 calcium assays in the presence of angiotensin

II as well as immunocytochemistry staining were performed on pericytes extracted from 7-day-old C57BL/6 wild type mouse spinal cords (n=6). All animal studies were reviewed by Nottingham Trent University AWERB and performed in line with ASPA legislation. Immunocytochemistry demonstrated that a large proportion of isolated spinal cord cells were neuron-glia 2 (NG2) positive, indicating isolated cells were pericytes (99.6% (****p<0.0001, Unpaired T Test comparing percentage of cells with and without NG2 expression)). In addition, angiotensin II dose dependently led to a pronounced increase in intracellular calcium immediately post application. 100nM angiotensin II led to the most prominent peak calcium response (****p<0.0001, One way ANOVA comparing peak calcium response across all angiotensin II concentrations) and greatest prolonged calcium response (**p<0.001, One way ANOVA comparing the area under the curve produced by all concentrations of angiotensin II when applied to pericytes). It can be concluded that angiotensin II produces contraction of spinal cord pericytes, which is explanatory of the increased calcium response. This suggests that this mechanism may be involved in constriction of microvessels, leading to nerve ischemia and diabetic neuropathic pain, however further research is needed to confirm this interaction and its mechanisms.

Acknowledgements :- M. Sheavyn L. Hardowar, and R.P. Hulse

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PC051

Investigation of effects of S5 mutation F557L on actions of selected non-canonical hERG potassium channel inhibitors

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The hERG potassium channel is well established to control ventricular repolarisation and to be the pharmacological (anti-)target of many drugs that cause acquired long QT syndrome. The aromatic S6 domain residues (F656 and Y652) are known to be important blocking determinants for many drugs; an S5 aromatic residue (F557) has recently been proposed to be an additional significant binding determinant (Saxena et al, 2016). F557 lies close to Y652 (Saxena et al, 2016; Helliwell et al, 2018). The aim of this study was to exploit drugs with unusual characteristics of hERG inhibition to further probe the role of the F557 residue.

Whole-cell patch-clamp recordings of hERG current (I_{hERG}) were made at 37°C from Human Embryonic Kidney (HEK 293) cells either stably or transiently transfected with wild-type (WT) or mutant hERG

channels. A voltage step protocol, comprised of a 2-second depolarization to + 20 mV, was followed by repolarization to –40 mV to elicit I_{hERG} tails, which were measured to quantify drug block (Helliwell et al, 2018). BeKm-1 scorpion toxin exhibits preferential closed channel block (Milnes et al, 2003), consistent with extracellular binding. At 100nM, BeKm-1 inhibited WT and F557L I_{hERG} tails by $57 \pm 7\%$ (mean $\pm$ SEM; n=7 cells) and $55 \pm 5\%$ (n=7 cells; $p > 0.05$ vs WT, unpaired t test). Two other concentrations (10 nM and 300 nM) of BeKm-1 also produced levels of I_{hERG} inhibition that did not differ between WT and F557L channels. The macrolide antibiotic erythromycin is large and appears only to have limited access to the S6 aromatic binding site (Duncan et al, 2006). At 100 μ M, erythromycin inhibited WT I_{hERG} tails by $75 \pm 5\%$ (n=10) and those carried by F557L by $75 \pm 4\%$ (n=6; $p > 0.05$ vs WT, unpaired t -test). A lack of significant difference of inhibitory actions between WT and F557 hERG was also observed for 1 mM erythromycin. I_{hERG} block by the antiarrhythmic propafenone depends on the F656 but not Y652 residue (Witchel et al, 2004). At 300nM propafenone inhibited WT I_{hERG} tails by $45 \pm 4\%$ (n=11) and those carried by F557L by $46 \pm 7\%$ (n=9, $p > 0.05$ vs WT, unpaired t -test). A lack of significant difference of inhibitory actions between WT and F557 hERG was also observed for 100nM, 1 μ M and 3 μ M of propafenone. As anticipated, a canonical inhibitor, cisapride was significantly less potent against F557L than against WT I_{hERG} (exhibiting a 9-fold greater half-maximal inhibitory concentration for F557L). Collectively, our findings complement and extend prior studies of the F557 residue, as they argue against a dependence of pharmacological block on this site independent of a compound's reliance on interaction with Y652 (as occurs with canonical inhibitors such as cisapride (Saxena et al, 2016)).

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PC052

Causes and Consequences of Preterm Birth

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INTRODUCTION

Globally PTB [<37 weeks gestational duration] is the major cause of death and disease in newborns, particularly in developing countries (Bonamy et al. 2005). Approximately 15 million babies with preterm birth (PTB) born each year and 28% of child deaths are related to prematurity (D'Silva et al. 2018).

METHOD

A subset of 15 appropriate studies to pool potential causes and 9 eligible studies considering possible effects of PTB from 2004 to 2021 was collected.

RESULTS

CAUSES: Yang et al. (2016) found the elevation in urinary Cadmium was associated with increased chance of PTB. Owen et al. (2017) described that anxiety and depression are the major factors predicting PTB. Zhang et al. (2017) discovered a significant association of four loci EBF1, EEFSEC, AGTR2 and WNT4 with gestational period. Tabatabaei et al. (2017) described that insufficiency of VTD increases the risk of PTB. Jue Liu et al. (2017) proved that HBV in mothers increases the risk of developing PTB. Stout et al. (2017) concluded that decreased vaginal microbiome community leads to PTB. Núria Baños et al. (2018) observed significant reduction in cervical consistency index in women who labored at <37 weeks. Jiang et al. (2018) found that approximately three times higher Thallium concentration was related to 0.99-day reduction in gestational duration. Liu et al. (2018) determined that risk of small gestational age increases by arsenic exposure in third trimester. Kolstad et al. (2020) estimated the risk for PTB by all autoimmune rheumatic diseases. Xiong et al. (2020) described the inverse relation of maternal plasma total protein level with PTB risk.

Anderson et al. (2021) determined that HIV exposed unaffected infants experience PTB and low birthweight more than HIV unexposed unaffected infants. Yuan et al. (2021) supported the link between maternal thyroid dysfunction and PTB. Vousden et al. (2021) found that risk of cesarean and PTB increases with impact of SARS-CoV-2 infection on pregnant females.

EFFECTS Paul et al. (2004) proved experimentally that children with PTB may face risk of type 2 diabetes mellitus. Anna-Karin et al. (2005) concluded that PTB face future cardiovascular risk. Mai luu et al. (2009) that more school services are required for preterm children in reading, writing and mathematics. Ball et al. (2012) evidenced the disruption of cerebral development due to PTB. Carr et al. (2017) described the strong association of heart failure with PTB. Crump et al. (2019) found the association of 20-30% increased risk of Chronic Kidney disease from birth to mid adulthood with PTB. He et al. (2020) indicated that PTB may cause obstructive lung diseases. Seppälä et al. (2020) described that PTB increases the risk of childhood cancer particularly germ cell tumors and acute myeloid leukemia. kumari et al. (2021) noticed the higher incident of cardio metabolic diseases in survivors of PTB.

CONCLUSION To our knowledge, this is the first comprehensive study that summarize the potential causes and effects of PTB. It will help clinicians to overcome the major causes of birth mortalities and to improve the outcomes of at-risk pregnancies.

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PC053

A novel dietary intervention reduces circulatory branched-chain amino acids by 50%: A pilot study of relevance for obesity and diabetes

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Abstract:

Background: Elevated circulating branched chain amino acids (BCAAs; isoleucine, leucine and valine) are associated with obesity, and type 2 diabetes (T2D). Reducing circulatory BCAAs by dietary restriction was suggested to mitigate these risks in rodent models, but this is a challenging paradigm to deliver in humans.

Aim: We aimed to design and assess the feasibility of a diet aimed at reducing circulating BCAA concentrations in humans, while maintaining energy balance and overall energy/protein intake.

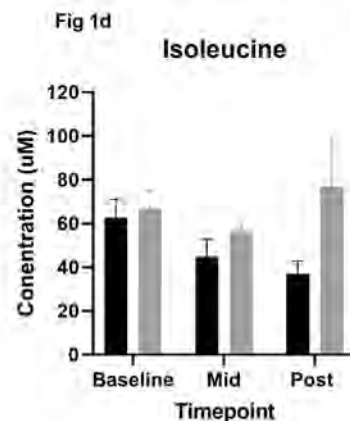
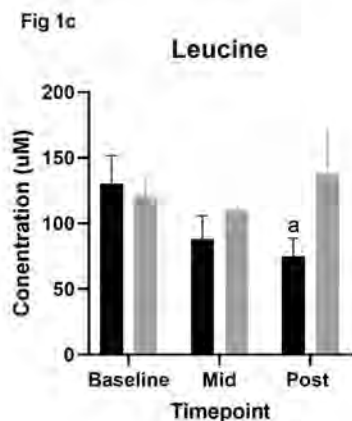
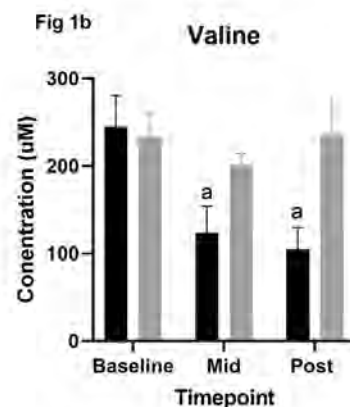
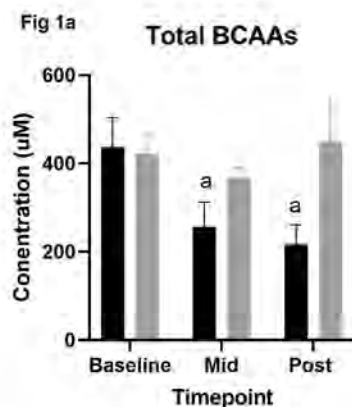
Method: Twelve healthy individuals were assigned to either a 7-day BCAA-restricted diet or a 7-day control diet. Diets were iso-nitrogenous and iso-caloric, with only BCAA levels differing between the two.

Results: The BCAA restricted diet significantly reduced circulating BCAA concentrations by ~50% i.e. baseline 437±60 to 217±40 µmol/L (p<0.005). Individually, both valine (245±33 to

105±23µmol/L;p<0.0001), and leucine (130±20 to 75±13µmol/L;p<0.05), decreased significantly in response to the BCAA restricted diet. The BCAA restricted diet marginally lowered Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) levels: baseline 1.5±0.2 to 1.0±0.1; (p=0.096).

Conclusion: We successfully lowered circulating BCAAs by 50% while maintaining iso-nitrogenous, iso-caloric dietary intakes, and while meeting the recommended daily allowances (RDA) for protein requirements. The present pilot study represents a novel dietary means by which to reduce BCAA, and as such, provides a blueprint for a potential dietary therapeutic in obesity/diabetes.

Ethics: All participants gave written informed consent before inclusion in the study in accordance with the Declaration of Helsinki, and approved by University of Nottingham Ethics committee, reference number 158-1711 approved on the 24th of November 2017.



■ BCAA restricted diet
■ Control diet

PC054

Identification of an epithelial cell line for two- and three-dimensional studies of intestinal ion transport.

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Cystic fibrosis (CF) is an inherited disease caused by loss of function of the epithelial anion channel cystic fibrosis transmembrane conductance regulator (CFTR). In addition to pulmonary symptoms commonly associated with CF, many individuals experience gastrointestinal tract complications, caused by impaired salt and water secretion across the intestinal epithelium. Intestinal organoids grown from rectal biopsies provide a powerful tool to investigate CFTR dysfunction in CF, and aid the identification of small molecule CFTR modulators for personalised medicine (1, 2). Here we sought an intestinal epithelial cell line suitable for use in the study of CFTR-mediated ion transport in the intestine. The cell line should possess both the ability to grow as a 2D epithelial monolayer for Ussing chamber studies, and as 3D organoids to optically measure CFTR-mediated lumen expansion.

Initial studies using the human colonic LIM1863 cell line, which expresses CFTR and forms organoids resembling colonic crypts (3), revealed that this cell line was unable to form polarised epithelia with a transepithelial resistance when seeded onto permeable filter supports ($n > 3$). Subsequently a panel of 15 human colorectal adenocarcinoma cell lines were screened to identify a cell line suitable for Ussing chamber and organoid studies.

Immunoblotting studies using the anti-CFTR-596 mouse monoclonal antibody (4) detected CFTR in 7 of the 15 cell lines screened. When the 7 CFTR-expressing cell lines were seeded onto permeable filter supports, only HCA7 (human colonic adenocarcinoma 7) cells developed a transepithelial resistance $> 0.5 \text{ k}\Omega \text{ cm}^2$ ($n > 20$). To investigate transepithelial ion transport by HCA7 cells, we mounted HCA7 epithelia in Ussing chambers, imposed a large Cl^- concentration gradient across the epithelium ($[\text{Cl}^-]_{\text{basolateral}}, 149 \text{ mM}$; $[\text{Cl}^-]_{\text{apical}}, 14.8 \text{ mM}$), clamped transepithelial voltage at 0 mV and recorded short-circuit current (I_{sc}) continuously (5). Forskolin ($10 \mu\text{M}$) stimulated a small increase in I_{sc} that was inhibited by CFTR_{inh}-172 ($10 \mu\text{M}$) ($n > 20$). Complementary to these studies, when HCA7 cells were grown in Matrigel for 14 days, they formed organoids with a lumen. When treated with forskolin ($10 \mu\text{M}$), organoid lumen increased ($n > 20$). By contrast, pre-treatment of HCA7 organoids with CFTR_{inh}-172 ($10 \mu\text{M}$) reduced the lumen expansion stimulated by forskolin ($10 \mu\text{M}$) ($n > 20$). We interpret these data to suggest that CFTR mediates cAMP-stimulated ion transport by HCA7 cells. The data also suggest that HCA7 cells might prove a useful model of intestinal ion transport because they form both polarised epithelia and organoids.

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PC055

Adipose depot dependency of chloride channels expression in murine white fat.

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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) the expression of ion channels and their associated roles remain unclear. Given the importance of chloride channels in WFA membrane potential (Pulbutr et al., 2007; Bentley et al., 2014), our aim was to identify these at the molecular level. We explored this issue in adipocytes isolated from different adipose depots of adult Wistar rats, CD-1 mice, as well as in a common adipocyte cell line model: 3T3-L1. Animal care and experimental procedures were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act (1986) and were locally approved. Deepseq revealed the expression of various chloride channels isoforms in the visceral WFA of adult male rat epididymis. Among these, putative plasma membrane chloride channels were the volume regulated chloride channels *Lrrc8a/b/c/d* and *Ttyh2/3*, and the calcium-activated chloride channel, *Ano1*. Relative expression was confirmed by RT-qPCR in WFA from visceral (rVWFA), mesenteric (rMWFA), perirenal (rPWFA) and subcutaneous (rSWFA) fat depots of rats, mouse subcutaneous (mSWFA) as well as differentiated 3T3-L1 cells. Statistical significance as $p < 0.01$, to account for repeated use of data, was determined by ANOVA with Dunnett's multiple comparison test relative to rSWFA for between depots, and relative to *Ano1* for within depots. Data are given as ratios (95% Confidence intervals, number of determinations) relative to rSWFA or *Ano1*.

Comparison between depots showed no difference in expression for *Lrrc8a*, *Lrrc8c*, *Lrrc8d*, *Ttyh3* and *ANO1* relative to rSWFA, whereas for 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. For 3T3-L1 cells, *Lrrc8c* was expressed at 2% (1 to 57, $n=4$) of that seen in rSWFA.

Comparison within depots showed that for rVWFA and rMWFA, *Lrrc8a* was expressed greater than *Ano1* by 7.4 (3.3 to 43, $n=5$) and 9 (5.1 to 18, $n=5$) fold respectively. In pSWFA; *Lrrc8a*, *Lrrc8c* and *Ttyh2* were all expressed greater than *Ano1* by 11 (5.7 to 92 (90%), $n=5$), 6.9 (3.2 to 56 (90%), $n=5$) and 7.5 (3.9 to 61, $n=5$) fold, respectively. Whereas for rSWFA and mSWFA only *Lrrc8c* was expressed greater by 9.6 (2 to 174 (90%), $n=5$) and 73 (11 to 173, $n=5$) fold, respectively. For 3T3-L1 cells, *Ttyh2* was expressed at ~2000% (CI interminable, $n=4$).

Our results show that the expression levels of chloride channel related genes differ both between adipose depots and within 3T3-L1 cells. The *Lrrc8x* chloride ion channel family had the greatest

expression. The consequences of these expression profiles for depot-dependent adipose function are being investigated.

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PC056

An investigation of the effects and mechanism of Adenocin triphosphate on pregnant human myometrial contractility.

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Background: Dystocic labours, also known as slow to progress labours, represent an important clinical and research challenge (Wiberg-Itzel et al., 2018). The uncoordinated myometrial contractions associated with dystocia cannot dilate the cervix, and thus ultimately, these labours end with an unplanned caesarean delivery. Adenosine triphosphate (ATP) can act extracellularly as a signalling molecule regulating numerous physiological and pathophysiological conditions (Burnstock, 2017). In response to hypoxia, cells can release ATP into the extracellular milieu of rat ventral medulla (Gourine et al., 2005). A study on rat myometrial tissue have shown that extracellular ATP increases uterine contractions (Zafrah et al., 2017). Further studies on human and rat myometrium have suggested that ATP stimulates P2X1 or P2X7receptos (P2X7R) (Miyoshi et al., 2012, Alotaibi, 2018, Ziganshin et al., 2006). Moreover, evidence has been established that extracellular ATP appears critical for the initiation of human myometrial contraction and controls their frequency; however, the underline mechanism is unknown (Hutchings et al., 2009).

Objective: I hypothesized that the expression of purinergic 2X receptors, P2X1Rs or P2X7Rs, would increase towards term pregnancy, and ATP binding to one of these receptors could potentiate the myometrium contraction, which would help in contractile augmentation during the onset of labour. On the other hand, the depletion of one or both receptors would lead to dysfunctional labour. **Methods:** human myometrial tissues were obtained from non-pregnant women undergoing hysterectomy, term pregnant labouring normal women but undergoing emergency CS deliveries due to fetal distress, term pregnant labouring women undergoing emergency CS deliveries due to dysfunctional labour, and term pregnant non-labouring women undergoing elective CS deliveries (n=3-10). Immunoblotting studies measured the level of expression of P2X1Rs & P2X7Rs. The effect of ATP and its analogues, ATPγS (a non-hydrolyzing form of ATP) and BzATP (a more potent agonist at the P2X7R), on labouring and non-labouring human myometrial contractility, were also examined using tissue baths (n=7-10). To further determine P2X7R roles in mediating this action, selective

antagonists, A-438079 & A-740003, were used (n=4). The human uterine tissue taken from tissue bank in accordance with Liverpool Women's Hospital ethical committee approval. **Results:** P2X1Rs were expressed in non-pregnant human myometrium, and their abundance increased in term pregnant myometrial tissues with no significant difference between labouring and non-labouring women. P2X7Rs expression in the normal labouring group (fetal distress) was significantly greater than all other groups (P= 0.0025). All three agonists increased contraction frequency significantly with the rank order of ATPyS> BzATP>ATP (472.7±82.8%> 253±30.8%> 209.8±28.5%), respectively. No significant differences were found in the agonists' stimulatory effects in the presence of the P2X7 antagonist compared to their control response. Furthermore, no significant differences in the effect of ATP on myometrial activities between non-labouring and dysfunctional labour women. **Inconclusion:** Expression data clearly showed the presence of P2X7R in human myometrial tissue, and their abundance might reflect the ATP response in the non-labouring and dysfunctional labouring human myometrium. However, investigating the effect of ATP on labouring normal myometrial tissue would be the next step.

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PC057

Systemic sex hormones do not explain resting fat oxidation or peak fat oxidation during exercise in women with varying menopausal states

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Menopause is considered to reduce whole-body fat oxidation due to systemic oestrogen deficiency. The premise is based on a few human studies, and no data exist if hormonal status explains peak fat oxidation (PFO) during exercise in middle-aged women. Menopause is linked to an increase in cardiometabolic disease incidence. One underlying mechanism could be insulin sensitivity loss due to fat oxidation capacity decline and subsequent accumulation of lipid intermediates in oxidative tissues. Ageing is often a confounder in menopause studies. Therefore, we studied if serum sex hormone profile explains resting fat oxidation (RFO) and PFO among similar age women with varying menopausal states. We also investigated associations between the fat oxidation measures and oral glucose tolerance test (OGTT) outcomes.

Our sample was 42 women (mean age 55.3 [*SD* 1.6] years), of which seven were pre-or perimenopausal, 26 were postmenopausal, and nine were postmenopausal oestrogen-containing hormone therapy users. Four postmenopausal women were excluded from between-group comparisons due to premenopausal range oestradiol levels. We measured RFO and PFO with indirect calorimetry at separate laboratory visits. PFO was determined during a bicycle-ergometry test using four-minute stages and 20 W workload increases. Cardiorespiratory fitness, quantified as maximum workload, was then measured with a ramp protocol to voluntary exhaustion. Standard 2-h OGTT was performed after RFO measurement. Blood-based biomarkers were measured using clinical chemistry. Lean body mass (LBM) and fat mass were determined with dual-energy x-ray absorptiometry. Physical activity was assessed with a questionnaire and accelerometers. Diet was recorded with two-day food diaries before measurements. We analysed between-group differences using ANOVA with Tukey's *post hoc* test and individual-level associations using multivariable linear regression. The Ethics Committee of the Central Finland Health Care District approved the study (Dnro 9U/2018). Participants gave informed consent.

The menopausal groups had discordant oestradiol ($F [2, 35] = 44.74, P < 0.0001$) and follicle-stimulating hormone FSH ($F [2, 35] = 18.24, P < 0.0001$) levels but similar LBM relative RFO ($F [2, 32] = 1.67, P = 0.2041$) and PFO rates ($F [2, 32] = 0.10, P = 0.9047$) (Fig. 1). We did not observe statistically significant associations between the measured sex hormones and fat oxidation (Table 1). RFO was positively associated with fat mass (standardised regression coefficient [β] = 0.44, $P = 0.0057, n = 39$) and negatively with energy intake before assessment ($\beta = -0.41, P = 0.0188, n = 39$). PFO was positively associated with cardiorespiratory fitness ($\beta = 0.59, P = 0.0019, n = 39$), LBM ($\beta = 0.48, P = 0.0022, n = 39$) as well as self-reported ($\beta = 0.41, P = 0.0116, n = 39$) and accelerometry-measured physical activity ($\beta = 0.36, P = 0.0196, n = 37$). RFO was positively associated with OGTT glucose and insulin areas under the curves; however, we observed no statistically significant associations with PFO and OGTT outcomes (Table 2).

In conclusion, menopause may not have a meaningful impact on RFO or PFO over their well-known determinants. Higher whole-body fat oxidation rates do not necessarily reflect better insulin sensitivity.

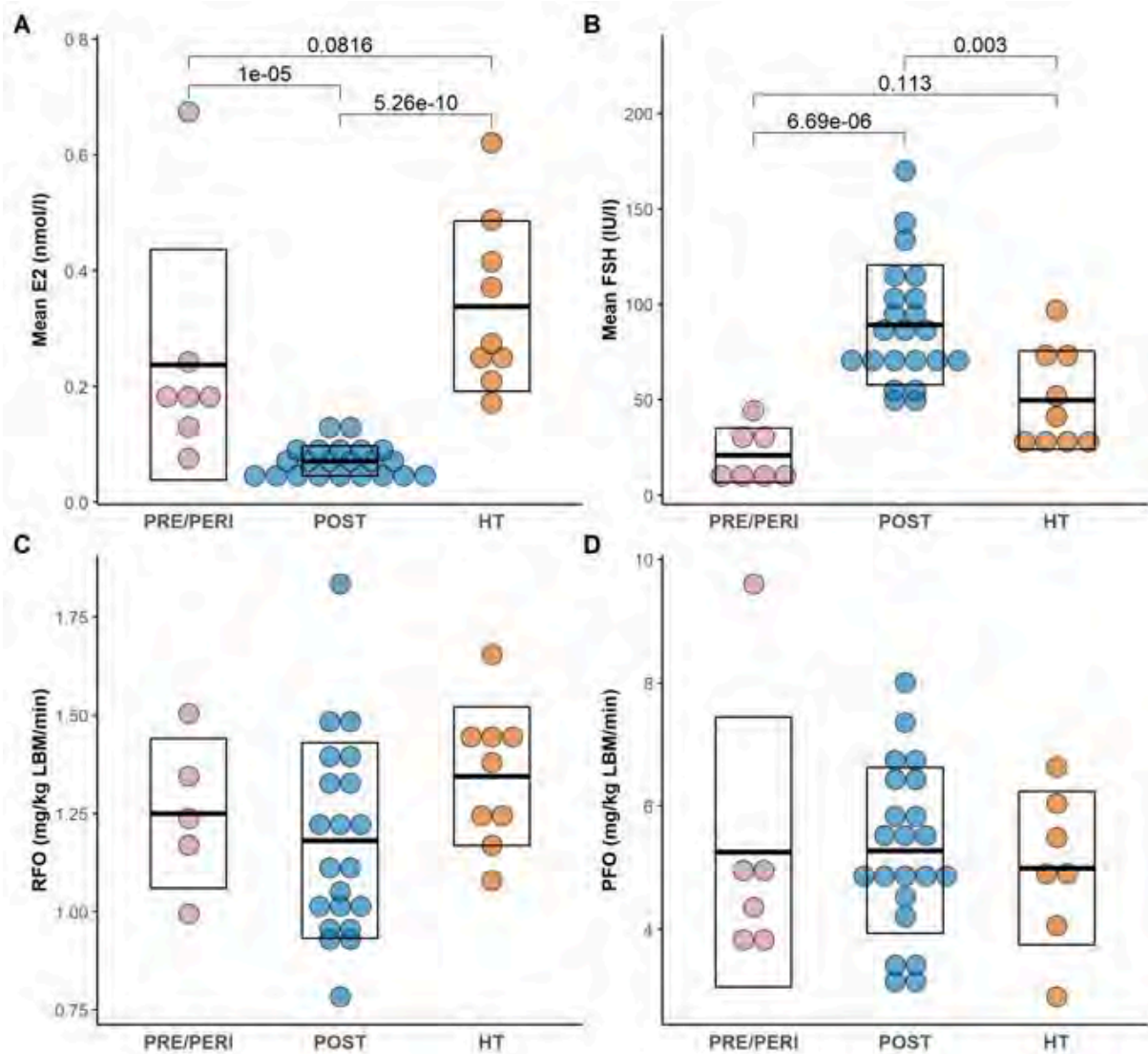


Fig 1. The figure presents individual data with group means, standard deviations, and between-group comparison *P*-values. Figs 1A. and 1B. show that serum oestradiol (E2) was lower and follicle-stimulating hormone (FSH) was higher in postmenopausal women (POST) compared with pre- or perimenopausal women (PRE/PERI) and postmenopausal hormone therapy (HT) users. However, lean body mass (LBM) relative resting fat oxidation (RFO, Fig 1C.) and peak fat oxidation (PFO, Fig 1D.) were similar across the studied groups. RFO measurement was unreliable in two PRE/PERI women and one POST woman. PFO determination was unreliable in one PRE/PERI and two HT using participants. Data were excluded from analyses.

Table 1. Pooled associations between serum sex hormones and resting fat oxidation (RFO) or peak fat oxidation (PFO) during exercise in the whole study sample. E₂ and progesterone were log-transformed to improve residual normality. Note that the adjusted R²s represent the variance proportion all the explanatory variables in the model explain together. The results show that serum sex hormones provide little value in explaining RFO or PFO over their main determinants.

	Lean body mass adjusted model					Fully adjusted model [#]				
	β	95% CI	P-value	R ² adj	P-value	β	95% CI	P-value	R ² adj	P-value
RFO (n = 39)										
Model without the studied sex hormone				0.03	0.1324				0.35	0.0004
E ₂ ^a	0.22	-0.10 to 0.53	0.1705	0.06	0.1263	0.10	-0.17 to 0.37	0.4392	0.34	0.0010
FSH	-0.21	-0.54 to 0.12	0.2000	0.05	0.1424	-0.09	-0.38 to 0.19	0.5037	0.34	0.0011
Progesterone ^{a,§}	0.20	-0.15 to 0.56	0.2509	0.01	0.3014	0.13	-0.17 to 0.43	0.3815	0.32	0.0038
Testosterone	0.27	-0.06 to 0.59	0.1049	0.08	0.0864	0.12	-0.17 to 0.40	0.4120	0.34	0.0010
SHBG	0.07	-0.26 to 0.40	0.6675	0.01	0.2982	-0.03	-0.34 to 0.27	0.8209	0.33	0.0013
PFO (n = 39)										
Model without the studied sex hormone				0.21	0.0022				0.39	0.0002
E ₂ ^a	0.02	-0.27 to 0.31	0.8917	0.18	0.0099	0.04	-0.22 to 0.31	0.7355	0.37	0.0005
FSH	0.16	-0.13 to 0.45	0.2587	0.21	0.0052	0.14	-0.11 to 0.40	0.2660	0.39	0.0003
Progesterone ^a	0.23	-0.05 to 0.51	0.0988	0.24	0.0025	0.19	-0.06 to 0.44	0.1296	0.41	0.0002
Testosterone	0.18	-0.11 to 0.46	0.2189	0.22	0.0046	0.20	-0.05 to 0.45	0.1162	0.41	0.0002
SHBG	0.06	-0.27 to 0.38	0.7276	0.19	0.0094	0.01	-0.28 to 0.30	0.9500	0.37	0.0005

^a log-transformed in the analysis, [§] n = 34 as in five postmenopausal women the values were below the detection limit.

β, standardised regression estimate; CI, confidence interval; E₂, 17β-oestradiol, FSH, follicle-stimulating hormone, SHBG, sex hormone-binding globulin

[#] RFO: lean body mass, fat mass and energy intake; PFO: lean body mass, maximum workload, and self-reported physical activity.

Table 2. Lean body mass adjusted associations between resting fat oxidation (RFO) or peak fat oxidation (PFO) and log-transformed oral glucose tolerance test outcomes. The sample size was 39 unless stated otherwise. The adjusted R²s represent the variance proportion lean body mass and the fat oxidation measure explain together.

	β	95% CI	P-value	R ² adj	P-value
HOMA-IR					
RFO	0.21	-0.15 to 0.56	0.2438	0.04	0.4958
PFO, n = 38	-0.32	-0.75 to 0.11	0.1393	0.09	0.1860
Matsuda index					
RFO, n = 38	-0.33	-0.68 to 0.02	0.0627	0.10	0.1653
PFO, n = 37	0.25	-0.22 to 0.72	0.2871	0.04	0.5566
Glucose tAUC					
RFO	0.52	0.21 to 0.82	0.0015	0.27	0.0037
PFO	0.00	-0.37 to 0.36	0.9852	0.01	0.8365
Insulin tAUC					
RFO	0.42	0.10 to 0.75	0.0123	0.18	0.0268
PFO	-0.10	-0.47 to 0.28	0.6102	0.06	0.3478

P-values < 0.05 are on bold.

tAUC, total area under the curve; β, standardised regression estimate; CI, confidence interval;

HOMA-IR, homeostatic model assessment of insulin resistance

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PC058

Inhibitory activities of n-hexane fraction of *Moringa oleifera* leaves(Lam.) against phospholipases isolated from *Naja haje* and *Naja nigricollis*venoms: *in vitro* and *in silico*

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Phospholipases are one of the principal toxic components of snake venom inducing a wide variety of pharmacological activities after envenomation. Natural inhibitors are known to inhibit toxic effects of snake venom enzymes. In this study, ethanol crude extract of *M. oleifera* was partitioned using n-hexane and ethyl acetate after which fractionation was done using column and thin layer chromatography. Subsequently, the inhibitory activities of the crude extract and sub-fractions of *M. oleifera* were investigated against phospholipase enzymes isolated from *Naja haje* and *Naja nigricollis* venoms *in vitro* and *in-silico* while EchiTab-PLUS polyvalent antivenom was used as the standard drug. The molecular weight of isolated *N. Haje* phospholipase (NH-PL) and *N. nigricollis* phospholipase (NN-PL) were 24.11 and 35.22 kDa respectively while NH-PL enzymes had specific activity of 2.70 $\mu\text{M}/\text{min}/\text{mg}$ substrate, NN-PL activity was 2.10 $\mu\text{M}/\text{min}/\text{mg}$ substrate. Furthermore, K_m of NH-PL was 0.330 μM with V_{max} of 0.085 $\mu\text{M}/\text{mL}\cdot\text{min}$ while NN-PL had V_{max} of 0.198 $\mu\text{M}/\text{mL}\cdot\text{min}$ and K_m of 0.670 μM . *M. oleifera* n-hexane sub-fraction 5 (MOLH5) displayed a complete inhibition of NN-PL enzyme at high concentrations and achieved total inhibition against NH-PL enzyme activity at all concentrations used. Molecular docking of the phytoconstituents of n-hexane sub-fraction (MOLH5) against venom phospholipase A2 showed 2-Hydrazino-8-hydroxy-4-phenylquinoline, the lead with a docking of -6.789 kcal/mol. Further *in-silico* studies revealed the lead as a potential drug candidate. Results indicated that the presence of natural inhibitors of phospholipases in *M. oleifera* n-hexane sub-fraction could assist in the development of alternative therapy in the treatment of snake envenoming.

PC059

Effect of a single session of exercise on salivary cytokines and salivary cortisol in young male judoists

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INTRODUCTION: Cytokines are important regulators of physiological growth through their direct interaction with the growth hormone - insulin-like growth factor-I axis, through that are mediated anabolic effects of exercise (Scheett et al., 1999; Nemet et al., 2002). Moreover, cytokines provide a link between the immune and endocrine systems to modulate adequate responses to different stressors (Elenkov, 2008). While acute changes in systemic pro-/anti-inflammatory cytokines occur with exercise in children (Rosa et al., 2007), effects of exercise on salivary cytokines and their relationship with cortisol in young athletes remain poorly studied. The purpose of present study was

to examine effect of a single session of exercise on salivary cytokines and salivary cortisol in young male judoists.

METHODS: Nine prepubertal boys aged from 8 to 10 years (Group 1) and eighteen adolescent boys aged from 13 to 16 years (Group 2) practicing judo were involved in the study. Mothers of all participants signed a voluntary informed consent. None of the participants was suffered from acute or chronic diseases of oral covary. Saliva samples were obtained before and after a routine judo session (mixed muscle type of training, 1 h 30 min, motor density of the session is 80%). The concentrations of IL-6, IL-8 and cortisol were determined using ELISA. Values are medians and interquartile intervals (Me; Q1-Q3), compared by non-parametric models. Statistical significance was accepted at $P<0.05$.

RESULTS: It was found that salivary concentrations of IL-6 and IL-8 were higher in adolescents when compared with children before a session of judo (IL-6: 31.2; 17.9-51.9 vs. 17.05; 8.17-23.1 pg/mL, $P<0.05$; IL-8: 1417.5; 706.0-1899.0 vs. 158.2; 104.6-502.0 pg/mL, $P<0.05$, respectively). A single session of judo did not affect the salivary IL-6 content in both adolescents and children. In contrast to IL-6, IL-8 levels increased after judo session in both children and adolescents. Thus, the content of IL-8 in saliva in children after exercise raised in 2.5 times and was 390.0; 222.0-494.0 pg/mL ($P<0.05$). In adolescents, the post loading level of IL-8 in saliva increased in 1.4 times to 1998.0; 1299.0-2234.0 pg/mL, $P<0.05$. There were no significant age-related differences in salivary cortisol levels either before or after exercise (children: 18.0; 12.13-28.5 vs. 19.65; 13.2-31.88 ng/mL, $P>0.5$; adolescents: 15.9; 9.49-24.9 vs. 24.05; 14.4-33.2 ng/mL, $P>0.5$, respectively).

CONCLUSION: Taken together, our results indicate that a single session of judo does not lead to a change in salivary concentration of cortisol in children and adolescents. This is likely due to the adaptation of body to regular training. We also demonstrated that the concentrations of IL-6 and IL-8 in saliva depended on the age of children, and the effects of exercise on these cytokines were different: the level of IL-6 did not change, and the concentration of IL-8 in saliva increased after training. Probably, it is due to the fact that the stress reactivity of salivary cytokines can reflect not only the processes in the immune system and in the hypothalamus-pituitary-adrenal system but also depends on the activity of the sympathetic nervous system.

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The modification of choline and betaine metabolism to TMA by the gut microbiota using polyphenol rich foods

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Introduction: Plasma levels of trimethylamine oxide (TMAO), a metabolite of TMA which is exclusively produced from choline, l-carnitine and betaine by the gut microbiota, has been shown to predict the risk of death from heart failure [1]. Plasma TMAO has also been reported to correlate with a range of non-communicable diseases such as cardiovascular disease (CVD), diabetes and neurodegenerative diseases which pose serious health, social and economic burdens worldwide [2, 3]. However, there are no current sustainable treatments to reduce TMAO levels in those at risk. Polyphenol-rich diets have been shown to protect against metabolic diseases such as CVD [4].

Aims and methodology: We investigated the potential and the capacity to which foods rich in polyphenols can alter TMA production from choline and betaine using the *in-vitro* batch fermentation human colon model [5], followed by metabolite analysis using LC-MS. We tested a black rice (BR) anthocyanin extract, an ellagitannin-rich pomegranate peel extract (Dermogranate™) and purified resveratrol. A written informed consent was obtained from all participating subjects, the trial is registered at [http:// www. Clinical trials. gov](http://www.ClinicalTrials.gov) (NCT02653001) and the study was approved by the London—Westminster Research Ethics Committee (15/LO/2169) and the Quadram Institute Bioscience (Human Research Governance committee (IFR01/2015)).

Results: We demonstrate that BR anthocyanins (predominantly cyanidin-3-Glc) reduced TMA production from choline by 22% in a manner that is consistent with inhibition of the choline TMA-lyase pathway ($F(1645, 1158) = 487.4, P < 0.05, N = 5$). The Dermogranate®, which contains mainly ellagitannins including β -punicalagin, reduced TMA production from choline in a manner consistent with the inhibition of both the TMA lyase ($F(914.7, 886.6) = 193.2, P < 0.0001, N = 4$) and the betaine reductase pathway ($F(173.5, 409.1) = 235.5, P < 0.001, N=4$). We substantiated this notion by showing inhibition of betaine metabolism in the presence of choline and a lack of betaine production from choline in experiments in which no betaine was supplemented. Dermogranate™ treatment also increased the production of lactic acid, suggesting a prebiotic activity ($F(2.1, 15.36) = 13.26, P < 0.05, N=4$). Resveratrol had no significant effect on TMA production from choline.

Conclusion: We have demonstrated the potential for BR and Dermogranate™ to be developed as functional foods that may be of benefit to people who have a high capacity to produce TMA/TMAO and are therefore at greater risk of developing chronic diseases or of extreme events after heart failure. The Dermogranate™ may also have prebiotic activity.

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PC061

(-)-Epicatechin and its colonic metabolite hippuric acid protect against dexamethasone-induced atrophy in skeletal muscle cells.

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INTRODUCTION: Cocoa flavanols have been shown to improve muscle function¹ and exercise capacity² and thus may offer a novel approach to protect against muscle atrophy. However, whether cocoa flavanols directly protect against atrophic conditions remains to be determined. (-)-Epicatechin (EPI) is the primary bioactive compound of cocoa flavanols, but it has a poor bioavailability and is ultimately metabolised into smaller bioactive compounds³. Hippuric acid (HA) is a colonic metabolite

of EPI that is present in circulation from 12-48h³ following cocoa ingestion and may be responsible for the associations between chronic cocoa supplementation and muscle metabolic alterations.

OBJECTIVE: Accordingly, we investigated the effects of EPI and HA upon skeletal muscle morphology and metabolism within an *in vitro* model of skeletal muscle atrophy.

METHODS: Differentiated C2C12 myotubes were exposed to 24h ± dexamethasone (DEX; 100 µM) to create ± atrophic conditions. To examine whether cocoa flavanol compounds confer protection against dexamethasone-induced atrophy, cells were concomitantly co-incubated with one of the following treatments: vehicle control (VC), EPI (25 µM) or HA (25 µM). Following the 24h treatment, myotube diameter was assessed using fluorescence microscopy and analysed using a two-factor mixed model ANOVA (*n* = 5). To assess basal and leucine (LEU)-stimulated myotube protein synthesis (MPS) using the surface sensing of translation technique, cells underwent a further 90-min incubation period with LEU (5 mM) or a volume-matched control. MPS data are presented as fold change relative to control conditions (VC, no DEX) and were analysed using a 3-factor mixed model ANOVA (*n* = 6). Data are presented as mean ± SEM.

RESULTS: Under atrophy-inducing conditions (DEX), myotube diameter was significantly greater in HA (11.19 ± 0.39 µm) and EPI (11.01 ± 0.21 µm) treated cells compared to the VC (7.61 ± 0.16 µm, both *P*<0.001). In basal and LEU-stimulated MPS measurements, there was a significant reduction in MPS rates following DEX-treatment in VC (fold change: 0.62 ± 0.07, *P*=0.024). Interestingly, concomitant treatment with EPI (fold change: 0.89 ± 0.16, *P*=0.148) and HA (fold change: 1.34 ± 0.40, *P*=0.205) abrogated the dexamethasone-induced reductions in MPS rates.

CONCLUSION: EPI and HA exerted anti-atrophic effects on skeletal muscle cells. Accordingly, our data suggests that HA could be partly responsible for the association between cocoa flavanols and skeletal muscle cellular metabolism and highlights the importance of colonic metabolites in the association between flavanol supplementation and health.

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Phoenixin 14 Ameliorates Pancreatic Injury In Streptozotocin Induced Diabetic Rats By Alleviating Oxidative Damage

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Phoenixin (PNX) is a neuropeptide, has a role in reproduction, food intake, energy balance behaviour, and response to stress. PNX immunostaining has been shown in periphery of islets of Langerhans(1). It stimulates insulin secretion, promotes glycolysis, prevents gluconeogenesis (2,3), reduces inflammation and ROS formation in mice model of fatty liver (4).

Aim of the study to investigate in vivo effect of PNX on experimental rat model of diabetes induced by streptozotocin (STZ) and nicotinamide (NA) which causes oxidative stress in B cells of pancreatic islets.

Experimental protocols were approved by the Marmara University Animal Care and Use Committee. Male, 12 weeks old, Sprague-Dawley rats were injected PNX-14/vehicle in dosages of 0.45 and 45 nmol/kg intraperitoneally (ip) 7 day before and 8-9-10 days after induction of diabetes by NAD 110 mg/kg and STZ 65 mg/kg ip administration. Glucose tolerance were measured before STZ injection. Fasting blood glucose and, gastric emptying rate of methyl cellulose was measured at the last injection date. Rats were decapitated. Blood, pancreas, ileum and liver were harvested. Plasma insulin, testosterone, tissue myeloperoxidase activity (MPO), luminol and lucigenin enhanced chemiluminescence, tissue insulin mRNA was measured. Histological evaluation was done. Insulin immunoreactivity was expressed as percentage of total islet area in insulin immunolabeled slides.

Results were summarized in Table.1.

Table.1.Effects of PNX on blood glucose, gastric emptying rate and pancreas*

	FBG mg/dL	% gastric emptying	Luminol rlu/mg tissue	Lucigenin rlu/mg tissue	% area of Insulin IR cell
NC (n=5)	77±6.7	52,6±17.2	15.3±1.9	21.8±10.5	34.8±17.3

N-PNX-0.45 n=6	74±9.8	49,1±10.52	27,4±4.99	16.0±3.2	54.0 ±6.8
N-PNX-45 n=6	73±15.8	53,3±8,99	21.4±6.4	19.4±5.2	66.4±6.8
DC n=6	189.9±124	68,1± 5.4	56.4±16.8	59.9±16.2	18.8±10.2
D-PNX-0.45 n=6	176.8±86.2	75,6±7.3	32.4 ± 13.2	33.5± 12.4	49.2 ± 11.8
D-PNX-45 n=5	139.8±66.2	68, ±14.45	29.3±11.1	34.8 ± 11.6	29.6 ± 7.7
Two-way ANOVA					
Interaction	0.6	0.06	0.0001	0.0195	0.002
Diabetes	0.0004	0.0002	<0.0001	<0.0001	<0.0001
PNX	0.6	0.59	0.045	0.001	<0.0001

*mean± standart deviation, N, normal, D Diabetic, C saline PNX, Phoenixin IR, immunoreactive, FBG fasting blood glucose

PNX treatment increased insulin immunoreactive cells in pancreatic islets of normal rats. It regenerated organization of pancreatic islets, reduced number of degenerated cells in diabetic rats, increased percent area of insulin immunoreactive cells. PNX reduced oxidative injury in pancreas without altering MPO activity. PNX lowered fasting blood glucose at the end of experiment even in the presence of increased blood testosterone level. Plasma insulin level and expression of insulin mRNA in pancreas was not changed by PNX. Gastric emptying rate of semisolid meal decreased only in nondiabetic rats treated with PNX.

Our findings suggest that PNX reduces pancreatic injury and lowers blood glucose in advance by reducing oxidative burden. Further studies are needed to clarify if PNX may be an attractive alternative in the treatment of diabetes.

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PC063

Pilot Study on Metabolic Flexibility in Elderly Women: Preliminary Results

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Introduction: Metabolic flexibility (MF) is the ability of individuals to switch between energy substrates in response to changing physiological conditions (1), being affected by factors such as sex, age and training level (2). For instance, men reported 28% higher Maximum Fat Oxidation (MFO) compared to women (2). Age could also mediate this process due to a higher energy expenditure at rest as well as a poor mitochondrial function with aging, leading to higher lactatemia for a similar load and limiting the uptake of free fatty acids (1). Finally lower levels of training may also affect MF since sarcopenia and reduced insulin action may compromise lipid metabolism. This study aims to analyse the behaviour of MF in active older women by means of fatty acid oxidation (FATox) and carbohydrate oxidation (CHOox) values.

Methodology: Nine older women who met the inclusion criteria (> 60 years and moderately active) started this pilot study. 7 participants (73±7.23 years, 64±24.18 kg, and 30±11.33kg of muscle mass) completed the protocol. Participants performed an incremental cycling test which started at 30w and increased progressively 10w each three minutes until volitional exhaustion or technical criteria: visual

analogue scale of Pain (VAS) >5; rating of perceived effort (RPE) >7; respiratory exchange ratio (RER) >1.1.; and/or arterial oxygen saturation (SpO₂) <92%. Gas exchange, heart rate (HR) and power output completed these outcomes which were continuously recorded during the test, in addition to lactate pre- and post-test (3-5min).

FATox and CHOox calculation was performed by indirect calorimetry, analysing oxygen uptake (VO₂) and carbon dioxide production (VCO₂). From these data, Frayn's equations (3) were applied, taking the values of the last 60s of each level of intensity (4) with the assumption that urinary nitrogen excretion was 0, as previously stated (1).

Results: Table 1 and Table 2 show the main descriptives, accompanied with an increase in blood lactate (BLa_{baseline}: 1.4±0.4 mmol·L⁻¹; BLa_{3min} 5.87±3.33 mmol·L⁻¹; BLa_{5min} 4.87±2.8 mmol·L⁻¹).

Conclusions: Preliminary results reflect a remarkable metabolic inflexibility in older women, which is possibly a consequence of poor mitochondrial function (1), as supported by current literature (5). Moreover, it appears that their MFO (0.10±0.11 g·min⁻¹) could correlate with greater mitochondrial dysfunction in this population with respect to others with greater literature, i.e., obese or sedentary middle-aged, with markedly higher values: 0.36±0.12 g·min⁻¹ and 0.24±0.03 g·min⁻¹, respectively (2).

On the other hand, Table 2 displays that these participants reach their MFO at the relative power output of 60%, higher than moderately active middle-aged by San-Millan and Brooks (1). More than a remarkable improvement in MFO, it could be due to the short number of periods completed in our sample (100% very closed to the test starting intensities). In addition, current literature (3) already suggest that shorter periods of 10min, between 3 and 4 min, may be more appropriate for assessing MF in unfit population, due to the limited benefits of longer periods and the associated fatigue that may limit the performance of the test in populations with mitochondrial dysfunction.

Table 1.

Subjects characteristics at Peak Power Output

	PEAK									
	SpO ₂ (mmHg)	RPE	VAS	HR (bpm)	VO ₂ (ml/min/kg)	FATox (g·min ⁻¹)	CHOox (g·min ⁻¹)	RQ	Power (W)	Power (%W)
Mean	96.33	5.5	3.67	131	21.49	0.00	2.37	1.26	71.67	100
SD	1.03	1.64	3.14	24.40	5.50	0.00	0.48	0.30	19.41	0

Table 2.

Subjects Characteristics at Maximal Fat Oxidation

	MFO										
	SpO ₂ (mmHg)	RPE	VAS	HR (bpm)	VO ₂ (ml/min/kg)	FATmax (%VO ₂)	FATox (g·min ⁻¹)	CHOox (g·min ⁻¹)	RQ	Power (W)	Power (%W)
Mean	96.8	2.4	1.4	99.2	15.26	71.19	0.10	1.38	0.94	42	60
SD	1.64	1.52	1.52	16.16	2.96	16.66	0.11	0.99	0.05	10.95	0.19

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PC064

Retinoid X Receptor A (Exon 9) gene polymorphisms among healthy individuals of southern Pakistani population

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Retinoic X receptor (RXRs), comprising family of nuclear retinoids activated transcription factors, play myriad role in tissue homeostasis, embryonic development, different metabolic pathways, antiproliferation and chemoprotective effects in certain pathologies. Retinoic X receptor alpha (RXRA), member of cis-retinoic acid receptor family, encoded by the gene located on Chromosome 9q34.2 consists of 10 exons, heterodimerizes/ homodimerizes with other nuclear receptors via ligand binding domain and binds to DNA effectively via DNA binding domain, is a differentially expressed stereoisomer of RXRs in brain, skin, thyroid gland, cardiac muscles, prostate gland and nephrons in kidneys under normal physiological and pathological conditions. The expression variation is caused by SNPs, present in RXRA gene associating with number of pathologies. The promotor region of RXRA has been previously characterized. The present study is aimed to amplify the seven novel single nucleotide polymorphisms (SNPs) in exon 9 of RXRA gene, its sequencing and validation among normal disease free premenopausal females of Karachi, Pakistan.

A cross sectional study including 50 healthy females aged between 20 and 40 years was recruited from different areas of Karachi. Peripheral blood was collected for extraction of genomic DNA. PCR was performed using forward and reverse primers designed for exon 9 SNPs (rs368586400, rs1006838045, rs901061760, rs11542209, rs755448808, rs183422344 and rs779356437) of RXRA gene. The amplified products were electrophoresed via agarose gel electrophoresis and sequenced

from outsource. Hardy-Weinberg equilibrium was calculated to check the normal distribution of all SNPs and P value >0.05 were considered as consistent with HWE.

Results showed SNPs present on exon 9 of RXRA gene are consistent ($p > 0.05$) with HWE indicating that these SNPs were normally distributed among population. The genotype frequency of these SNPs in normal population is as rs368586400 CC (60%), rs1006838045, CT (50%), rs901061760 GG (60%), rs11542209 CT (60.86%), rs755448808 CC (50%), rs183422344 CA(62%) and rs779356437 AA (46.60%). The allele frequency of rs368586400 "C" vs "T", rs1006838045 "C" vs "T", rs901061760 "A" vs "G", rs11542209 "C" vs "T", rs755448808 "C" vs "T", rs183422344 "A" vs "C" and rs779356437 "A" vs "C" was 32 vs 9, 15.5 vs 26.5, 9.5 vs 30.5, 9 vs 14, 31 vs 13, 18 vs 14 and 10 vs 5 respectively in our population.

Hence it is concluded that the all studied SNPs of exon 9 of RXRA gene are normally distributed among healthy population.

Key words: RXRA, VDR, Polymorphism, SNPs

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Retinoid X Receptor A (Exon 9) gene polymorphisms among healthy individuals of southern Pakistani population

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Abstract

Retinoic X receptor (RXRs), comprising family of nuclear retinoids activated transcription factors, play myriad role in tissue homeostasis, embryonic development, different metabolic pathways, antiproliferation and chemoprotective effects in certain pathologies. Retinoic X receptor alpha (RXRA), member of cis-retinoic acid receptor family, encoded by the gene located on Chromosome 9q34.2 consists of 10 exons, heterodimerizes/ homodimerizes with other nuclear receptors via ligand binding domain and binds to DNA effectively via DNA binding domain, is a differentially expressed stereoisomer of RXRs in brain, skin, thyroid gland, cardiac muscles, prostate gland and nephrons in kidneys under normal physiological and pathological conditions. The expression variation is caused by SNPs, present in RXRA gene associating with number of pathologies. The promotor region of RXRA has been previously characterized. The present study is aimed to amplify the seven novel single nucleotide polymorphisms (SNPs) in exon 9 of RXRA gene, its sequencing and validation among normal disease free premenopausal females of Karachi, Pakistan.

A cross sectional study including 50 healthy females aged between 20 and 40 years was recruited from different areas of Karachi. Peripheral blood was collected for extraction of genomic DNA. PCR was performed using forward and reverse primers designed for exon 9 SNPs (rs368586400, rs1006838045, rs901061760, rs11542209, rs755448808, rs183422344 and rs779356437) of RXRA gene. The amplified products were electrophoresed via agarose gel electrophoresis and sequenced from outsource. Hardy-Weinberg equilibrium was calculated to check the normal distribution of all SNPs and P value >0.05 were considered as consistent with HWE.

Results showed SNPs present on exon 9 of RXRA gene are consistent ($p > 0.05$) with HWE indicating that these SNPs were normally distributed among population. The genotype frequency of these SNPs in normal population is as rs368586400 CC (60%), rs1006838045, CT (50%), rs901061760 GG (60%), rs11542209 CT (60.86%), rs755448808 CC (50%), rs183422344 CA(62%) and rs779356437 AA (46.60%). The allele frequency of rs368586400 "C" vs "T", rs1006838045 "C" vs "T", rs901061760 "A" vs "G", rs11542209 "C" vs "T", rs755448808 "C" vs "T", rs183422344 "A" vs "C" and rs779356437 "A" vs "C" was 32 vs 9, 15.5 vs 26.5, 9.5 vs 30.5, 9 vs 14, 31 vs 13, 18 vs 14 and 10 vs 5 respectively in our population.

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Feedback loop switch model of fibromyalgia pathophysiology, first assessment by patient questionnaires

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Introduction: Fibromyalgia (FM) is an idiopathic chronic widespread pain syndrome accompanied by a broad range of symptoms encompassing fatigue, sleep disturbances, mood disorders, cognitive dysfunction, irritable bowel, and chronic dizziness, thus deeply worsening the patients' quality of life. Despite a better understanding of the disease in recent years, which led to recognize FM as a central rather than peripheral disorder of pain processing, the diagnosis, pathogenesis and therapy are still challenging issues (1, 2).

Aim: We believe that Systems and Control Theory and Psycho-Neuro-Endocrine-Immunology (PNEI) represent the most suitable framework for the study of such a complex condition (3, 4, 5). We aim at providing a unifying model for FM pathogenesis, based on a loop neural network involving thalamocortical regions, i.e. ventroposterior lateral thalamus (VPL), somatosensory cortex (SC), and thalamic reticular nucleus (TRN). Besides, we aim at finding significant stimuli within the PNEI network able to switch the system from physiological to pathological functioning.

Methods: The dynamics of the loop system were described by three differential equations having neuron mean firing rates as variables and containing Hill functions to model mutual interactions among thalamocortical regions. The computational analysis was conducted with MATLAB. As a first assessment of the model, we designed a cross-sectional questionnaire study enrolling FM patients aged 18-65 years. The characterization of the type and intensity of pain was assessed by painDETECT questionnaire. Behavioral problems, psychosocial stress, and self-rated emotional intelligence were explored by Cognitive Behavioral Assessment-Hospital (CBA-H) and Self-Rated Emotional Intelligence Scale (SREIS). Statistical analysis was performed with the software R and included the computation of the correlations between quantitative variables.

Results: The thalamocortical loop displayed a transition from monostability to bistability for a weakening of GABAergic transmission between TRN and VPL. This involved the appearance of a high-firing-rate steady state in SC that is assumed to represent pathogenic pain processing giving rise to chronic pain. The questionnaire analysis was based on 173 respondents (88% females), and revealed

a prevalence of neuropathic pain (chi-squared test, $p < 0.05$), consistent with a supraspinal origin of pain. FM patients reported a low efficacy of pharmacological medications, whereas complementary mind-body therapies seemed more effective in alleviating pain (Mann-Whitney test, $p < 0.05$). Based on CBA cutoffs, participants revealed a high ability in perceiving emotions but a low capacity to understand and self-manage them, leading to situational anxiety and critical emotional stability.

Conclusion: We suggested a thalamocortical feedback loop system and its GABAergic/glutamatergic strength ratio as a crucial hub within the PNEI network underlying FM pathogenesis. The consistency of our model with the clinical features of FM patients suggests that critical targets for FM treatment are to be found among PNEI pathways leading to GABA/glutamate imbalance.

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PC066

A *de novo* variant in *CROCC* identified in a Chinese family implies the potential association with Atlanto-occipital Fusion (AOF)

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Atlanto-occipital fusion (AOF) is a rare skeletal malformation causing nerve compression with high risk of fatal. Its genetic information is currently lacking. Through whole-exome sequencing (WES) on a Chinese family having a sporadic proband son of AOF but other healthy family members, we identified a novel variant (chr1: c.4702C>T: p.R1568C) in ciliary rootlet coiled-coil (*CROCC*). The variant had different genotypes between the proband and healthy family members but with high conservations of “damage” to protein structure based on MutationTaster and SIFT forecast. *CROCC* gene can be obtained in both healthy (n=220) and non-mutated AOF patient samples (n=68) but absented in five sporadic patients with the novel variant. Furthermore, abnormal of cilia was observed after editing the target sequence on *CROCC* using CRISPR-Cas9. These results suggested that AOF might be caused by the mutation of the variant c.4702C>T:p.R1568C in *CROCC*. With strong amino acid conservation and interaction regulation, the variant mutation could cause the signal

disorder of skeletal development which may lead to the defective bone formation and finally cause the development of AOF.

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PC067

Phenotyping of human induced pluripotent stem cell derived atrial cardiomyocytes and determination of responsiveness to a small conductance, calcium-activated potassium channel inhibitor

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The role of small conductance, calcium-activated potassium (SK) channels in atrial repolarisation has been debated due to contradictory effects of SK channel inhibitors. We proposed that the native SK current that contributes to atrial repolarisation is carried by a heteromer comprised of both SK2 and SK3 subunits. This heteromer displays a unique but poorly understood pharmacology (Hancock et al., 2015). As cardiac SK channel expression may change in disease states, evaluation of SK channel function in the *healthy* human myocardium is difficult (Darkow et al., 2021). Consequently, this study was undertaken to evaluate the use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as a potential model system to probe human SK channel composition and function. Human iPSCs (REBL-PATs; a gift from Professor Chris Denning; Mosquiera et al., 2018) were differentiated in the presence or absence of retinoic acid, to produce atrial (Atr-hiPSC-CM) or mixed atrial/ventricular (AV-hiPSC-CM) lineages, and cultured in 2D monolayers for up to 90 days before dissociation onto coverslips. Experimentation occurred at three separate timepoints (1: day 35±5; 2: 65±5 & 3: 95±5). Whole-cell voltage clamp was used to evaluate single-cell responses to the SK channel inhibitor UCL1684 at room temperature. Additionally, the presence of acetylcholine (ACh)-activated K⁺ current (I_{K,ACh}) was probed as an atrial-selective response. Immunocytochemistry was used to evaluate expression of MLC2v, a ventricular-specific myosin light chain isoform; and TNNI3, a troponin isoform absent in the foetal heart (Lee et al, 2017). Finally, whole-cell current clamp was performed to measure maximum diastolic potentials (37°C). Data are presented as mean ± S.E.M. Initial patch clamp experiments on Atr-hiPSC-CMs at timepoints 1 and 2 of culture showed that UCL1684 had no effect on membrane current evoked during a voltage ramp from -100mV to

+100mV ($n=6$ & 5 respectively) despite an increased magnitude and incidence of $I_{K,ACh}$ in response to $1\mu M$ ACh (1.46 ± 0.40 pA/pF in 88% of Atr-hiPSC-CMs vs. 0.50 ± 0.18 pA/pF in 38% of AV-hiPSC-CMs; $n=8$ for each; $p<0.05$, Student's t-test) and the absence of the ventricular marker MLC2v (1% positive Atr-hiPSC-CMs vs. 29% AV-hiPSC-CMs). The immature phenotype of hiPSC-CMs is an established limitation of their use (Feyen et al., 2020). Prolonging time in culture (to timepoint 3) led to a more mature cell phenotype, significantly increasing the expression of TNNI3 and hyperpolarising the maximum diastolic potential (-54.4 ± 1.7 mV [timepoint 1, $n=13$] vs. -61.6 ± 2.3 mV [timepoint 2, $n=8$] vs. -74.2 ± 2.2 mV [timepoint 3, $n=4$]; $p<0.001$, one-way ANOVA). Prolonged culture also decreased the spontaneous action potential firing rate of single cells over the 3 timepoints (2.7 ± 0.2 Hz [~ 35 , $n=13$] vs. 0.9 ± 0.2 Hz [~ 65 , $n=8$] vs. 0 Hz [~ 95 , $n=4$]; $p<0.0001$, one-way ANOVA). Extending culture time to timepoint 3 resulted in a proportion of Atr-hiPSC-CMs (3 of 7 cells) responding to SK channel inhibition (1.80 ± 1.0 pA/pF at +80mV). In conclusion, although a UCL1684-sensitive current could not be recorded *consistently* from Atr-hiPSC-CMs cultured under these conditions, our data suggest a potential link between cell maturity and UCL1684 responsiveness.

Acknowledgement: This work was funded by the British Heart Foundation (FS/17/60/33474).

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Acknowledgements :- This work was funded by the British Heart Foundation (FS/17/60/33474).

PC068

Signaling pathways activated by G protein coupled receptors in essential hypertension: differential contribution of G_{q11} and G_{12-13} proteins

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Vascular tone, a key determinant of arterial pressure, is set by the contractile state of vascular smooth muscle cells (VSMCs). There is a variety of mechanisms that regulate VSMC contractility and can represent possible targets for antihypertensive therapy. Among these mechanisms, membrane potential and $[Ca^{2+}]_i$ are of paramount importance, and changes in the way VSMCs control them are

at the core of the development of arterial hypertension. Using a hypertensive mouse strain (BPH) and its corresponding normotensive control (BPN), we have previously identified changes in the expression and function of ion channels (voltage-dependent Ca^{2+} and K^{+} channels and TRPC channels) contributing to the increased vascular tone of the hypertensive vessels. However, changes in the contribution of different G-protein coupled receptors (GPCRs) could also be relevant. Therefore, here we explored if changes in some of the molecular constituents of several GPCRs pathways could contribute to increased vascular tone in BPH mice. Mice were anesthetized by isoflurane inhalation (5% O_2 at 2.5 Lmin⁻¹) and sacrificed by cervical dislocation, following the EC guiding principles regarding the care and use of animals (Directive 2010/63/UE). Endothelial-denuded mesenteric arteries and isolated VSMC from BPN and BPH mice were used to analyze the expression of several components of the contractile machinery, including receptors (purinergic and $\alpha 1$ -adrenergic), second messengers and ion channels and pumps. Their functional contribution was analyzed by exploring the contractile responses to Phenylephrine (Phe) and Uridine triphosphate (UTP) using pressure and wire myography. The involvement of primary effectors of G_{q11} and G_{12-13} pathways in agonist-induced contractions was evaluated with specific blockers of Phospholipase C ($\text{PLC}\beta$), Protein Kinase C (PKC) and Rho-associated protein kinase (ROCK). Dose-response curves for Phe showed a larger vasoconstrictor effects in BPH arteries (maximal tension (T_{max}) of 2.85 ± 0.06 mN in BPN and 8.56 ± 0.06 mN in BPH, $p < 0.001$) with increased affinity (EC_{50} of 1.74 ± 0.15 μM in BPN and 0.58 ± 0.02 μM in BPH, $p < 0.05$). In the case of UTP, there was also a significant increase in T_{max} in BPH (2.63 ± 0.45 mN in BPN and 5.65 ± 0.33 mN in BPH, $p < 0.001$) together with a large leftward shift of the dose-response curve. While the EC_{50} for UTP in BPN was of 424.05 ± 137.09 μM , UTP dose response curves in BPH were best fitted to a two-binding site model with EC_{50} of 1.79 ± 0.43 and 126.70 ± 141 μM respectively. These data suggested a different functional expression of P2Y receptors in the hypertensive animal, confirmed by expression studies and pharmacological characterization of the responses. We have also found a larger contribution of ROCK and PLC to Phe and UTP responses in BPN arteries, as well as a differential role of Ca^{2+} -activated Cl^{-} channels to Phe and UTP effects in both preparations. Our results demonstrate not only a greater reactivity of the arteries from hypertensive mice, but also a significant change in the relative contribution of the different effectors involved in each signaling pathways. These data evidence how the marked non-linear character of the contractile responses hinders the prediction of the global effects produced from changes in individual elements.

PC069

Changes in aortic resistance and small artery compliance during vasoconstriction

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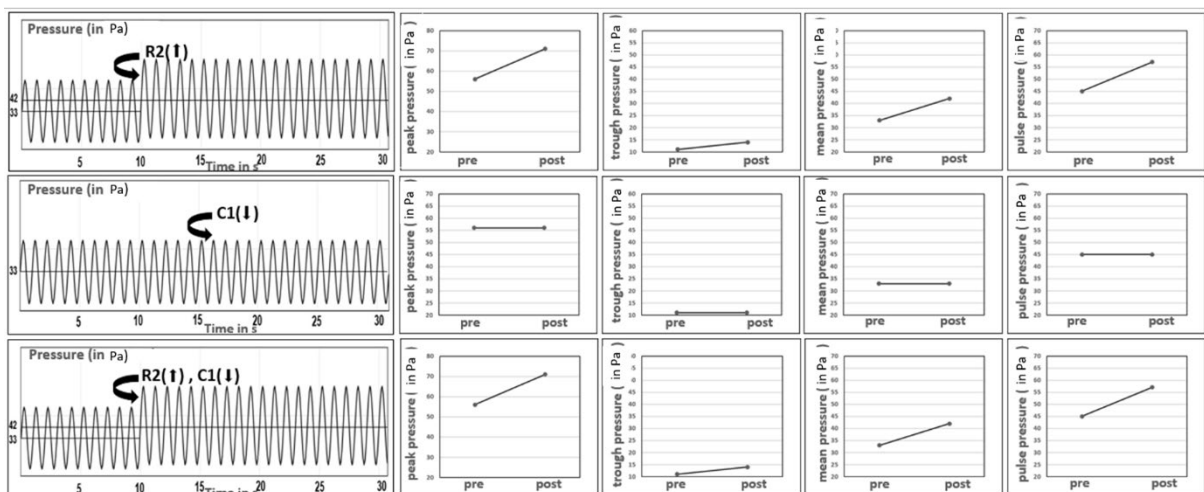
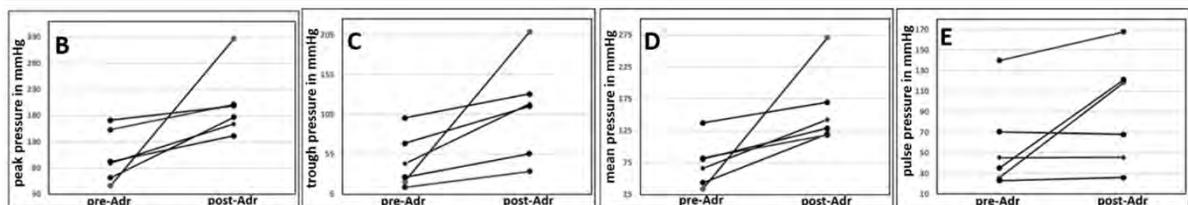
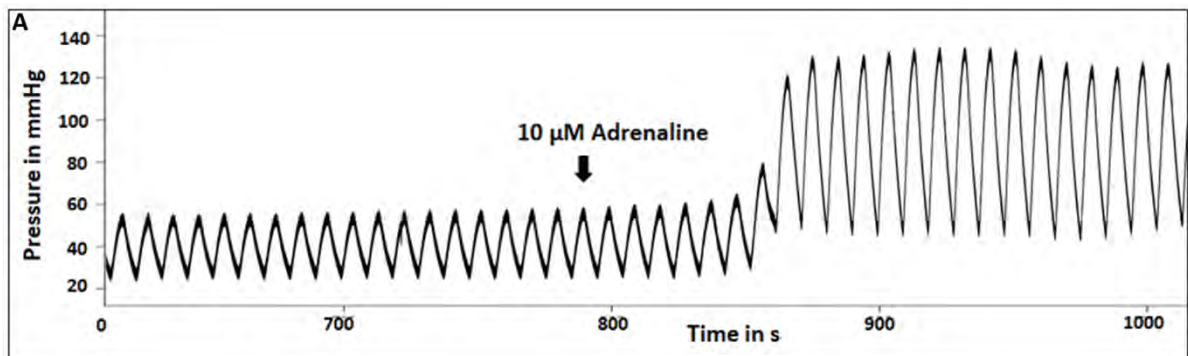
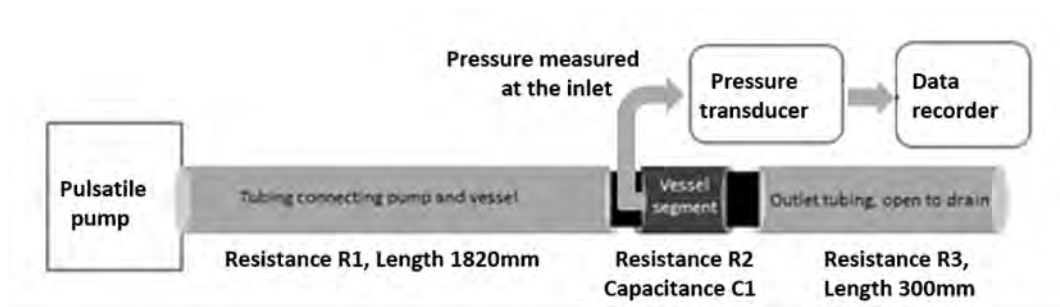
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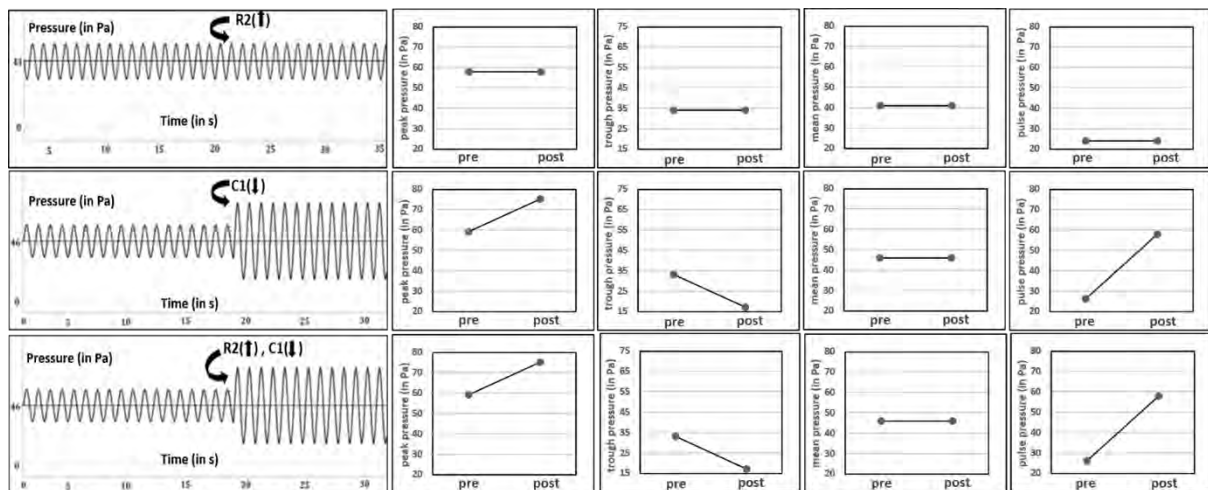
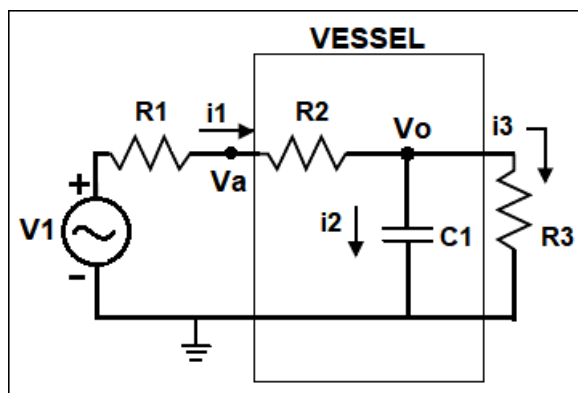
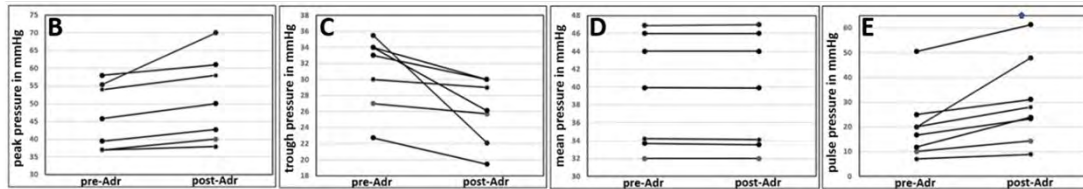
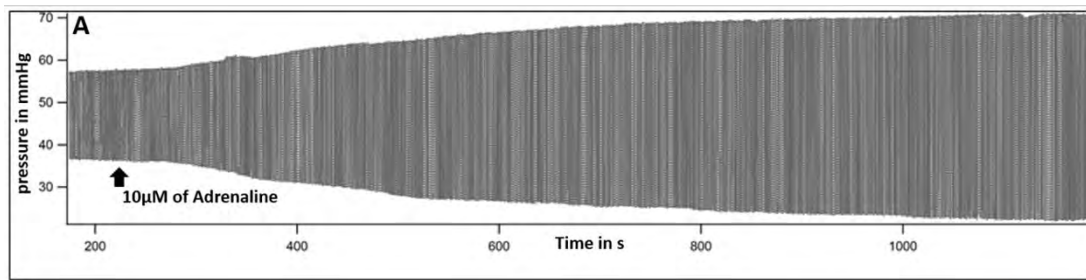
Background: Smooth muscle contraction in small arteries is known to decrease the lumen diameter and therefore increase the resistance, whereas the large artery compliance is known to decrease [1]. Besides this information, there is no categorical evidence on what happens to the large artery resistance and small artery compliance during vasoconstriction, which has been addressed in the presented study. This is the first of its kind to present a comparative assessment of aorta and small artery (isolated vessel model) response to adrenaline, a potent vasoconstrictor, in terms of resistance (R) and compliance (C) through physiological in-vitro experiments as well as equivalent physiological modeling. Understanding the exact location of R and C can be used to see how drugs affect vascular resistance and compliance, thereby allowing us to conclude if there is a differential action of drugs on small arteries versus large arteries by assessing the changes in R and C. This understanding would enable the tailoring of anti-hypertensive therapies for essential hypertension (in which resistance is high) and isolated systolic hypertension (in which compliance is low) using targeted drugs that act on specific vessels [2,3,4].

Methods and Results: The study was approved by the Institutional Review Board of Christian Medical College Vellore, IRB no: 9930, 17/02/2016. Goat aorta/legs were freshly procured from a registered slaughterhouse (*Co-ordinates 12.93°N 79.13°E accreditation number: 309/13*) and transported in ice-cold extra-cellular solution. Isolated cylindrical segments of goat aortae and small arteries were perfused with a pulsatile pump (*Harvard Apparatus, rodent blood pump model 1407*) at a preset flow rate under physiological conditions, and lumen pressures were recorded before and after the addition of adrenaline (10 μ mol/L) using a pressure transducer connected to an intra-arterial cannula (Fig 1). In aortae, adrenaline caused a rise in peak pressure, a drop in trough pressure, but did not change mean pressure ($p = 0.357$, WSR*) (Fig 3D, $n = 7$). Whereas in small arteries, vasoconstriction caused an increase in all the pressures (systolic, diastolic, and mean pressures, $p = 0.028$, WSR) (Fig 4D, $n=6$). Using dimensions from the tissues and the tubings of the experimental set-up, equivalent electrical models were simulated (Fig 2). Changes in lumen pressures in response to adrenaline were compared with equivalent voltage responses in the simulation to changing values of R2 and C1 of the vessel model (Fig 2). Simulation results show that vasoconstriction in aorta does not lead to a significant reduction in lumen diameter sufficient enough to cause an increase in resistance and mean pressure (Fig 5, panels 2, 3). Whereas during small artery vasoconstriction, there is a significant reduction in lumen diameter causing an increase in vascular resistance, but a decrease in compliance if any, does not affect any of the pressures measured (Fig 6).

Conclusion: The inference is that large artery vasoconstriction does not lead to a significant reduction in lumen diameter sufficient enough to cause a rise in resistance and mean pressure. Whereas small artery vasoconstriction increases resistance but does not change compliance.

* Wilcoxon's signed-rank – WSR





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Ethics Approval: Institute of Review Board, Christian Medical College Vellore

PC070

Ventricular myocyte action potential responses to adrenergic stimulation and changes in rate

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Purpose: The adrenergic response is fundamental to modulation of cardiac function during exercise or stress. At the cellular level, past research has primarily investigated responses at one activation frequency and very high doses of adrenergic stimuli, often at ambient temperature. Data on rate-dependent and non-rate-dependent responses to adrenergic stimulation on ventricular myocyte repolarisation under physiological temperatures are key for being able to understand and reliably model cardiac responses to stress. We therefore investigated the impact of rate of pacing frequency and adrenergic stimulation on the rat ventricular action potential at physiological temperatures and levels of adrenergic stimulation.

Methods: Adult (3 months) male Wistar rats (N = 13) were sacrificed in accordance with Schedule One methods stated in the Animals (Scientific Procedures) Act, 1986 and approved by the University of Leeds ethics committee. Whole-cell patch-clamp recordings on ventricular myocytes were made at physiological temperature (35-37°C). Action potentials were triggered by current injections in current-clamp mode using 2ms pulses. The intracellular solution consisted of (in mM): KCl 135, EGTA 10, HEPES 10 and glucose 5, pH 7.2. The extracellular solution consisted of: NaCl 136, KCl 4, MgCl 2, CaCl 1, HEPES 10 and glucose 10, pH 7.4. Action potentials were recorded with myocytes paced at 1, 2 and 4Hz before and after isoproterenol infusion (5nM). Action potential duration at 25, 50, 75 and 90% of full repolarisation (APD₂₅, APD₅₀, APD₇₅, APD₉₀) were compared between activation frequencies and resting and isoproterenol conditions. Changes in action potential duration (APD) are expressed relative to the initial APD recorded during pacing at 1Hz with no isoproterenol present. Data expressed as mean ± SEM.

Results: Increasing activation frequency prolonged APD₂₅₋₉₀ (p = < 0.01, repeated measures ANOVA). Action potentials recorded at 4Hz demonstrated a prolongation in APD₂₅₋₉₀ of 7-59% (p = < 0.015), with the greatest prolongation observed at APD₂₅ (59%) followed by APD₅₀ (23%), APD₇₅ (13%) and

APD₉₀ (7%). Action potentials recorded at 2Hz displayed a similar trend (Figure 1). Isoproterenol shortened APD₅₀₋₉₀ but not APD₂₅, by magnitudes of 17-25% and 13-22% at 1Hz and 4Hz pacing, respectively ($p < 0.05$). Isoproterenol also stimulated APD₅₀₋₉₀ shortening by 14-15% at 2Hz pacing, though not statistically significant. The greatest Isoproterenol-induced APD shortening was observed at APD₅₀ followed by APD₇₅ and then APD₉₀ (Figure 1).

Conclusion: This work displays the separate and combined effects of frequency and adrenergic stimulation on rat ventricular action potentials under simulated physiological conditions. The findings of this study suggest APD prolongs with increased rate and shortens with adrenergic stimulation. Rate-dependent changes in APD were greatest during early phase repolarisation (APD₂₅), whilst isoproterenol-dependent changes in APD predominantly influenced APD₅₀₋₉₀ indicating a greater role in late repolarisation and plateau phases. Though this study included a physiologically relevant dose of adrenergic stimuli and examined activation frequency toward a physiologically relevant basal heart rate for a rat (4Hz), the use of even greater rates (up to 10Hz) in future work will provide further insight into the effects of adrenergic stimulation on repolarisation across a physiologically relevant range.

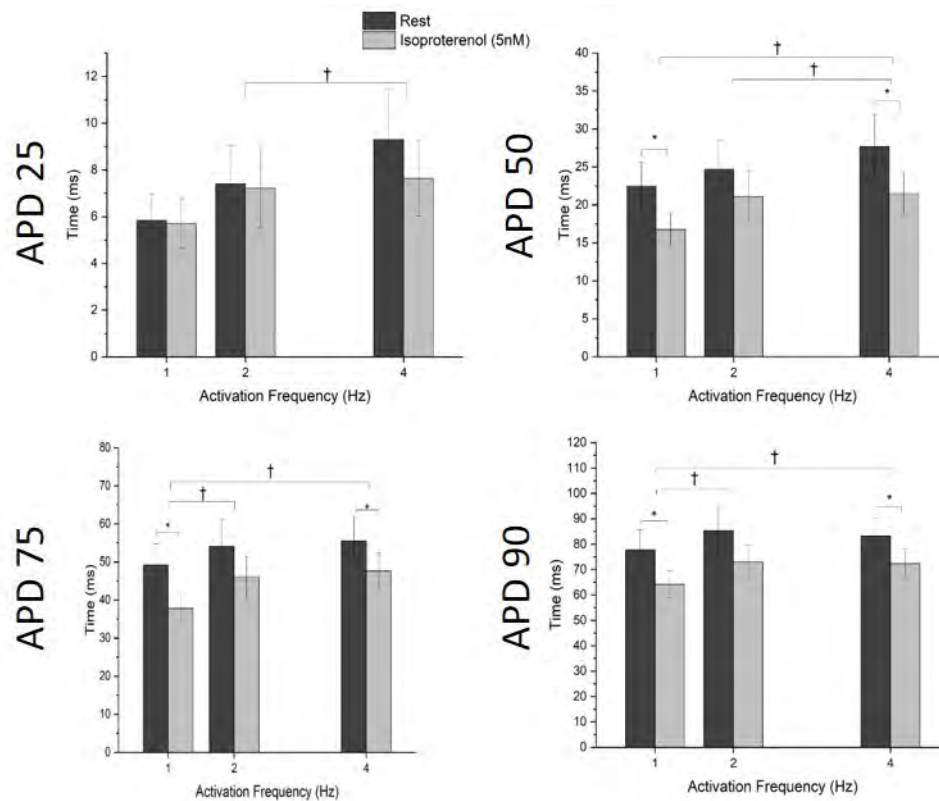


Figure 1. Effect of activation frequency and adrenergic stimulation on action potential duration (APD) as measured at 25, 50, 75 and 90% repolarisation. Data presented as mean $\pm$ SEM. * indicates a significant difference between control conditions and isoproterenol. † indicates a significant difference between activation frequencies.

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PC071

Microcirculation in a murine model of pregnancy: novel technique for endoscopic blood flow visualisation and optimised endothelial assessment

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Objectives

Endothelial dysfunction is important in the pathophysiology of preeclampsia often preceding the onset of the clinical disease, suggesting a major contributor to placenta dysfunction. Animal models offer distinctive opportunities to study both placental insufficiency and maternal endothelial function during pregnancy. New blood flow visualisation techniques are needed for phenotyping of murine models. We developed an endoscopic probe for real-time perfusion imaging of the placenta. We also miniaturized the hardware for transdermal drug delivery by iontophoresis to facilitate the assessment of maternal skin microvascular function in mouse models.

Methods

A minimally invasive probe featuring an endoscopic Laser Speckle Flow Imaging (LSFI) system to visualise real-time perfusion in organs was developed. LSFI is a real-time wide-field scanning technique, used clinically for assessment of skin microvascular function. Laser illumination of moving erythrocytes generates a dynamic pattern (speckles) based on backscattering properties, which quantify blood flow activity.

For the application of iontophoresis in rodents we miniaturized the ion chamber, a device which is attached to the skin and contains the ionic drugs, houses the electric wiring, and allows optical blood flow imaging. The novel chamber has been designed, manufactured, and finally validated in a vasculature model of C57Bl/6 mice.

Results

Our LSFI-device combines a miniature camera (speckle capture) with a fibre-coupled near-infrared laser (illumination) in the tip of the endoscopic probe. A micro camera (< 2x2mm cross-section) successfully visualized speckle contrast on different flow phantoms.

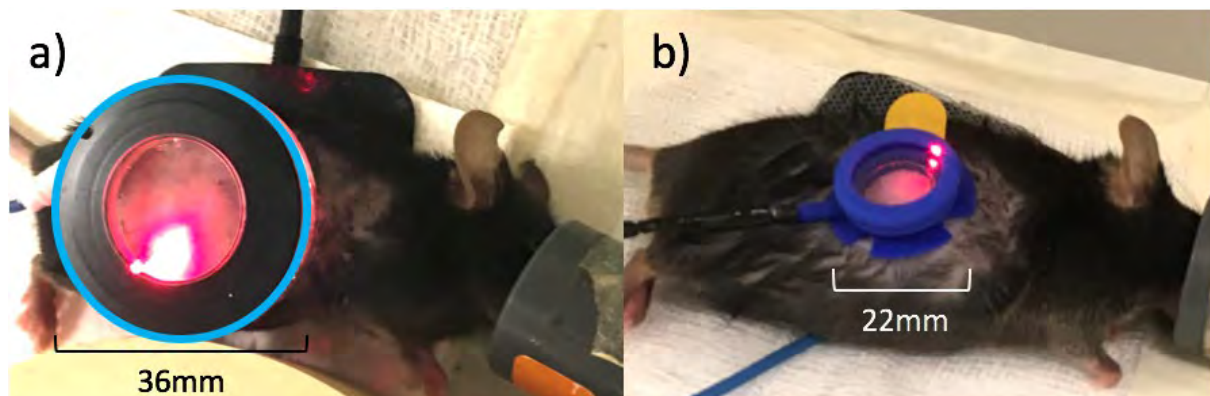
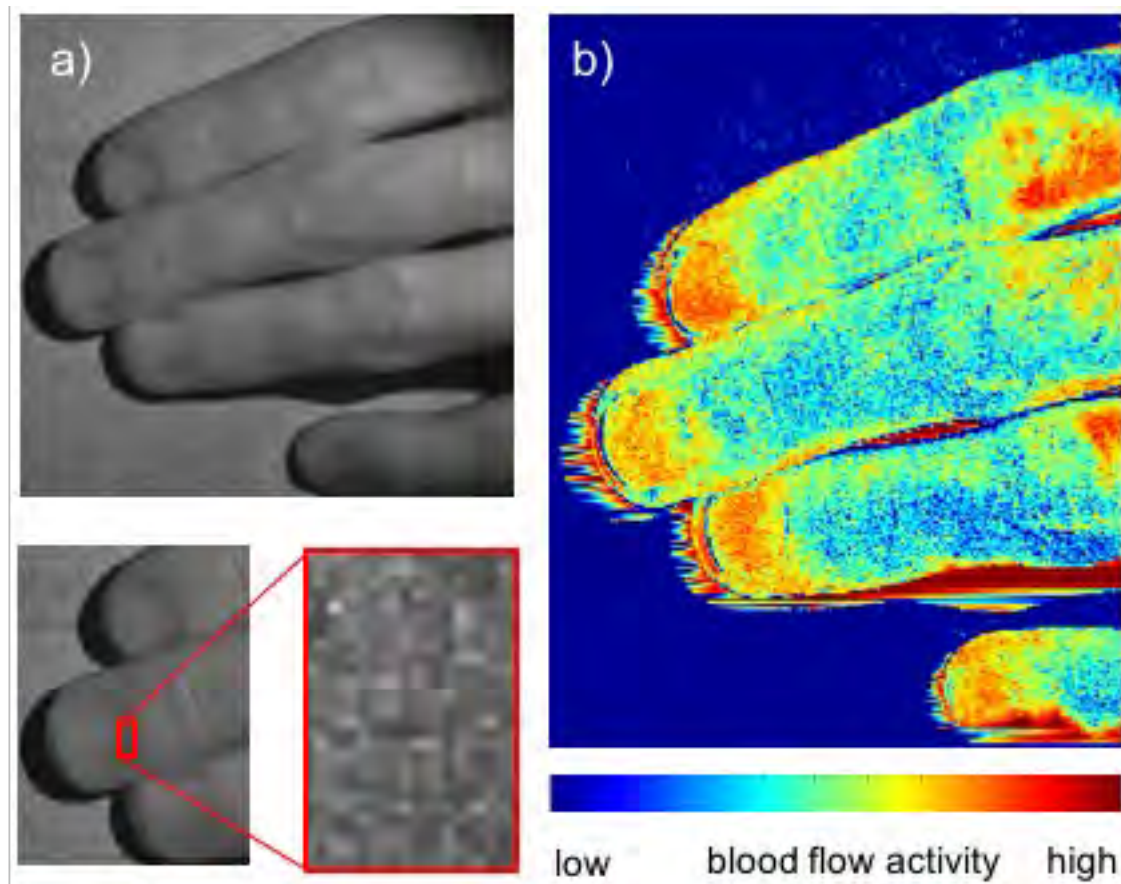
Ion-mini is a circular (23mm-diameter) flexible chamber which allows for faster measurements, reduces the strain on the tissue and allows for more cost-effective application. Compared with some commercially-available chambers, the reduced footprint area (by >50%) and the use of a flexible material, it attaches better to small rodents. The weight reduction (by 80% to 2.5g total weight) is a significant refinement in pressure on the skin of small sedated animals. The smaller treatment area allows for faster and higher resolved LDI imaging and reduces the dose of delivered drugs. Hence, the systemic drug load on the animal is lowered and measurements are more cost effective.

Conclusion

The hardware for LSFI has been successfully miniaturized and tested in vitro. Use of the endoscopic laser to assess placental vascular insufficiency will be further validated in a mouse model of pregnancy.

New iontophoresis hardware has been adapted and validated for the use in rodents. This technology will provide a platform to assess animal models of pregnancy and compare against clinical parameters to validate model characteristics.

Endoscopic LSFI could provide wider clinical impact to visualize blood vessels and organ perfusion in real-time during surgery without the need for different imaging modalities or injection of dyes.



Acknowledgements :- This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765274. Further supported by funding from Tenovus Scotland. Acknowledge Moor Instruments collaboration.

PC072

Ion Channel Expression in the Equine Heart - In-silico prediction utilising RNA sequencing data from mixed tissue samples.

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Neurons, muscle fibres and cardiomyocytes are the only three excitable cell types in any mammals. Their electrophysiologic properties are determined by specific proteins controlling the ion flux through the cellular membrane. These ion channels are essential for many physiologic and pathologic processes and constitute the targets for many drugs and are responsible for both their therapeutic and adverse effects. Knowing their expression across cardiac, cerebral and muscular tissue is the first step for understanding electrophysiological and pathological mechanisms and developing new treatments. Up to now, equine tissue-specific gene expression data is available for normal brain and muscle but not for the heart. Only one study sequenced a multi-tissue mix including cardiac tissue. Our work aims to predict the cardiac gene expression of ion channels based on open-source RNA sequencing data obtained from this multi-tissue mix.

Material and methods

Raw RNAseq data from normal horses from 9 datasets were retrieved from ArrayExpress and European Nucleotide Archive and reanalysed. These 9 datasets included RNA sequences from 13 tissues plus a mix of 43 tissues including cardiac muscle. Data was processed in R-Studio for quality control, alignment, read counts and data analysis using the Bioconductor packages RSubread, ShortRead. To predict the cardiac-specific gene expression, the normalized (FPKM) read counts G_{ik} , for a gene i , in a sample k , was hypothesised to be the average of the expected expression in the tissue j α_{ij} weighted by the proportion of the tissue in the mix p (Figure 1, equation 1). The cardiac-specific expression was calculated by estimating the mean expression in each other tissues (Figure 1, equation 2). When the data was unavailable the median expression in all tissues was used. To test the validity of the model, predicted gene expression values were compared to the expression in human cardiac tissue determined by RNA sequencing.

Results

Ion channel expression exhibited tissue-specific expression patterns. Samples originating from the brain, muscle, uterus, kidney and testis showed the highest and most diverse expression of ion channels. The cardiac-specific expression could be predicted for 92 ion channels, calcium-handling proteins, pumps and exchangers. Comparison with human RNA sequencing (Ref1) and qPCR (Ref2)

data showed a significant correlation with the experimental equine values (Pearson's test of correlation, $p\text{-value} < 0.001$) and with the predicted values after removal of the outliers (Figure 2, Pearson's test of correlation, $p\text{-value} < 0.001$).

Conclusion

This work gives the first insight into the cardiac-specific ion channels expression in the horse and could serve as a base for targeting genes for future differential gene expression analysis.

$$G_{ik} = \sum_j^n (\alpha_{ij} p) + \varepsilon_{ik}$$

(equation 1)

$$H_{ik} = \frac{G_{ik}}{p} - \sum_j^{n-1} (\alpha_{ij}) + \varepsilon_{ik}$$

(equation 2)

Figure 1— Model for cardiac-specific gene expression prediction.

The normalized (FPKM) read counts G_{ik} , for a gene i , in a sample k , was hypothesised to be the average of the expected expression in the tissue j α_{ij} weighted by the proportion of the tissue in the mix p (equation 1). The cardiac-specific expression H_{ik} was calculated by estimating the mean expression in each other tissues (equation 2). When the data was unavailable the median expression in all tissues was used. ε_{ik} represents the error term.

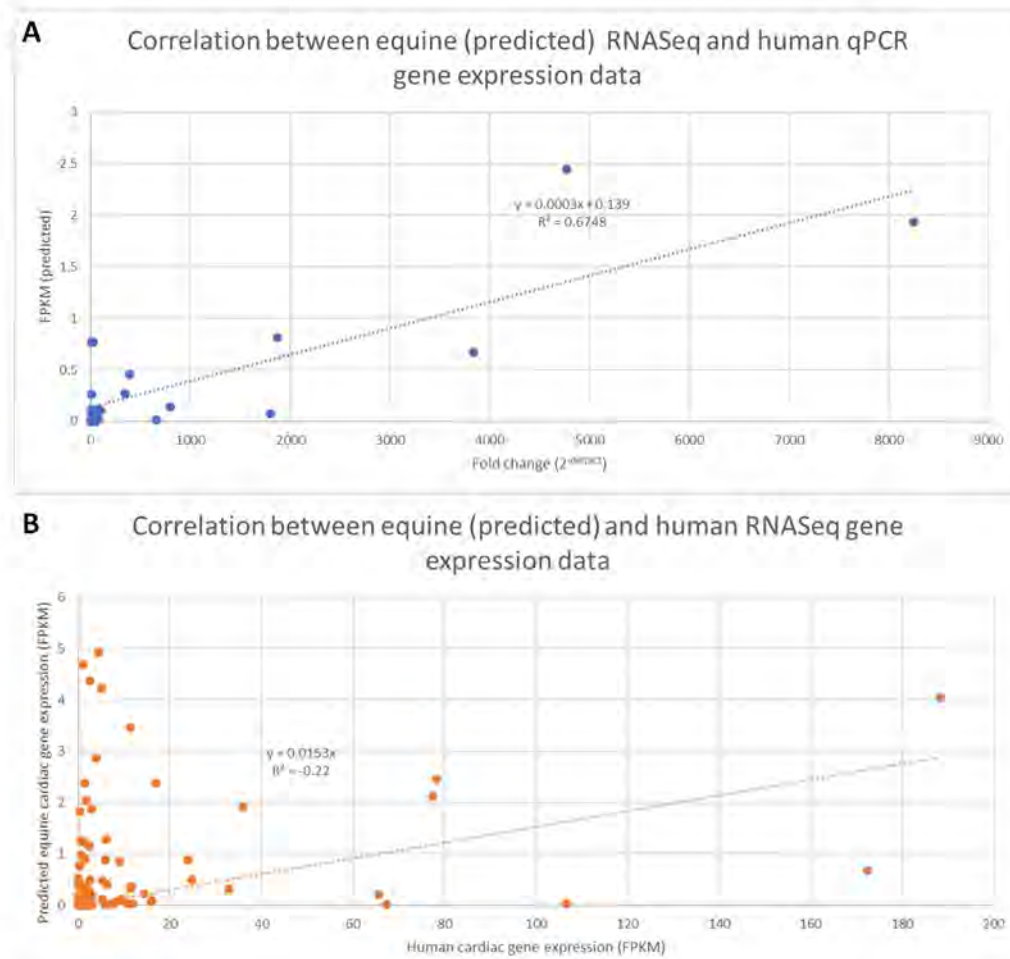


Figure 2— Correlation between human cardiac ion channel expression and equine predicted values

(A) Correlation between equine (predicted) RNA sequencing and human qPCR cardiac ion channel expression data. A significant correlation was observed with a Pearson's correlation test (coefficient =0.821, T-statistic = 6.756, DF=22, p-value<0.001). (B) Correlation between equine (predicted) and human RNA sequencing cardiac ion channel expression data. A significant correlation was observed with a Pearson's correlation test (coefficient =0.478, T-statistic = 4.809, DF=78, p-value<0.001).

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hENT1-mediated adenosine uptake is reduced by intracellular alkalization in human umbilical vein endothelial cells from gestational diabetes mellitus

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Introduction: Human umbilical vein endothelial cells (HUVECs) from gestational diabetes mellitus (GDM) pregnancies show reduced adenosine transport via the human equilibrative nucleoside transporters 1 and 2 (hENT1/2) (Westermeyer et al., 2011). Increased nitric oxide (NO) generation is seen in an alkaline intracellular medium, and high NO reduced the hENT1/2 expression and activity in HUVECs. Furthermore, GDM associates with intracellular alkalization due to a higher activity of the Na⁺/H⁺ exchanger 1 (NHE1) (Fuentes et al., 2019).

Aim: To evaluate whether GDM alters hENT1/2 transport activity due to intracellular alkalization.

Materials & Methods: HUVECs were isolated (collagenase digestion) from full-term normal (n = 11) or GDM (n = 8) pregnancies collected at the Clinical Hospital CHRISTUS-UC (Chile) and conformed to the principles outlined in the Declaration of Helsinki. HUVECs were cultured (passage 3) in medium 199 plus sera (20%) under standard conditions (5% O₂, 5% CO₂). The pHi was measured in cells loaded with the fluorescent probe 2',7'-Bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF-AM, 12 μmol/L, 10 min) and exposed to ammonium chloride (NH₄Cl, 20 mmol/L). Basal and pHi recovery rate ($dpHi/dt$) were estimated (up to 360 s) in cells exposed to 5 μmol/L 5-N,N-hexamethylene-amiloride (HMA, Na⁺/H⁺ exchangers (NHE) general inhibitor), 0.1 μmol/L zoniporide (Zn, NHE1 inhibitor), 0.1 μmol/L concanamycin A (V-ATPases inhibitor), or 10 μmol/L Schering (H⁺/K⁺-ATPase inhibitor). Uptake of 2,3-[³H]adenosine (0-500 μmol/L, 3 μCi/mL, 37°C, 10 s after NH₄Cl removal) was measured in the absence or presence of 1 or 10 μmol/L S-(4-nitrobenzyl)-6-thio-inosine (NBTI), inhibitory concentrations for hENT1 or hENT1+hENT2 transport, respectively. The difference between adenosine uptake in the presence of 1 and 10 μmol/L NBTI corresponded to the hENT2-mediated uptake.

Results: HUVECs from GDM showed higher basal pHi compared with normal pregnancies (pHi = 7.7 ± 0.2 vs 7.1 ± 0.1, respectively) (mean ± SEM, unpaired ANOVA, P<0.05 as significant). The $dpHi/dt$ in

GDM was higher (4.2 ± 0.2 fold) than in normal pregnancies. Zn and HMA reversed the GDM-increased $dpHi/dt$ to values in normal pregnancies. The reduced hENT1-mediated adenosine uptake in GDM at basal pHi was reversed by Zn but increased (2.2 ± 0.7 fold) in the presence of NH_4Cl + Zn. However, hENT2-mediated uptake was increased (2.9 ± 0.3) in an acidic intracellular medium compared with basal pHi, but unaltered by Zn. hENT1-mediated transport was increased in the presence of NH_4Cl in cells from normal pregnancies.

Conclusion: NHE1-mediated alkaline pHi inhibits hENT1 and hENT2-adenosine transport in HUVECs from GDM pregnancies.

Reference 1 :- Westermeier F et al. (2011). Diabetes 60, 1677-1687

Reference 2 :- Fuentes G et al. (2019). J Physiol

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PC074

Does the small conductance Ca^{2+} -activated K^+ current (I_{SK}) flow during atrial action potential repolarisation under physiological conditions?

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Background: The small conductance Ca^{2+} -activated K^+ current (I_{SK}) is considered a potential target for preventing new onset atrial fibrillation (AF). The contribution of I_{SK} to atrial repolarisation under physiological conditions is unclear, however, and substantial I_{SK} activation may require $[Ca^{2+}]_i$ exceeding typically measured global average diastolic-to-systolic levels. **Aim:** To test the hypothesis that I_{SK} flows during the atrial action potential (AP), when local (sub-sarcolemmal, trigger) $[Ca^{2+}]_i$ is expected to exceed global average levels, either at physiological or supra-physiological stimulation rates. **Methods:** Atrial myocytes were isolated from hearts excised from rabbits (anaesthetised with Na^+ -pentobarbital, 100 mg/kg I.V.), and from atrial tissues obtained from consenting patients who were in sinus rhythm and undergoing elective cardiac surgery. APs were recorded by whole-cell ruptured-patch clamp, at 35-37°C, and 1-3 Hz stimulation, before and after acute superfusion of an I_{SK} blocker, ICA (1 or 10 μM). A +ve control, 4-AP (4-aminopyridine; K^+ current blocker) was used initially since I_{SK} , and thus the effect of ICA on APs, might be small. **Results: 1)** In rabbit atrial myocytes, stimulated at 1 Hz, 2 mM 4-AP significantly increased AP duration (APD): by 72% at APD₃₀ (6.2 ± 0.6 vs 3.6 ± 0.3 ms; $P < 0.05$), and by 31% at APD₇₀ (21 ± 4 vs 16 ± 3 ms; $P < 0.05$); with no significant effect on APD₉₀ ($n = 6$ cells, 2 rabbits). Drug responses were rapid (≤ 30 s), stable in all cells, and reversible in 5 of 6 cells studied. **2)** By contrast, ICA, at 1 μM ($\sim 2 \times IC_{50}$ for I_{SK}) had no significant effect

on APD₃₀ (3.4±0.7 vs 3.7±0.6 ms), APD₇₀ (27±9 vs 32±8 ms), or APD₉₀ (82±14 vs 87±11 ms; n=10 cells, 4 rabbits), including relative to time-matched controls (TMC; 6 cells, 3 rabbits); nor on maximum diastolic potential (MDP) or AP maximum upstroke velocity (V_{max}). **3)** However, ICA at 10 µM (potentially non-selective for I_{SK}) increased (non-reversibly) both APD₇₀ and APD₉₀ vs TMCs (by +2.9±1.2 ms vs -1.8±0.7 ms, and by +22±9 ms vs -3.9±2.1 ms, respectively; P<0.05 for each; 6-7 cells, 3 rabbits), also with no effect on MDP or V_{max}. **4)** In human atrial myocytes, also stimulated at 1 Hz, 1 µM ICA had no significant effect on APD₃₀, APD₇₀, or APD₉₀ (n=9 cells, 4 patients), including relative to TMCs (4 cells, 2 patients), nor on MDP or V_{max}. **5)** At higher stimulation rates (2 or 3 Hz, for 80-110 s), intended to elevate [Ca²⁺]_i and thus potentially enhance I_{SK}, 1 µM ICA again had no significant effect on these AP parameters (n=7-10 cells, 3 patients), including vs TMCs (performed at 2 Hz; 6 cells, 3 patients). **Conclusion:** In ruptured-patch-clamped rabbit or human atrial isolated myocytes, I_{SK}, assessed as any AP response to acute ICA at 1 µM, may not flow during AP stimulation at physiological rates, nor during short bursts of supra-physiological (up to 180 beats/min) stimulation. I_{SK} activation (and thus its potential pharmacological inhibition during AF) may require changes to cellular electrophysiology or cell signalling systems to develop a sensitivity to I_{SK} block.

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PC075

Functional exercise increases perfusion disparity in healthy human lower limb in both sexes

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Introduction:

Among the multiple consequences of our bipedal condition, lower limb perfusion asymmetries during rest are one of the most intriguing. Although referred as non-significant in most of the studies, this unevenness is still poorly understood. It has been referred to be related with performance posture (and limb dominance) and it is believed that exercise eliminates (or blurs) those differences.

Aims/objectives:

To understand the origin of this phenomena characterizing the impact of exercise on this lower limb perfusion unevenness.

Methods:

Perfusion in previously selected participants (n=34, 28.4 ± 9.2 y.o. was measured with LDF (laser Doppler flowmetry) at the 3rd metatarsophalangeal dorsal region in both feet and polarized spectroscopy (TiVi) on the dorsal region of both feet. Median age of the participants was 23.5 years (IQR: 22.0-32.0). Blood pressure (systolic and diastolic) and Body Mass Index (BMI) were recorded and categorized (normal weight BMI <25; Excess weight BMI >25). Median BMI was 23.29 (IQR: 20.94 – 25.19). Procedures respected all the principles of good clinical practice for human studies research. There was a previous submission to the Institutions' Ethical Commission and all procedures complied with the good scientific practice for clinical studies involving human participants. Experimental protocol included three phases. In the first, volunteers remained in the standing position for 5 minutes; in the second phase, a 2 minute squat, single leg squat, or isometric plantar flexion were made as a challenge; the third and final phase was the recovery period (5 minutes, in the standing position). Statistical analysis was performed in SPSS version 22.0. Parametric or non-parametric tests to assess differences between variables were performed. All tests used a 5% significance level.

Results and Discussion

Every individual presented asymmetric perfusions values between limbs. Women had higher perfusions values (mean of both feet) in the three stages of the protocol, from baseline to recovery, but the differences were non-significant (Mann-Whitney; baseline, p=0.658; challenge, p=0.306; recovery, p=0.610). The ratio between the higher and lower LDF value at baseline showed that the intra-individual differences could range from 2.77 to 132.88% (median: 21.56; IQR 9.20 – 38.26). Women had lower ratios at baseline and at recovery (baseline median: Women 14.28 vs. Men 23.10; recovery median: Women 23.88 vs. Men 32.25) and higher at exercise (Median: Women 41.93 vs. Men 29.39), but all differences between sexes were non-significant (Mann-Whitney: baseline p=0.658; exercise p=0.973; recovery p=0.375).

Results suggest that in functional exercise increase perfusion asymmetries in the lower limbs, since the ratios between limbs increase in both sexes. However, this asymmetry seems to remain longer in men after the exercise with the ratios at baseline and recovery being significantly different (Wilcoxon; p=0.013). Regarding BMI categories no significant differences between sexes were found (p=0.865). However, the higher perfusion decrease in the contralateral limb seems to be driving for the detected differences.

Conclusions

Movement even if quasi static seems to increase perfusion, magnifying asymmetries between both limbs.

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PC076

Long term local vibrations affect skin oxygenation and motor nerve conduction in the arm of workers reporting wrist discomfort during computer mouse work

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Introduction: Computer mouse work (CMW) is characterized by forced posture of the wrist causing discomfort, often resulting in carpal tunnel syndrome. Local vibration has been proposed as a promising strategy to avoid these adverse effects.

Aim: The aim of our study was to evaluate the effect of regular local vibration of affected wrist on the median motor distal latency (t_{lat}), laser Doppler skin blood flow (LDF) and transcutaneous oxygen partial pressure (tcpO₂) in computer workers reporting carpal discomfort during CMW.

Methods: 10 secretaries aged 47±10 years with carpal discomfort during CMW were educated to use specially designed vibrator placed under the wrist of the working arm. With working arm on the computer mouse, LDF was measured (PeriFlux 6000) on glabrous (LDFg) and nonglabrous (LDFng) skin of vibrated and contralateral arm, tcpO₂ was obtained distally and proximally from the vibration site and t_{lat} was assessed (PowerLab 2/26, AdInstruments) on working arm before and after four weeks of regular local vibration of working wrist for two hours a day during CMW (4WV).

Results: No difference was found in LDF of glabrous as well as nonglabrous skin of vibrated (LDFg=199.38 (30.41) before and 205.33 (36.22) after; LDFng=8.56 (1.20) before and 6.61 (0.79) after) and contralateral arm (LDFg=234.97 (32.50) before and 238.34 (47.04) after; LDFng=6.46 (0.48) before and 7.90 (1.84) after; all in perfusion units) after 4WV compared to before. Distal to the vibration point tcpO₂ was significantly greater after 4WV compared to before (45.28 (4.54) mmHg compared to 35.18 (2.66) mmHg, p<0.001) but no changes were obtained proximally (40.10 (6.49) mmHg compared to 38.70 (4.85) mmHg). t_{lat} was significantly decreased after 4WV (4.3 (0.2) ms compared to 5.2 (0.5) ms, p=0.003).

Conclusion: Our results revealed that long term local vibrations improve the conduction of medial nerve motor branch and cutaneous oxygenation distal to local vibration site, however skin blood flow remains unaffected in glabrous as well as in nonglabrous skin. Local vibrations may be beneficial in prevention and treatment of posture-related patophysiological changes in CMW.

PC077

Exploring the effect of a single meditation session on cutaneous microcirculation – a pilot study

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Adopting a healthy lifestyle is paramount to the prevention of cardiovascular disease. In recent years there has been a steep increase in terms of available strategies for health promotion, ranging from technology-driven to more naturalistic and holistic approaches. Meditation is one of such strategies, with recent studies highlighting a wide range of benefits, including improvement of cardiovascular physiology. However, few studies have addressed the role of microcirculation on the cardiovascular response to meditation. Our aim was to explore the effect of a single meditation session on the cutaneous microcirculation in healthy subjects. A group of twelve subjects (38.9 ± 17.7 y.o.) of both sexes, participated in this study after giving informed written consent. None of the subjects had previous experience in meditation practices and were properly instructed prior to carrying out the experimental procedure, which was carried in a temperature-controlled room. Subjects performed the procedure while seated and following a 10 min stabilization period. The procedure consisted of three phases – 2 min baseline (phase I), 10 min of focused attention meditation (phase II) and 2 min recovery (phase III). Cutaneous microcirculation was assessed on a randomly chosen earlobe with a photoplethysmography sensor, which quantified local blood flow. The raw blood flow signal was decomposed into its spectral components with the wavelet transform – cardiac, respiratory, myogenic, sympathetic, endothelial dependent and independent of NO. Statistical comparisons between phases were carried out with the t-test for related samples ($p < 0.05$). During phase II no significant change in blood flow was observed. Similarly, the cardiac, respiratory, myogenic, sympathetic and endothelial NO-dependent activities also remained stable during this phase. However, the endothelial NO-independent activity decreased significantly during phase II, and remained significantly lower than baseline during phase III. These preliminary results suggest that a single meditation session might influence the mechanisms regulating microcirculatory perfusion.

PC078

Maternal high fat high sugar diet induces metabolic alterations related to hepatic and pancreatic changes that are independent of changes in body composition

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Introduction: Western diets, high in fat and sugar (HFHS), are contributing to the increased number of women entering into pregnancy overweight/obese. In turn obesogenic diets/obesity are risk factors for metabolic conditions in the mother during pregnancy¹. The liver and pancreas are key to metabolic regulation. Previous studies principally focused on the effect of long-term exposure to an obesogenic diet or established obesity on these organs in males or non-pregnant females^{2,3}. This study aimed to determine if short-term exposure to a HFHS diet induces structural changes in the liver and pancreas that correspond to increased maternal body weight or adiposity during pregnancy.

Methods: Animal experimentation was carried out under the UK Home Office Animals (Scientific Procedures) Act 1986, following ethical review by the University of Cambridge. 6 week old female mice were fed a control (RM3, SDS) or customized HFHS diet (35.9% fat, 29.5% sugar) (n=10/group) from three weeks prior to, and during, pregnancy (mated with control diet-fed males). Maternal body composition was assessed by TdNMR. On pregnancy day (D)17.5, tail nick blood samples were collected from 4-hour fasted dams for glucose and insulin measurements. On D18.5, dams were schedule 1 killed and liver and pancreas were weighed and processed for analysis. Livers were snap frozen for determination of lipid and glycogen content and pancreas and liver were PFA-fixed for histology. Data were analysed by 2-Way ANOVA or Mann-Whitney U tests where appropriate (significance considered at $p<0.05$).

Results: Pre-pregnancy, despite only moderate changes in bodyweight ($p=0.0714$), HFHS dams had higher adiposity than controls ($p<0.0001$). However, by the end of gestation, there were no differences in body weight or adiposity between groups (Table 1). Liver weight, fat content, lipid droplet size and steatosis area were increased in HFHS dams compared to controls, although hepatic glycogen content was unaltered (Table 1 & Figure 1A-E). Fasting insulin, but not glucose concentrations were decreased in HFHS dams (Table 1). Moderately reduced pancreas weight in HFHS dams was seen, although average number of islets, islet area and β -cell mass were not affected. Frequency distribution analysis revealed a decrease in the number of islets with a diameter of 20-40 μm in HFHS compared to control dams (Figure 1F-J).

Conclusion: A short-term HFHS diet is associated with maternal insulin deficiency during pregnancy, which is related to mild impairments in pancreatic islet morphology and likely defects in β -cell insulin secretion. It is also associated with excessive lipid accumulation in the maternal liver, which may compromise its functional capacity. Importantly, these HFHS diet-induced alterations occurred even without increasing maternal adiposity during pregnancy. These data suggest that short-term HFHS diets, as well as pre-pregnancy adiposity may cause metabolic derangements/disease in the mother during pregnancy even in the absence of changes in pre-pregnancy bodyweight or excessive gestational weight gain – which are current clinical risk parameters.

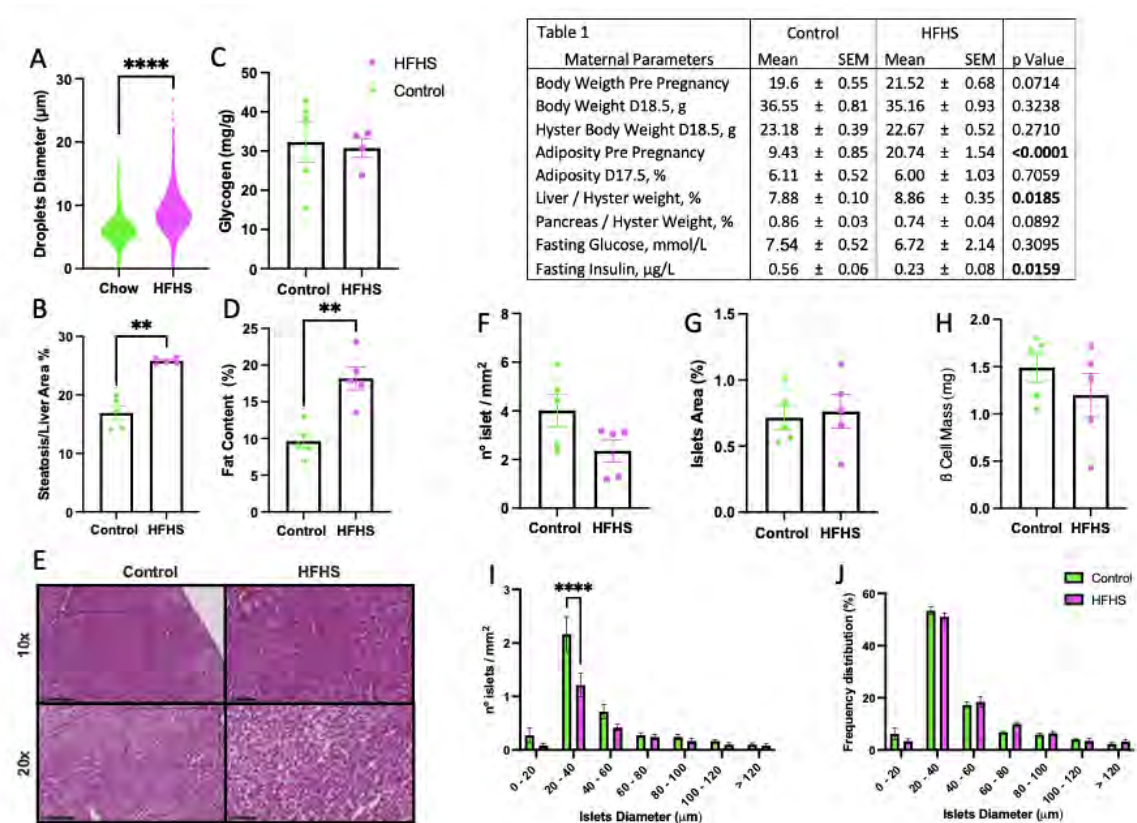


Figure 1. Lipid droplets diameter (A), percentage of steatosis (B), glycogen content (C), fat content (D), and representative images of liver tissue (E). Islet number (F), total islets area (G), β cell mass (H), number (I) and percentage (J) of islets distributed per size. Distribution data were analysed by 2-way ANOVA. All other data were analysed by Mann-Whitney U test. * $p<0.05$, ** $p<0.002$, *** $p<0.0001$. Scale bar size: 10x=250 μ m, 20x=100 μ m.

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PC079

Characterization of CaV1.2 calcium voltage-gated channels of rat visceral white fat adipocytes using recombinant expression cloning

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Calcium plays a key role in the functions white fat adipocytes (WFA). Recently we verified the existence of L-type calcium voltage-gated channels (CaVs) in WFA, (Fedorenko *et al.*, 2019a, 2019b);

however, their ion channel properties remain unknown. We have used recombinant expression cloning of the identified transcriptomic sequence to biophysically and pharmacologically characterise the CaV1.2 variant expressed in WFA.

Rat visceral WFA has the *cacna1c* gene with transcription variant x3 (accession number XM_006237175.3); interestingly, this gene encodes a unique Ca_v1.2 sequence with an extension between exons 9 and 10. This variation was confirmed by PCR with exon-specific primers in comparison with cDNA generated from RNAs extracted from heart and brain samples of adult male rat.

To study the electrophysiological and pharmacological properties of the adipocyte CaV1.2, the sequence of the *cacna1c* gene, obtained from RNA sequencing of visceral WFA of male rats, was cloned into a vector construct and expressed in CHO cells. The characteristics of L-type Ba²⁺ and Ca²⁺ inward currents of CaV1.2 were determined with the whole-cell patch-clamp method and were compared with currents carried by brain-type CaV1.2. When no differences were observed between adipocyte and brain CaV1.2, data is given just for adipocytes as mean (95% confidence intervals; number of determinations).

Currents were observed when CaV1.2 α 1 subunit was expressed alone or was co-expressed with β and α 2 δ subunits (co-transfection of *cacna1c*, *cacna2d1* and *cacnab3* genes at 1:1:1 ratio); as co-transfection increased the current density ~10 fold, co-expression was used throughout this study. The kinetics of the currents in Ba²⁺ (*I*_{Ba}) or Ca²⁺ solutions were similar, with activation and inactivation time constants of 6.2 ms (4 to 8.3; n=8) and 322 ms (158 to 486; n=8) respectively at 10mV in 10 mM Ba²⁺, the half maximal voltage for *I*_{Ba} activation was -6.5 mV (-11 to -1.6; n=8) (n=8); values typical for CaV1.2 channels (Tuckwell, 2012).

At +10 mV, the Cav1.2 antagonists: nifedipine, verapamil and calciseptine irreversibly blocked *I*_{Ba} with IC₅₀s of 2.2 μ M (0.9 to 6; n=5), 49 nM (27 to 94; n=7) and 3.3 nM (1.7 to 6.7; n=6) respectively. At +10 mV, the Cav1.2 agonist BAYK 8644 enhanced *I*_{Ba} by 218 % (195 to 246) with an EC₅₀ of 0.1 μ M (0.03-0.4; n=8) whereas FPL 64176 potentiated *I*_{Ba} by 403 % (300 to 600) with an EC₅₀ of 0.9 μ M (0.11 to 12; n=7). These values were insignificantly different to those for brain CaV1.2 except for verapamil, had an IC₅₀ of 330 nM (140 to 800; n=4) similar to that previously published of 339 nM for this brain channel isoform (Johnson et al., 1996).

This study extends our knowledge about the properties of CaV1.2 in adipocyte. One possible consequence of the extension between exons 9 and 10 of the WFA CaV1.2 α 1 subunit is the 10 fold enhanced potency to verapamil.

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Reference 2 :- Fedorenko et al. (2019b). *J Endocrinol*; DOI: 10.1530/JOE-19-0493.

Reference 3 :- Tuckwell HC (2012). *Prog Neurobiol* 96, 1–31.

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PC080

The effects of adropin on adipokine expression in rodent adipocytes

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Adipose tissue is a specialized connective tissue composed mostly of fat cells (adipocytes), which play a pivotal role in the storage of excess energy as a fat. Moreover, adipose tissue is known as a metabolic organ which regulates energy homeostasis and serves as an endocrine gland. Adipocytes express and release several biologically active molecules, known as adipokines [1]. The proliferation and differentiation of adipocytes is regulated by, among others, adropin [2]. Adropin is a peptide encoded by *Enho* gene [3]. It is known that adropin affects the modulation of body mass as well as lipid metabolism [4]. However, the potential role of adropin on rodent mature adipocyte biology was unknown. Therefore, our research aimed to evaluate the effects of adropin on the expression of adipokines in rodent adipocytes.

To conduct the study we utilised two different cell types, namely 3T3-L1 cells (a cell model to study white adipocyte function [5]) and rat primary preadipocytes. Rat primary white preadipocytes were collected from epididymal adipose tissue depots of male Wistar rats. After reaching confluency, cells were differentiated into mature adipocytes, and then incubated in the presence or absence of adropin (10 or 100 nmol/L) for 3 or 24 h. To evaluate the expression of selected adipokines (adiponectin, resistin and visfatin), we used real-time PCR. Data was analysed using one-way ANOVA followed by the Bonferroni post hoc test, and results are shown as mean $\pm$ SEM, n = 6. All experiments were conducted at least two times.

We found that adropin (10 and 100 nmol/L) promoted the expression of adiponectin mRNA in rat adipocytes after 3 h (3.81 ± 0.58 and 4.97 ± 0.20 vs. 2.14 ± 0.37 , $p < 0.05$, respectively), but not after 24 h (1.11 ± 0.07 and 0.81 ± 0.08 vs. 0.96 ± 0.05 , $p < 0.05$, respectively). By contrast, adropin (10 and 100 nmol/L) downregulated the expression of resistin (after 3 h – 0.34 ± 0.08 and 0.34 ± 0.07 vs. 1.13 ± 0.05 ; after 24 h – 0.69 ± 0.09 and 0.31 ± 0.02 vs. 1.03 ± 0.08), and visfatin (after 3 h – 0.43 ± 0.08 and 0.41 ± 0.04 vs. 0.96 ± 0.04 ; after 24 h – 0.64 ± 0.09 and 0.39 ± 0.02 vs. 0.92 ± 0.10 , $p < 0.05$, respectively) in rat adipocytes. Furthermore, adropin (10 and 100 nmol/L) effectively upregulated adiponectin mRNA expression in 3T3-L1 adipocytes exposed to adropin for 3 h (1.38 ± 0.05 and 1.49 ± 0.06 vs. 0.95 ± 0.05) and 24 h (1.27 ± 0.09 and 1.29 ± 0.05 vs. 1.04 ± 0.02 , $p < 0.05$, respectively). No effects were found in the expression of resistin (1.13 ± 0.21 and 0.96 ± 0.10 vs. 1.23 ± 0.08) and visfatin (1.07 ± 0.18 and 1.06 ± 0.08 vs. 0.91 ± 0.05 , $p < 0.05$) in 3T3-L1 adipocytes exposed to adropin (10 and 100 nmol/L, respectively) for 3 h. However, adropin (100 nmol/L) suppressed mRNA expression of resistin (0.72 ± 0.03 vs. 0.90 ± 0.04) and visfatin (0.63 ± 0.04 vs. 0.95 ± 0.02 , $p < 0.05$) in 3T3-L1 adipocytes after 24 h of incubation.

To conclude, we found that adropin stimulates adiponectin mRNA expression, but suppresses the expression of resistin and visfatin in rodent adipocytes. Our results indicate that adropin may modulate adipokine expression in rodent adipocytes.

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Reference 2 :- Jasaszwili M, Wojciechowicz T, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Effects of adropin on proliferation and differentiation of 3T3-L1 cells and rat primary preadipocytes. Molecular and cellular endocrinology 2019, 496, 110532.

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PC081

CYCLOTIDE FRACTION OF *CAJANUS CAJAN* IMPROVES INSULIN SIGNALING IN A MODEL OF TYPE 2 DIABETIC RATS

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Background: Plant cyclotides are chemically stable and could be beneficial for medicinal application if they possess such properties. *Cajanus Cajan* exhibits anti-diabetic activity; however whether its cyclotide fraction also exhibits anti-diabetic effects is unknown. The present study was thus designed to determine the anti-diabetic effect of *Cajanus Cajan* Cyclotides (CCC) in type 2 diabetic rats.

Method: CCC fraction was obtained by solvent extraction and peptide purification. Adult male wistar rats weighing 180-200 g were divided into six groups (n=7). Group 1 animals served as control and were untreated. Group 2-6 were induced with diabetes. Group 3 was administered with metformin for 14 days after induction of diabetes, Groups 4-6 were administered with CCC 10, 20 and 40 mg/kg respectively for 14 days after induction of diabetes. Diabetes was induced in rats by 3 weeks high sucrose diet followed by a single i.v low dose of streptozotocin (50mg/kg). Markers of glycaemic control, liver function test, liver histology, immunohistochemistry and biochemical markers were determined.

Result: Diabetic presentation in rats included a significant increase in fasting blood glucose from 87.5 ± 4.95 to 106.5 ± 9.2 mg/dl, increased area under the cover for oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) from 9488 ± 668 to 11693 ± 301.8 and 4020 ± 520 to 5940 ± 485 mg/dl min. respectively; significant increases in plasma triglyceride from 61.64 ± 4.79 to 146.6 ± 15.22 mg/dl when compared with control. Treatment of diabetic animals with CCC at various doses mitigated diabetic presentations which were associated with up regulation of hepatic IRS-1 and skeletal muscle GLUT 4 expressions.

Conclusion: CCC possesses remarkable anti-diabetic activity which was associated with improved insulin signalling.

PC082

The colonic catabolism of black rice anthocyanins by the gut microbiota

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Introduction: Anthocyanins (ANCs) are a major subclass of polyphenols and give the natural colour to many plant foods such as bilberries and black rice. There is considerable interest in their putative health benefits and people consuming high quantities of ANCs have lower risk of developing chronic diseases. However, studies of ANC bioavailability have shown that ANC derived phenolics are the main forms found in the circulation, while intact ANCs are very poorly bioavailable [1]. The availability of several human metabolites reported previously suggest that there are additional ANC degradation routes, presumably involving the gut microbiota [2].

Aim: this work was to investigate the role of gut microbiota on ANC metabolism and to identify microbial metabolites that may be responsible for delivering the beneficial effects to human health.

Method and experimental design: An ANC extract from black rice was incubated with live-faecal as well as autoclaved-faecal inocula using a batch colon model [2] over 24 h (n=3). Control vessels was prepared by inoculating live-faecal and for matrix solution for standard curves preparation. HPLC was

used to quantify the rate of loss of anthocyanins over time and UPLC-MS/MS and UPLC-TOF methods were used to identify and quantify the ANC metabolites that appeared. The study investigated the inter- and intra- individual differences (n=3 each).

Faecal samples used in the colon model experiments were obtained from participants recruited onto the QIB Colon Model study. The study was approved by the Quadram Institute Bioscience (formally Institute of Food Research) Human Research Governance committee (IFR01/2015), and London - Westminster Research Ethics Committee (15/LO/2169). The informed consent of all participating subjects was obtained, and the trial is registered at <http://www.clinicaltrials.gov> (NCT02653001).

Results and conclusion: Loss of ANCs occurred over 24h. However, the rate of loss of ANC was considerably faster in the presence of live faecal inoculum compared to autoclaved faeces. The most abundant initial ANC metabolites produced in the presence of gut microbiota were 3,4-dihydroxyphenylacetic acid, protocatechuic acid, phloroglucinaldehyde, catechol, and phloroglucinol carboxylic acid. The production of the most abundant metabolite, catechol, was completely microbiota-dependent, providing strong evidence that gut microbiota metabolism of ANCs is important in delivering the health benefits of ANC-rich foods.

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PC083

Neurophysiological implications of CPT1C deficiency: from synapse to behavior

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CPT1C, a neuron-specific enzyme located at the endoplasmic reticulum, is widely expressed throughout the central nervous system (CNS). Intriguingly, dense expression has been found in discrete brain areas, including the hypothalamus, hippocampus and amygdala. Although its molecular functions remain partially unclear, CPT1c is well known to regulate ceramide metabolism and, more recently, its involvement in dendritic spine maturation and AMPA receptor synthesis and trafficking has also come to light. Consistently, its main roles described to date are related to hypothalamic control of energy homeostasis, motor function and hippocampal-dependent spatial memory. However, its widespread distribution along with the diverse molecular mechanisms attributed invites to consider its potential implication in additional functions associated to different brain regions. Here, we carried out systematic characterization of the physiological role of CPT1C at different levels -molecular, synapses, neural networks and behavior- of complexity by comparing CPT1C knock-out (KO) mice and wild-type littermates. First, the expression pattern of the protein was examined by fluorescence immunohistochemistry. In behaving mice, we explored the impact of CPT1C deficiency on locomotor activity, energy status, emotional state and different types of learning. Specifically, we evaluated motor learning, hippocampal-dependent spatial memory, and associative instrumental learning. In order to correlate memory processes that rely on hippocampal function with changes in synaptic plasticity, we analyzed dendritic spine maturation in the hippocampus as well as local field potentials from hippocampal slices *ex vivo* and electroencephalographic recordings obtained *in vivo*.

Our results confirmed the presence of CPT1C across almost all brain regions, with strong expression in the hippocampus and amygdala. When compared to WT mice (n = 25), CPT1C-deficient animals (n = 22) showed energy deficits (tail suspension test (TST); $p < 0.001$) and locomotor impairments (locomotion test; $p < 0.001$). More surprisingly, CPT1C deficiency is not associated with anxiety- nor depression-like behaviors (WT, n = 25, vs CPT1C KO, n = 22; elevated plus maze, $p = 0.443$; TST, $p = 0.259$). CPT1C-KO mice also exhibited deficits in motor learning (rotarod performance task; WT, n = 25, latency = $148 \pm 13.57\%$ of first session, $p < 0.001$; CPT1C KO, latency = $96.12 \pm 7.54\%$, $p = 0.951$) and instrumental learning (Skinner-box paradigm; WT, n = 14, vs CPT1C KO, n = 12: $p = 0.009$), as well as in spatial memory (novel object location test; WT, n = 25, vs CPT1C KO, n = 20: $p = 0.846$). The latter effects might be attributed to inefficient hippocampal dendritic spine maturation (WT, n = 3, vs CPT1C KO, n = 4: $p = 0.002$), long-term plasticity impairments observed at the CA3-CA1 synapse (magnitude of LTP: WT, n = 19, vs CPT1C KO, n = 17: $p = 0.049$) and aberrant cortical oscillatory activity (gamma rhythm: WT, n = 24, vs CPT1C KO, n = 14; $p = 0.006$). Together, our results not only support the notion that CPT1C regulates energy homeostasis and motor function, but also reveal that it is required for learning and memory processes taking place in brain areas that underlie motor, associative, and non-associative learning.

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Fasting-induced torpor in mice: implications for behavioural neuroscience research

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Food restriction (FR) protocols are a common strategy in neuroscience research to facilitate engagement and motivation within behavioural tasks. However, behavioural tasks often lack reproducibility due to a high degree of interindividual variability, thus limiting the impact and reliability of the data being generated. We hypothesised that a potential source of variability may be due to the occurrence of a hypometabolic and hypothermic state termed fasting-induced torpor (FIT), in laboratory mice. FIT is a profoundly altered state of physiology, associated with sleep disruption and changes to synaptic morphology. As a result, FIT may be impacting on cognition and subsequent behavioural performance. To address this, we investigated whether FR protocols used for behavioural tasks were sufficient to induce FIT. To this end, adult male C57Bl/6J mice (N=8) were fed once daily and maintained at ~85% of their free-feeding bodyweight. As FIT expression is under circadian control, we investigated whether feeding time would alter FIT characteristics. Accordingly, mice were randomly assigned to either a light-fed group (food given at ZT1) or to a dark-fed group (food given at ZT13) of equal size (n=4). Skin temperature (T_{skin}) was continuously measured using non-invasive thermal imaging cameras to detect FIT. FIT was operationally defined as when T_{skin} was reduced by >2SD from baseline levels for at least 1 hour. Bouts of FIT were consistently observed in all mice from day 8 of FR, which coincided with when mice had reached ~85% of their free-feeding bodyweight. A relationship between bodyweight and mean daily T_{skin} over the course of FR was confirmed using linear regression analysis (light-fed R²=0.7351; dark-fed R²=0.6703). The distribution of T_{skin} values shifted from unimodal to bimodal with prolonged FR, indicating an increased occurrence of hypothermia bouts, with lower body temperatures becoming more prevalent. Mean T_{skin} significantly dropped for all mice over FR (P<0.05) by ~1-2°C compared to ad libitum T_{skin}. Similarly, the minimum daily T_{skin} also dropped significantly during FR by ~6°C. The mean number of torpor bouts expressed by the light-fed group was 8 (SD 3.464), whereas the mean value for the dark-fed group was 7 (SD 1.00). An unpaired t-test revealed that there was no difference in the number of bouts expressed by each group (P=0.9165). However, the length of FIT bouts expressed by the light-fed group were significantly longer than the dark-fed group (P=0.0007) (light-fed: mean 5.40 hrs, SD 2.77; dark-fed: mean 3.14 hrs, SD 1.06). These results indicate that FR protocols typically used in behavioural neuroscience are sufficient to induce FIT bouts, regardless of feeding time. Further, differences in FIT characteristics depending on feeding time may be contributing to variability and the lack of reproducibility between studies. Having established that FR for behavioural tasks induces torpor, the effects of torpor on subsequent performance will be

investigated. As such, mice will perform a series of tasks during and after FIT bouts. These tasks will be used to assess locomotor ability, exploratory behaviour, and cognitive processes such as learning and memory.

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PC085

Atrial Granules as Acidic Calcium Stores in Atrial Cardiomyocyte Physiology and Disease

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Acidic calcium stores in the heart provide a significant contribution to basal calcium transient amplitude and β -adrenergic responses in both atrial and ventricular myocytes¹. In ventricular myocytes these acidic compartments, lysosomes, are positioned in close apposition to the sarcoplasmic reticulum and mitochondria, forming membrane contact sites which have the potential to act as signalling microdomains². Atrial, but not ventricular, myocytes express a second small acidic organelle, the atrial granules (AG). AG are lysosome-related organelles which secrete ANP. They are known to be acidic and contain a high calcium content³ however their number relative to lysosomes and position with relation to other calcium signalling sites has not been fully explored.

All animal experiments were performed in accordance with the United Kingdom Home Office Guide on the Operation of Animal (Scientific Procedures) Act of 1986. Guinea pig myocytes were isolated enzymatically and live cell imaging performed using spinning disk confocal microscopy. Human tissue samples (University of Freiburg) were collected from patients in sinus rhythm (SR) and atrial fibrillation (AF), fixed in Karnovsky fixative, embedded in Epon resin and imaged at the EMBL Heidelberg Electron Microscopy (EM) Core Facility.

Staining of acidic organelles (LysoTracker) in freshly isolated guinea pig atrial and ventricular myocytes revealed fluorescent acidic puncta throughout the cytosol. In atrial myocytes there was an additional concentration of acidic organelles at the nuclear poles, consistent with published

literature on the site of AG formation⁴. Inhibition of PAM (200 μ M PBA), an essential component of AG membranes⁴, abolished not only staining at the nuclear poles but the majority of acidic puncta in atrial cells. Application of PBA to ventricular myocytes had no impact on LysoTracker staining.

Consistent with our live cell results, EM of fixed human atrial tissue revealed the presence of both lysosomes and AG in atrial myocytes. In the SR sample, sarcomeres are regularly structured and surrounded by rows of mitochondria whilst in persistent AF, we observed myolytic areas. Qualitatively, both AG and lysosomes were significantly increased in samples from AF patients when compared to SR. Examples were seen of both lysosomes and AG in close apposition to the sarcoplasmic reticulum and mitochondria, as well as to each other.

In the 1960s EM studies suggested that patients and dogs with atrial septal defects (AF is a common complication in these patients) have increased lysosome numbers⁵. Our imaging studies suggest that AG comprise a large percentage of acidic calcium stores within atrial cardiomyocytes and our preliminary EM studies in human samples indicate an increase in number during human atrial disease. Acidic calcium stores are known to play a significant role in normal atrial physiology¹ and increase in number during atrial disease⁵. The strategic position of AG raises the question of whether they signal with other calcium containing organelles. It is an important next step to explore whether atrial granules contribute to physiological calcium signalling on top of their known hormone secretion role.

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Matrix metalloproteinase-9 expression levels in acute ischaemic stroke (AIS) clots of different etiologies.

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Introduction

The matrix metalloproteinase MMP-9 is involved in the integrity of the vascular wall. MMP-9 degrades denatured collagens, several types of native collagens as well as other extracellular matrix substrates such as laminin. As such they can degrade the basal lamina surrounding capillaries which contain collagen type IV, fibronectin and laminin.

Neutrophil infiltration into infarcted and haemorrhagic tissue along with elevated neutrophil derived MMP-9 expression has also been associated with basal lamina collagen IV degradation leading to blood-brain barrier breakdown.

We hypothesised that MMP-9 would be a component within the clots that cause acute ischaemic strokes and that there may be a potential difference in MMP-9 expression levels in AIS clots based on stroke etiology (cardioembolic v large artery atherosclerosis (LAA)).

Methods

Clots from 100 AIS patients (165 passes) were collected. These were separated based on etiology into two groups, Cardioembolic (50 cases, 73 passes) and LAA (50 cases, 92 passes). Immunohistochemical staining was performed on 3µm sections for MMP-9 on a Leica Bond III autostainer. Histological staining for red blood cells (RBC), white blood cells (WBC), fibrin and platelets/other components was performed using Martius Scarlet Blue (MSB) stain. Images were scanned using an Olympus VS120 slide scanner. Quantification analysis to determine percentage expression was performed using Orbit Image Analysis software. Data was not normal therefore the non-parametric Mann-Whitney test was used for statistical analysis (Graphpad 8).

Immunofluorescence was used to determine co-localisation of MMP-9 with key clot components, neutrophils (CD66b), fibrin, lymphocytes (CD3), red blood cells (GlyA), platelets (CD42b), neutrophil extracellular traps (NETs) (histone H3).

Results

Descriptive statistics and the Mann-Whitney test statistics are reported in table1.

Table 1: Descriptive statistics for MMP-9, RBC, WBC, fibrin and platelets/other for cardioembolic and LAA clots. Non-parametric statistics by Mann-Whitney test for comparison of components across etiologies.

There was a strong trend indicating that content of cardioembolic clots were lower in RBC's and higher in fibrin which is in agreement with published data. There was considerable expression of MMP-9 in AIS clots, however there was no significant difference in MMP-9 expression in AIS clots of cardioembolic and LAA etiologies.

Co-localisation studies showed that WBC's, specifically neutrophils were the main component in AIS clots that expressed MMP-9. There was also some evidence of MMP-9 in NET's. MMP-9 expression did not co-localise with RBC's, fibrin or platelets.

Conclusion

No significant difference in MMP-9 expression levels was observed between Cardioembolic and LAA. There was no significant difference in WBC's according to etiology. LAA clots were richer in RBC's but lower in fibrin and platelets.

Co-localisation studies showed that neutrophils were the main source of MMP-9 in AIS clots. Although MMP-9 is expressed to a significant extent in AIS clots, the level of expression is not connected to etiology.

Etiology	n	Median % expression	IQR	Mean (SD) % expression	Mann Whitney test statistic
MMP-9					
Cardioembolic	73	9.51	4.97-17.63	13.24 (11.08)	p=0.8431
LAA	92	10.49	6.30-14.60	12.63 (9.54)	U=3297
RBC					
Cardioembolic	73	39.34	21.92-57.34	39.81 (21.35)	p=0.0567
LAA	92	46.57	30.11-65.50	47.07 (23.49)	U=2777
WBC					
Cardioembolic	73	2.64	1.51-4.10	2.98 (1.92)	p=0.2286
LAA	92	2.41	1.35-3.56	2.70 (1.79)	U=2990
Fibrin					
Cardioembolic	73	27.15	16.95-37.02	28.29 (15.11)	p=0.1041
LAA	92	21.87	12.88-34.08	24.66 (14.58)	U=2862
Platelets/other					
Cardioembolic	73	22.26	12.95-46.48	28.90 (19.69)	p=0.4970
LAA	92	22.15	13.83-36.08	25.44 (15.59)	U=3150

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PC087

Rescue of homeostatic regulation: the effect of drug vs lifestyle approach

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Doxorubicin(DOX) is a highly effective anticancer agent that improved survival and patient's quality of live but causes dose-dependent cardiotoxic effects leading to severe and irreversible cardiomyopathy in many patients. Different preventive strategies, such as physical exercise and β -blockers, have been proposed to maintain physiological homeostasis. However, besides the extensive research that has been done to understand the mechanism and pathophysiology of DOX, it is not clear what is the most effective preventive approach to maintaining physiological homeostasis.

In the present work, we intended to compare the efficacy of two different approaches, one pharmacological intervention, using atenolol, a β 1-selective antagonist, and other non-pharmacological intervention using treadmill training in an animal model of DOX. Female Wistar rats were divided into 4 groups: Doxorubicin (DOX; ip. cumulative dose 8mg/kg, 1 time/week, for

4weeks), DOX with physical exercise (DOX + EX; treadmill, 22 cm/seg for 30minutes, 5 times/week), DOX with β -blocker (DOX + ATN: DOX; ip. cumulative dose 8mg/kg, 1 time/week and 4mg/ml, Atenolol; oral administration, 5 times/week, for 4weeks) and controls (ip. with saline solution).

At the end of the protocol, animals were anaesthetised with sodium pentobarbital and blood pressure(BP), electrocardiogram, heart rate(HR) and respiratory frequency(RF) were recorded. Baro and chemoreceptor reflexes were stimulated by phenylephrine(25 μ g/mL,iv) and lobeline(25 μ g/mL,ia), respectively and baroreflex gain and chemoreflex sensitivity calculated. Low frequencies(LF) and high frequencies(HF) were determined for sympathetic and parasympathetic activity estimation. One-way ANOVA with Tukey's multiple comparison between means were used (significance $p<0.05$) for statistical analysis.

All the experimental procedures were in accordance with the European Community legislation on animal experimentation and were approved by Ethical Committee of the Academic Centre of Lisbon.

Our results reveal that DOX treatment triggered a significant decrease in systolic (CTL: 156 ± 6 vs DOX: 114 ± 9 mmHg), diastolic (CTL: 105 ± 3 vs DOX: 83 ± 8 mmHg) and mean BP (CTL: 127 ± 3 vs DOX: 97 ± 9 mmHg) as well as in HR (CTL: 378 ± 24 vs DOX: 289 ± 28 bpm), caused hypopnea (CTL: 71 ± 2 vs DOX: 41 ± 3 cpm), decreased baro (CTL: 1.5 ± 0.3 vs DOX: 0.4 ± 0.1 bpm/mmHg) and chemoreflexes (CTL: 22 ± 2 vs DOX: 18 ± 3 cpm), without evidence of sympatho-excitation (LF-band, CTL: 2.93 ± 0.9 mmHg² vs DOX: 0.65 ± 0.17 mmHg²). These changes can be explained by the decline in cardiac function, respiratory muscle weakness, autonomic dysfunction and vascular changes induce by DOX. During treatment with DOX, the physical activity protocol countered some of the adverse effects caused by DOX. It normalized systolic (165 ± 5 mmHg), diastolic (123 ± 6 mmHg) and mean BP (141 ± 5 mmHg), HR (406 ± 6 bpm), and RF (65 ± 4 cpm) to physiological values and decreases the loss in baroreflex gain (0.5 ± 0.06 bpm/mmHg). Chemoreflex sensitivity, sympathetic and parasympathetic activities remained similar. Atenolol treatment, similar to the physical activity effect, also increased baroreflex gain (0.9 ± 0.2 bpm/mmHg) and RF (70 ± 4 cpm) to normal values, causing a clear tendency to maintain BP values.

Although complementary data is still needed, with these results we can conclude that treadmill training was more effective than atenolol in counteracting the adverse effects of the cumulative low dose of DOX administered, suggesting that physical activity is a good non-pharmacological alternative to atenolol for preserving the homeostasis during DOX therapy.

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Voltage-dependence of Na⁺ current inhibition by eleclazine in adult rat atrial and ventricular myocytes

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Atrial fibrillation (AF) is a fast and irregular activation of the atria that represents the most common clinical arrhythmia. AF is associated with significant mortality and morbidity. While sodium channel blockers can be used as part of a rhythm-control strategy, their use is limited due to the risk of potentially lethal ventricular tachyarrhythmias in patients with cardiac structural abnormalities (Hindricks *et al.*, 2020). It has been suggested that atrial-ventricular differences in Na⁺ currents (I_{Na}) may be exploited for atrial selective antiarrhythmic drug action without risk of ventricular side-effects (Hancox *et al.*, 2016). Eleclazine (GS-6615) is a putative anti-arrhythmic drug that has been shown to be safe and well-tolerated in patients and have properties similar to the prototypical atrial-selective Na⁺ channel blocker, ranolazine (Caves *et al.*, 2017; El-Bizri *et al.*, 2018). At a previous meeting, we presented data from rat isolated cardiac myocytes showing atrial-ventricular differences in I_{Na} and suggested an atrial-selective action of eleclazine, although the inhibitory mechanism was unclear (Caves *et al.*, 2019). The present study investigated the mechanisms of I_{Na} inhibition by eleclazine in left atrial and left ventricular myocytes isolated from adult male Wistar rat hearts. Procedures were approved by local ethics committee and performed in accordance with UK legislation. I_{Na} was recorded at room temperature (~22 °C) using whole-cell patch clamp recording techniques with symmetrical external and internal [Na⁺] (5 mM). The use-dependence of I_{Na} inhibition by eleclazine (10 μM) was examined at holding potentials (HP) of -120 mV and -100 mV using a series of 40 pulses of 20 ms duration to -30 mV at diastolic intervals (DI) of 40 ms and 110 ms. Data are presented as mean ± standard error of the mean, compared by unpaired *t*-test and the limit of statistical confidence is *P*<0.05. Eleclazine caused use-dependent inhibition of I_{Na} in both atrial (HP=-120 mV, *n*=12; HP=-100 mV, *n*=8) and ventricular (HP=-120 mV, *n*=9; HP=-100 mV, *n*=9) myocytes that was greater at DI=40 ms (HP=-120 mV: atrial 37.5±2.6%; ventricular 36.2±5.1%; HP=-100 mV: atrial 22.9±6.3%; ventricular 23.8±4.1%) than DI=110 ms (HP=-120 mV: atrial 17.1±1.9%; ventricular 15.7±3.2%; HP=-100 mV: atrial 8.6±2.0%; ventricular 10.4±1.9%). However, there was no difference between the two cell-types in the use-dependent inhibition of I_{Na} at either HP. Eleclazine also produced an instantaneous inhibition of I_{Na} , which was greater at HP=-100 mV than at HP=-120 mV in both cell types. Notably, at HP=-120 mV, the instantaneous inhibition was greater in atrial (9.1±1.9%) than in ventricular myocytes (-0.8±2.2%; *P*=0.0031) whereas at HP=-100 mV there was no difference between the two cell-types (atrial: 28.8±6.3%; ventricular: 29.1±4.7%). Eleclazine caused a negative shift in the voltage of half-maximal inactivation and slowed the recovery of I_{Na} from inactivation in both cell types. Increasing the duration of the test pulse to 200 ms had no effect on eleclazine inhibition in either cell type. In summary, the data are consistent with preferential activated state block of Na⁺ channels by eleclazine in atrial and ventricular myocytes (Caves *et al.*, 2020). Eleclazine warrants further investigation as an atrial-selective anti-arrhythmic drug.

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Reference 2 :- Caves RE, Cheng H, Choisy SC, Gadeberg HC, Bryant SM, Hancox JC & James AF. (2017). Atrial-ventricular differences in rabbit cardiac voltage-gated Na⁺ currents: basis for atrial-selective block by ranolazine. *Heart Rhythm* **14**, 1657-1664. doi: 10.1016/j.hrthm.2017.06.012

Reference 3 :- El-Bizri N, Xie C, Liu L, et al. (2018). Eleclazine exhibits enhanced selectivity for long QT syndrome type 3-associated late Na⁺ current. *Heart Rhythm* **15**, 277-286. doi: 10.1016/j.hrthm.2017.09.028

Reference 4 :- Hancox JC, James AF, Marrion NV, Zhang H & Thomas D. (2016). Novel ion channel targets in atrial fibrillation. *Exp Opin Therap Targets* **20**, 947-958. doi: 10.1517/14728222.2016.1159300

Reference 5 :- Hindricks G, Potpara T, Dagres N, et al. (2020). 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. *Eur Heart J* **42**, 373-498. doi: 10.1093/eurheartj/ehaa612

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PC089

Phase contrast X-ray CT for imaging of the entire circumferential structure of arteries under pulsatile pressure condition

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X-ray phase computed tomography (PCT) is highly sensitive in detecting density differences between structure components of biological soft tissue and well-suited to visualize the density-based tissue structure. Recently, PCT was successfully used to visualize repeated motions of soft tissue [1]. In this study, we applied PCT for visualizing arteries under pulsatile pressure condition and evaluated its feasibility in microstructure measurement of the entire circumferential arterial wall.

A porcine carotid artery, purchased from a slaughterhouse, was measured by PCT at SPring-8 synchrotron radiation facility. The arterial segment was closed at one end by a plug. The other end was connected to a T-shape connector that was attached to a rotational stage on one side and

connected on the other side to an overflow reservoir via a polyethylene tubing with an in-line check valve (Fig. 1). The tubing was filled with physiological saline. To induce pulsatile pressure waves, a programmable syringe pump was used to move the saline through a branch tubing in a to-and-fro motion over 2 s with 1-s rest insertion. The arterial segment was axially stretched by 30% and placed in a saline-filled container.

A PCT system used a Talbot grating interferometer for phase measurement. The grating interferometer was composed of a phase grating (G1) and an absorption grating (G2), both with the pitch size of $2.4\ \mu\text{m}$, and optimized for the 20-keV X-ray. A fiber coupled sCMOS camera equipped with a beam monitor and a P43 phosphor screen was used as an X-ray detector. The effective pixel size was $4.47\ \mu\text{m}$. Schematic drawings of the image acquisition procedure are shown in Fig. 2. Phase retrieval was achieved by 5-step fringe scan, where the G2 mounted on a piezo stage was scanned for phase stepping with sequential saw-tooth wave signals from a function generator. The trigger signal from the syringe pump generated at the end of each pulsatile pressure was used to synchronize the saw-tooth wave generation as well as the projection angle increment. Images were acquired with 40-ms exposure at 50-ms intervals, yielding 60 images during each pressure pulsation or equivalently at each angular position of sample rotation over 180° in 0.2° steps.

Cross-sectional tomographic images of the artery were shown at seven time points in a single pressure pulsation in Fig. 3, with the magnified image in the rectangle. The density resolution was estimated to be $2.5\ \text{mg}/\text{cm}^3$. When the pressure was increased from 70 to 90 mmHg, the equivalent inner diameter calculated from the luminal area increased by 2.5% from 2.81 cm to 2.88 cm, and the wall volume of 3.8-mm long segment decreased by 0.6%. The outer layer is higher in density than the inner layer; the latter presented light- and dark-gray bands (i.e., different density bands). Elastica van Gieson staining confirmed that the image contrast in PCT was attributed to different components of vascular wall (Fig. 4). The present PCT has the potential to promote better understanding of arterial wall dynamics as well as risks of arterial rupture and dissection.

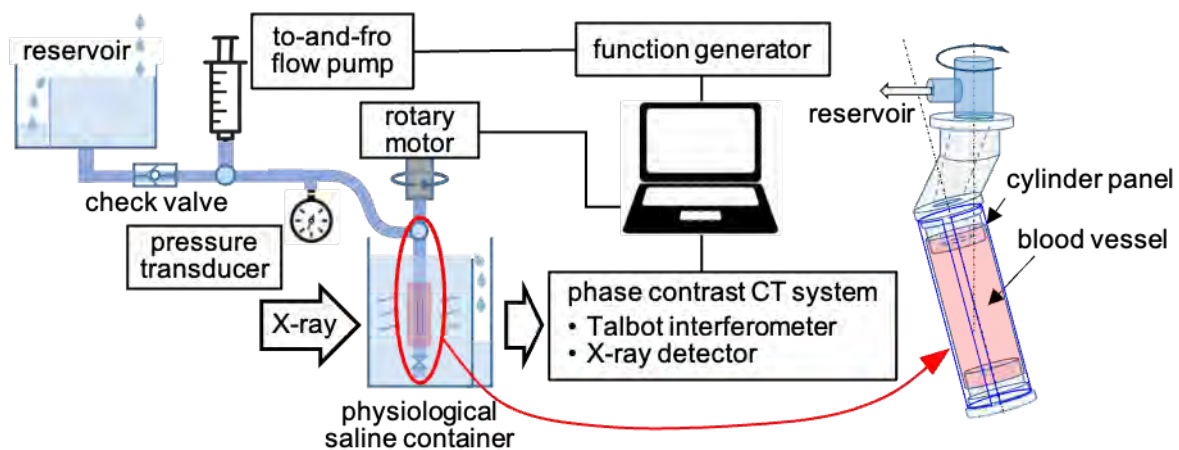


Fig. 1 Experimental setup

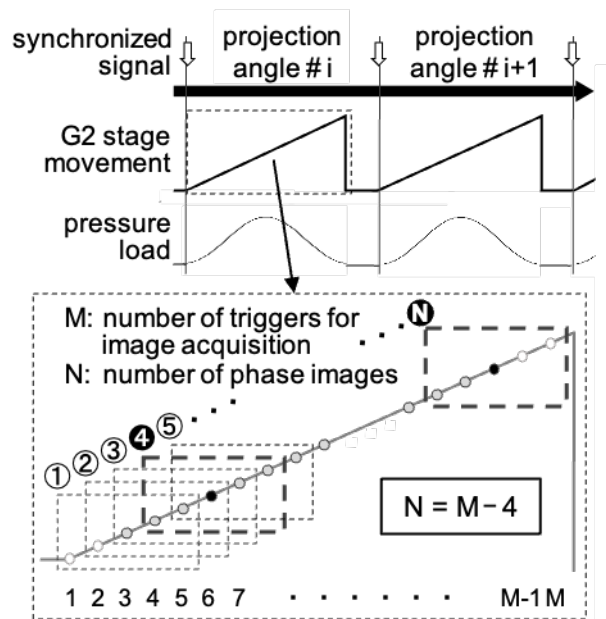


Fig. 2 Schematic drawings of X-ray phase CT under repetitive deformation of a sample.

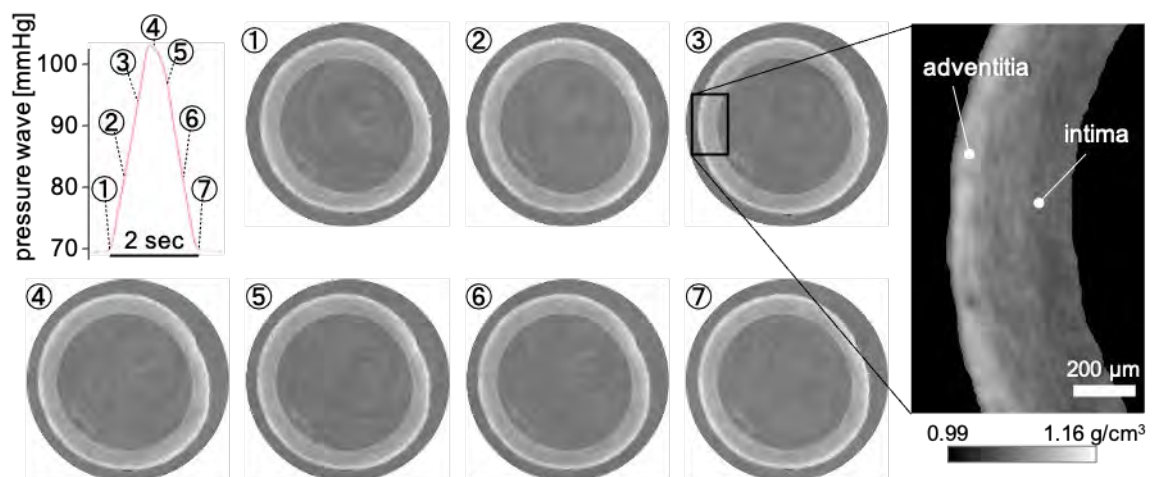


Fig. 3 Cross-sectional tomographic image of the entire arterial wall at seven time points. The magnified image in the rectangle is shown on the right.

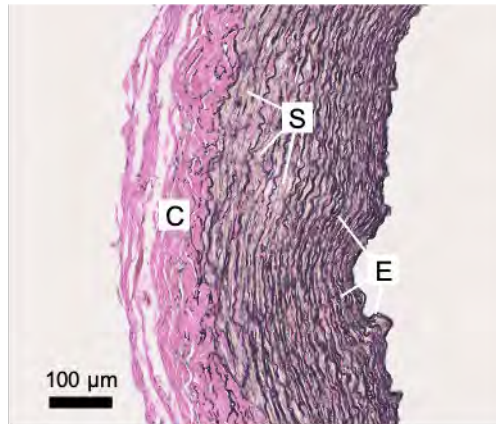


Fig. 4 Histological section of the same sample measured by PCT (Fig. 3), stained with Elastica van Gieson: elastin (black, E), collagen (pink, C), smooth muscle cells (yellow, S).

Reference 1 :- Hoshino et al. (2017). J Physics: Conf Ser 849, 012054.

Acknowledgements :- Part of this study was supported by Grants-in-Aid for Scientific Research (grant no. 20K21899) from the Ministry of Education, Culture, Sports, Science, and Technology.

PC090

Factors Contributing to the Discrepancies In 2D and 3D Fetal Echocardiography Strain Measurements

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Introduction

Echocardiographic strain measurements can be performed with 2D or 3D speckle tracking. Differences between 2D and 3D measurements are reported, but the exact mechanism for this is unclear [1]. Also, measured myocardial strain magnitudes in fetal hearts vary substantially across different studies [2], and it is unclear why this is so. Our study aims to demonstrate possible reasons for these discrepancies.

Methods

4D echocardiography images (STIC mode) were obtained from 10 healthy fetuses. A validated cardiac motion estimation algorithm [3] was used to track the motion of the fetal left ventricle (LV) in 3D and 2D, and was used to calculate myocardial strains. The same images were used for both 2D and 3D strain quantification to enable controlled comparisons.

Results

3D longitudinal strain (LS) was significantly lower than 2D LS by 2.7%. This is partially due to LV twist causing the longitudinal line in 3D to move out of plane while preserving its length after contraction. 2D quantification cannot capture this effect. By quantifying strains in a way to negate LV twisting (tracking motion in 3D but projected tracked points to the 2D plane before strain calculation), We found that 1.2% of the difference can be explained by LV twist motion. *In the circumferential direction*, 3D strain was found to be significantly higher than 2D circumferential strain (CS) by 2.0%. This can be fully explained by the systolic motion of myocardium towards the apex, which brings wider transverse cross-sections down to the imaging plane.

A timing mismatch was observed between when the longitudinal and circumferential lengths are at their peaks, caused by LV shape changes during the isovolumic contraction period. In 2D strain quantification, strain in each direction is assigned different zero-strain reference time; in 3D, a single reference time is used for both directions. Favouring any one direction when specifying this reference will reduce strain magnitude of the other direction. This accounted for another 0.7-0.8% difference between 2D and 3D strains.

A spatial variability of strains was also found. Strains at epi- and endo-cardial locations differed substantially, by 3.6% in the longitudinal direction and 9.3% in the circumferential direction. Since strain quantifications are manually controlled clinically, this could account for wide discrepancies between the different studies [2], in which reputable groups and publications reported strain values that differed by 6.3-7.1%. Different smoothing extent during motion tracking causes significant differences in strain values, and could be another factor for discrepancies between studies. Lastly, we find that 3D motion tracking can enable the quantification of LV twist ($7.8 \pm 3.3^\circ$) and the average myofiber orientations ($4.6 \pm 2.7^\circ$). Myofiber orientations was estimated via eigenvectors of the strain tensor.

Discussion

We demonstrated mechanisms for discrepancies between 2D and 3D strain measurements, and potential reasons for wide discrepancy in literature values of fetal myocardial strains. Our finding calls for caution in interpreting strain results in the literature before future standardization of strain is achieved. Although our study was conducted on fetal echocardiography, the results are likely applicable to adult echocardiography.

Reference 1 :- Muraru, et al. J. Am. Soc. Echocardiogr. 27:858, 2014

Reference 2 :- van Oostrum et al. Eur. J. Obstet. Gynecol. Reprod. Biol. 250:178, 2020

Reference 3 :- Wiputra et al. Sci. Rep. 10:18510, 2020

Acknowledgements :-

Funding for this study is provided by Singapore Ministry of Education, Tier 2 Grant (MOE2018-T2-1-003), and Imperial College startup funding.

PC091

Defective X-gating in the TASK-1 K⁺ channel caused by *de novo* mutations in *KCNK3* produces a developmental disorder with sleep apnea

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A recent study identified 28 novel genes with a high burden of *de novo* mutations associated with developmental disorders [1]. One of these genes was *KCNK3* which encodes TASK-1, a member of the Two-Pore Domain (K2P) family of K⁺ channels. The recent crystal structure of TASK-1 revealed a lower gate (X-gate) created by two crossed transmembrane helices (M4) at the vestibule entrance [2].

All nine patients in this cohort were heterozygous for a single *de novo* missense mutation in *KCNK3* and shared a common phenotype with developmental delay, abnormality of the limbs, face and jaw, and also sleep apnea. A total of six unique mutations were found in this cohort of nine patients. TASK-1 is expressed in many tissues associated with sleep apnea and has previously been implicated in many of the pathways which control breathing, chemosensitivity and oxygen sensing [3, 4].

In this study, we investigated the functional properties of the six novel mutations found in these patients to gain a deeper insight into the mechanisms which produce sleep apnea and potential therapeutic strategies.

Whole-cell recordings of heterologously expressed mutant TASK-1 channels revealed a markedly higher activity compared to wild-type TASK-1 with single channel recordings demonstrating up to a 10-fold increase in open probability for all the tested mutants. All six patient mutations are located within the M2 or M4 helices and cluster around the X-gate in TASK-1. Molecular dynamics simulations of both WT and mutant structures of TASK-1 also revealed that the mutations promote opening of the X-gate.

Furthermore, both coexpression with G-protein coupled receptors (GPCR) and activation of endogenous muscarinic receptors revealed a strongly reduced sensitivity to GPCR-mediated inhibition of all mutant channel currents compared to wild type. Overall these mutations give rise to a marked gain-of-function compared to wild-type TASK-1 and so we characterised a wide range of natural ligands and drugs known to inhibit TASK-1 that might be used as a potential treatment for these patients. Nearly all known TASK-1 inhibitors were found to work equally well on these mutants, including one specific drug (BAY1000493) used in clinical trials for the treatment of sleep apnea which exhibits highly efficacious block of these mutant channels in the nanomolar range.

Reference 1 :- Kaplanis, J., et al., (2020) Evidence for 28 genetic disorders discovered by combining healthcare and research data. **Nature**, 586: 757-762.

Reference 2 :- Rödström, K., et al., (2020) A lower X-gate in TASK channels traps inhibitors within the vestibule. **Nature**, 582: 443-447.

Reference 3 :- Trapp, S., et al., (2008) A Role for TASK-1 (KCNK3) Channels in the Chemosensory Control of Breathing. **Journal of Neuroscience**, 28: 8844-8850.

Reference 4 :- López-Barneo, J., et al., (2016) Oxygen sensing by the carotid body: mechanisms and role in adaptation to hypoxia. **American Journal of Physiology**, 310: C629-C642.

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PC092

Ferulic acid enhanced L-type Ca^{2+} channel function and insulin secretion in rat insulinoma cell line

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Introduction: Ferulic acid is a natural polyphenol richly found in rice bran, whole grains and citrus fruits. Studies showed that this compound induced *in vitro* insulin secretion and decreased blood glucose in diabetic animal model.

Aims: To study the effects of ferulic acid on cell viability, L-type Ca^{2+} channel, and insulin secretion, in INS-1 cells, a rat insulinoma cell line.

Methods: Cell viability was assessed with MTT assay. Whole-cell L-type Ca^{2+} currents were recorded using patch clamp technique. Insulin secretion was measured with ELISA assay.

Results: INS-1 cells exposure to 0.3–100 μM ferulic acid for 24 and 48 hours did not affect the cell viability. Electrophysiological experiments showed that ferulic acid significantly induced a rapid concentration-dependent increase in L-type Ca^{2+} currents (mean $\pm$ SEM; EC_{50} , $3.10 \pm 0.22 \mu\text{M}$; maximum increase, $70.98 \pm 9.64\%$). Moreover, 10 μM ferulic acid shifted the conductance-voltage curve in the hyperpolarized direction (control vs. ferulic acid: $V_{1/2}$, -15.16 ± 2.11 vs. $-20.75 \pm 2.97 \text{ mV}$, $n = 7$, $p = 0.03$, paired t -test), with decreased slope factor (8.56 ± 0.74 vs. $6.21 \pm 0.35 \text{ mV}$, $n = 7$, $p = 0.02$, paired t -test), while the voltage dependence of inactivation was not affected. Furthermore, 10 μM ferulic acid could significantly increase insulin secretion and this effect was inhibited by nifedipine and Ca^{2+} -free extracellular fluid: %Increase of insulin secretion in cells treated with ferulic acid, ferulic acid + nifedipine, and ferulic acid in Ca^{2+} -free solution were $41.79 \pm 1.46\%$, $1.93 \pm 5.34\%$, and $-3.96 \pm 3.18\%$, respectively ($n = 3$ each, $p < 0.05$, one-way ANOVA with Tukey's *post hoc* test).

Conclusion: We demonstrated that ferulic acid-induced insulin secretion in INS-1 cells was mediated by augmenting Ca^{2+} influx through L-type Ca^{2+} channel. This is the first electrophysiological demonstration that acute ferulic acid exposure could increase L-type Ca^{2+} current by enhancing the channel's voltage sensitivity, which may explain the rapid ferulic acid-induced insulin secretion and *in vivo* lowering of blood glucose levels.

No conflict of interest.

PC093

Increased expression of (pro)renin receptor by anti-cancer drugs in cultured cancer cells: Relation to apoptosis and autophagy

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(Pro)renin receptor [(P)RR] is a receptor for renin and its precursor, prorenin. (P)RR plays physiological roles, not only in the renin-angiotensin system, but also in the activity of vacuolar H^{+} -ATPase (V-ATPase)(Nguyen G. 2011). V-ATPase is responsible for the active transport of protons and maintains the acidity of intracellular compartments and the extracellular environment (Forgac, 2007). (P)RR is expressed in various types of cancer cells, including breast cancer and lung cancer. We have recently reported that suppression of (P)RR expression by (P)RR siRNA reduced cell proliferation and migration with suppressed autophagy in A549 lung cancer cells, suggesting that (P)RR plays a role in the cell proliferation and progression of cancer cells through the regulation of autophagy (Ohba et al., 2020). The aim of the present study is to clarify the effects of anti-cancer drugs on (P)RR expression, and the relation to apoptosis and autophagy in cancer cells. MCF-7 breast cancer cells and A549 lung cancer cells were treated with anti-cancer drugs, carboplatin or paclitaxel, and the expression of (P)RR, apoptosis markers and autophagy markers were studied by RT-qPCR, western blot analysis and immunocytochemistry. Expression levels of (P)RR mRNA were elevated 1.4-fold ($p < 0.0001$) at 48 hours in MCF-7 cells and 1.3-fold ($p < 0.01$) at 24 hours and 1.8-fold ($p <$

0.0001) at 48 hours in A549 cells by the treatment of carboplatin (100 μ M)(n = 4). Expression levels of (P)RR mRNA were also elevated 1.3-fold ($p < 0.01$) at 48 hours in MCF-7 cells and 1.4-fold ($p < 0.01$) at 48 hours in A549 cells by the treatment of paclitaxel (10 nM) (n = 4). Western blot analysis showed that carboplatin increased expression levels of full-length (P)RR at 100 μ M (1.2-fold, $p < 0.05$ compared to control) and soluble (P)RR at 50 and 100 μ M (2.2-fold, $p < 0.05$ and 3.0-fold, $p < 0.01$ compared to control) in MCF-7 cells (n = 4). Carboplatin increased expression levels of soluble (P)RR at 100 μ M (3.8-fold, $p < 0.05$, compared to control) in A549 cells (n = 4). Paclitaxel increased expression levels of soluble (P)RR at 10 nM in both MCF-7 and A549 cells (4.5-fold, $p < 0.05$, and 2.4-fold, $p < 0.05$, compared to control, respectively) (n = 4). Immunofluorescence staining of (P)RR was enhanced in both MCF-7 and A549 cells treated by carboplatin or paclitaxel. Apoptosis induction was shown by elevated BAX/BCL2 mRNA levels and increased active caspase3-positive cells. Moreover, autophagy induction was confirmed by increased levels of autophagy-associated mRNAs and LC3B-II proteins. The present study showed the expression of (P)RR mRNA and soluble (P)RR was increased by anti-cancer drugs with apoptosis and autophagy. Upregulated (P)RR and autophagy may act in protection of cancer cells from apoptosis.

Reference 1 :- Forgac M. Nat Rev Mol Cell Biol, 8:917-929; 2007. doi:10.1038/nrm2272

Reference 2 :- Nguyen G. Clin Sci (Lond), 120:169-178; 2011. doi:10.1042/cs20100432

Reference 3 :- Ohba K et al. Genes Cells 25:782-795; 2020. doi:10.1111/gtc.12812

Acknowledgements :- This study was supported in part by Grants-in-Aid for Scientific Research (19H03677, 19K17661, 19K21596, and 20K08612) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and in part by Takeda Science Foundation.

PC094

Animation for the Hypothesis that the Cervix Transforms into the Lower Uterine Segment through the TYVU and an inverted U Pattern due to Direct and Indirect Uterine Cervical Interaction and its Subsequent Reversal

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Abstract

This hypothesis accepted for iposter presentation at the RCOG world congress 9-12 June 2021.

It is also, accepted for publication in the online World Congress edition of the BJOG journal.

This submission is 3D-animation for the hypothesis.

<https://www.youtube.com/watch?v=dyyG-Jhxr7o>

Objective:

Support the hypothesis that the cervix transforms into the lower uterine segment (LUS) through TYVU and inverted U pattern with its subsequent reversal.

Methods:

1. This study investigates the current evidence base that may support Roederer and Mauriceau's hypothesis (the 1800s), that the LUS is derived from the cervix. (1)
2. A 40-second 3D animation supporting the hypothesis was developed in conjunction with Houston-based Baulsen Medical Company depicting the mechanism of transformation of the cervix into the LUS and its reversal.

Conclusions:

- During pregnancy and labor, due to the complex uterine system with the presence of fetal resistance, each uterine contraction creates two opposing forces that cause direct and indirect uterine cervical interaction (DIDUCI). (2,3)
- The first force is a direct force that pulls the cervix upward against the cervical ligaments, causing direct uterine cervical interaction.
- The second force is secondary to the first force and pushes the fetus down, causing circumferential cervical stretching and indirect uterine cervical interaction.
- In the third trimester, DIDUCI causes chronic upward cervical traction, which chronically transforms the cervix cranially into the LUS through a TYVU pattern (4) with simultaneous pushing the fetal head down through the pelvic inlet.
- By the end of the first stage of labor, the cervical tissue disappears completely through an inverted U pattern with complete transformation into the LUS.
- The cervix regains its full deformed shape instantly after the baby's delivery due to a reversal of these changes.
- The cervix contributes to the uterine volume when it is transformed into the LUS.
- The progressive loss of the cervical strength through a TYVU pattern is secondary to its transformation into the LUS, and it is due to DIDUCI.
- The cervix keeps its strength to the last fibers, and the U pattern initiates labor.
- The TYVU pattern dictates the duration of pregnancy.
- DIDUCI dictates the duration of the TYVU pattern through the interactive relationship between the uterine force and the cervical strength, and cephalon-pelvic proportion.

Unanswered Questions:

1. Cervical integrity is essential for a successful pregnancy, what is the primary function of the cervix during pregnancy?
2. Loss of cervical strength initiates labor, what is the mechanism that initiates labor when the cervix loses resistance?

The answer to these questions is submission no. **0264**

Reference 1 :- Smyly. W. The Ingleby lecture on the lower uterine segment and the contraction ring:. delivered before the medical faculty of the University of Birmingham.: Br Med J; 1901. p. 1(2107):1189–92.

Reference 2 :- Mahmoud H, Wagoner Johnson A, Chien EK, Poellmann MJ, McFarlin B. System-level biomechanical approach for the evaluation of term and preterm pregnancy maintenance. J Biomech Eng. 2013;135(2):021009.

Reference 3 :- Allman AC, et al. Head-to-cervix force: an important physiological variable in labor. The temporal relation between head-to-cervix force and intrauterine pressure during labor. Br J Obstet Gynaecol 1996;103(8):763-768.

Reference 4 :- Iams JD. Prediction and early detection of preterm labor. Obstet Gynecol 2003;101:402–12.

Acknowledgements :- The author has nothing to declare.

PC095

Animation for the Hypothesis that the Cervix Dictates the Pregnancy Interval and Circadian Timers through Exponential Uterine Wall Tension with Light-dark Cycle Modulation

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Abstract

This hypothesis is accepted for iposter presentation at the RCOG world congress 9-12 June 2021.

It is also, accepted for publication in the online World Congress edition of the BJOG journal.

This submission is 3D-animation for the hypothesis.

<https://www.youtube.com/watch?v=0e00RQFdj6w>

Background:

The smooth muscle of the uterus during pregnancy presents a unique circumstance of physiological mechanotransduction because the tissue remodels in response to stretching imposed by the growing fetus, yet the nature of the molecular and functional adaptations remains unresolved. (1)

We hypothesize that the exponential uterine wall tension (EUWT) (2) is the functional component of the uterine mechanotransduction and the intrinsic myometrial cell character (IMCC) is the molecular component.

IMCC enables the uterus to control its function autonomously and intrinsically secondary to tension change. Where high tension induces relaxation and low tension induces contraction. (3-5)

Objective:

To answer:

1. What is the primary function of the cervix during pregnancy?
2. What is the mechanism that initiates labor when the cervix loses strength?

Methods:

1. Review the evidence-based that supports this hypothesis.
2. A 40-second 3D animation supporting the hypothesis was developed in conjunction with Houston-based Baulsen Medical Company.

Conclusions:

- Uterine wall tension (UWT) is created and maintained autonomously through a complex interaction between the gestational sac, the uterus, and cervical support.
- UWT followed an exponential curve, with results increasing throughout pregnancy, and later preterm birth was associated with a lower UWT.(2)
- Pregnancy is a balance between two opposing interactive forces, mechanisms, and systems.
- The first force, EUWT, is the inhibitory system that maintains pregnancy, through a stretch-dependent (mechanotransduction) mechanism, in addition to direct relaxants.
- The second force is the stimulatory system, which terminates pregnancy by transforming the cervix into the lower uterine segment through the TYVU pattern.
- Both the inhibitory and stimulatory systems are induced secondary to exponential uterine wall tension (EUWT) mechanotransduction and direct progesterone/estrogen stimulation.
- Autonomic two-system interaction secondary to light-dark cycle modulation divides gestation into five phases: growth, maturation, transition, parturition, and involution.
- Maternal cortisol modulation of the inhibitory system, and melatonin, and oxytocin exerted on the stimulatory system is synchronized and synergized, making the cervix lose its strength completely at the end of the maturation phase, secondary to its transformation into the lower uterine segment, which signals EUWT failure and initiating parturition.
- The clock metering the duration of pregnancy has been suggested to consist of two interacting timers; an interval timer measuring the overall length of gestation, and a circadian timer defining when within a 24-hour cycle birth takes place.(6)
- The pregnancy interval and circadian timers are achieved by a single mechanism, EUWT failure, secondary to the complete loss of the cervical resistance nocturnally.
- Gestation is Genetically Controlled through the Complex Interactive EUWT components which include the paternal side as well.
- The Creation, the Autonomic Maintaining, and eventually the Autonomic Termination of the EUWT through light-dark cycle modulation make Gestation an Autonomic Cycle with a Constant Interval and Circadian Timers.

- Malfunction of any interactive EUWT component terminates the Gestation prematurely or post-term or leads to Dystocia in Labor.

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Reference 3 :- Hurd WW, Gibbs SG, Ventolini G, Horowitz GM, Guy SR. Shortening increases spontaneous contractility in myometrium from pregnant women at term. Am J Obstet Gynecol. 2005;192(4):1295-301; discussion 301-3.

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Acknowledgements :- The author has nothing to declare.

PC096

Effects of Acute and Chronic High intensity muscle contractions on irisin and related factor expression in mice skeletal muscle

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• Title

Effects of Acute and Chronic High intensity muscle contractions on the expression of irisin and related factors in mice skeletal muscle

Riku Tanimura¹, Kazuki Uemichi¹, Katsuyuki Tokiunoya^{2,3}, Takanaga Shirai^{1,3}, Tohru Takemasa¹

- **Introduction**

Irisin is an exercise-induced myokine which regulates adipocyte browning and thermogenesis (Boström et al., 2012). Peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α), which plays a central role in oxidative metabolism in skeletal muscle, leads to the processing of irisin from its precursor, fibronectin type III domain-containing protein 5 (FNDC5) (Reza et al., 2017). It is known that blood irisin concentrations in mice are increased by endurance exercise (Fatouros et al., 2017), but it is unclear what type of exercise is effective for irisin expression in skeletal muscle. The aim of this study was to investigate changes in the expressions of irisin signals in mouse skeletal muscle following acute and chronic high intensity muscle contractions.

- **Methods**

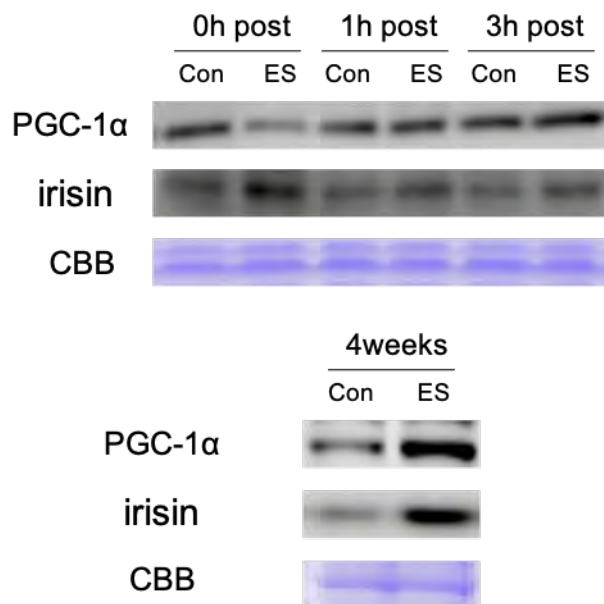
All experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba (20-407). Male ICR mice aged 7 weeks were used in this study. We conducted electrical stimulation (ES) as a model to induce high intensity muscle contractions (Shirai et al., 2020). Gastrocnemius muscle was obtained 0, 1, and 3 h after single bout of ES, or 48 h after chronic ES (3 times a week for 4 weeks) (n = 5-6 in each time point). All tissues were rapidly frozen with liquid nitrogen and stored at -80 °C until use. We used Western blotting as the method of analysis for protein expressions in skeletal muscle. A one-way analysis of variance (ANOVA) was performed using the SPSS software (IBM Corp., New York, NY, USA) to determine whether a significant interaction exists between two paired factors.

- **Results**

We found that the expression of irisin was significantly increased by acute and chronic ES ($p < 0.05$). The expression of PGC-1 α was significantly increased by chronic ES ($p < 0.05$), but we found no significant difference in acute ES groups compared with that in control (no ES) legs.

- **Conclusion**

We demonstrated that acute and chronic high intensity muscle contractions increased the expression of irisin in skeletal muscle, but the expression of PGC-1 α showed different results between acute and chronic ES. These results may suggest that acute high intensity muscle contractions augment the expression of irisin through a PGC-1 α -independent pathway.



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Reference 3 :- Boström, P et al. (2012). *Nature*, 481(7382), 463–468.

PC097

Effects of mechanistic/mammalian target of rapamycin complex 1 inhibition on mitochondrial dynamics-related signaling induced by acute or chronic resistance exercise in mouse skeletal muscle

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• Introduction

Resistance exercise (RE) induces skeletal muscle hypertrophy mainly by stimulating a muscle protein synthesis pathway called mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling. On the other hand, mitochondrial dynamics, which involves mitochondrial fusion and fission, is an important mechanism for maintaining healthy skeletal muscle quality. It has been

reported that RE not only increases skeletal muscle mass through mTORC1, but also activates mitochondrial dynamics (Lee et al., 2018; Mesquita et al., 2020). However, the participation of mTORC1 in the quality control through mitochondrial dynamics has not been clarified. In our previous study, we suggested that mTORC1 may participate in promoting mitochondrial fusion during skeletal muscle hypertrophy using synergist ablation model (Uemichi et al, 2021). However, the effect of mTORC1 on mitochondrial dynamics during acute or chronic muscle contraction-induced RE is unclear.

- Methods

All experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba (20-407). Male ICR (Institute of Cancer Research) 8-week-old mice were divided into two groups: vehicle-treated control mouse (Vehicle, n=5-8), Rapamycin-treated mouse (RAPA, n=6-8). mTORC1 inhibitor, rapamycin (2.5mg/kg) or vehicle control (equal volume of 0.5%DMSO) was injected intraperitoneally 1h before muscle contraction. Under anesthesia with isoflurane inhalation, the right gastrocnemius muscle was contracted isometrically using percutaneous electrical stimulation, as described previously (Ogasawara et al., 2020). The left gastrocnemius muscle was served as a control. In the acute RE groups, muscle samples were obtained 1h after RE. In the chronic RE groups, mice were exercised every other day for four weeks (12 exercise sessions in total) and muscle samples were obtained 48h after the last session. Western blotting was performed to analyze protein expression levels. Data were shown as means $\pm$ SEM. We performed two-way ANOVA followed by Bonferroni's post hoc test. The significance level was set up to $p < 0.05$ for all cases.

- Results

We measured expression levels of mitochondrial dynamics-related signaling molecules and found no main effect of RE or Rapamycin injection in the acute RE group. On the other hand, the main effect of RE was confirmed at the expression levels of Optic atrophy 1 (OPA1), which is involved in mitochondrial fusion, and Dynamin related protein 1 (DRP1) and Mitochondrial fission protein 1 (FIS1), which are involved in mitochondrial fission in the chronic RE group. However, we found no main effect of rapamycin injection.

- Conclusion

Chronic resistance exercise increases mitochondrial dynamics-related signaling expression in mTORC1-independent manner.

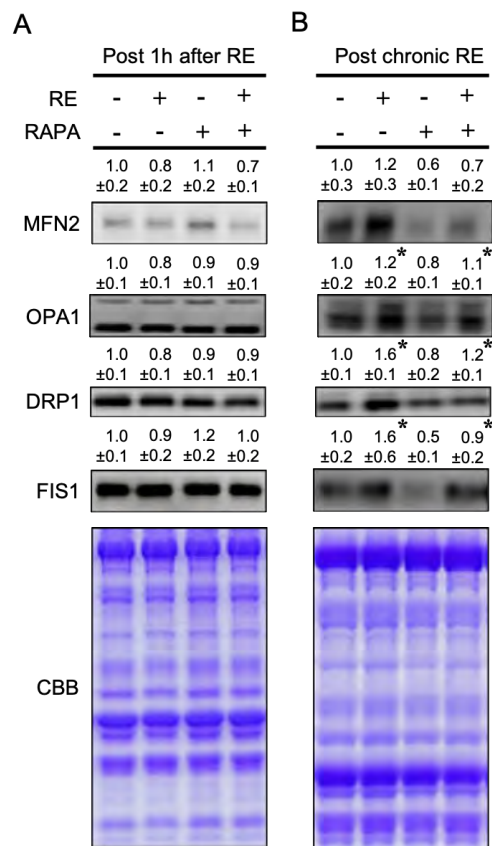


Figure 1. Effect of acute or chronic RE and rapamycin injection on the mitochondrial dynamics-related signaling in the gastrocnemius muscle. Representative immunoblots are shown in A and B. Data are presented as means $\pm$ SEM. * $p < 0.05$ significant effect of RE.

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Reference 2 :- Mesquita et al. (2020). Physiol Rep **8**, 1–10.

Reference 3 :- Ogasawara et al. (2020). J Physiol **598**, 5453–5466.

Reference 4 :- Uemichi et al. (2021). Physiol Rep **9**, 1–14.

PC098

Maternal and fetal p110 α deficiency induces sex-specific changes in feto-placental growth and placental mitochondrial function

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Introduction:

The placenta is vital for the transport of nutrients from mother to the fetus for growth. The phosphoinositol 3-kinase (PI3K) pathway regulates growth in relation to nutrient supply. Previous

work using gene manipulations in mice have demonstrated that loss of the PI3K-p110 α isoform in either the fetus or mother alters placental morphology and transport capacity in line with fetal growth outcomes [1, 2]. Placental growth and transport are fueled by energy provided by mitochondria [3]. However, little is known about whether maternal and fetal PI3K-p110 α deficiency may affect placental mitochondrial function, and how this may relate to growth of the female and male fetus.

Aims/ objectives:

In mice, determine whether loss of PI3K-p110 α in the mother versus the fetus affects feto-placental growth and placental mitochondrial function in each fetal sex.

Methods:

Virgin 4-months-old C57BL/6 wildtype (WT) and heterozygous PI3K-p110 α deficient (α /+) female mice were mated to α /+ and WT males, respectively to generate litters of mixed genotype (WT and α /+). On day 18 of pregnancy, dams (WT, n=5 and α /+ n=7) were Schedule-1 killed and each fetus and placenta in the litter was weighed. The transport labyrinth zone of the placenta (LZ) was micro-dissected, weighed and subjected to high resolution respirometry to analyze mitochondrial function. Conceptuses were genotyped and sexed by PCR. Data for each sex were analyzed using two-way ANOVA followed by Tukey's test to evaluate the effect of maternal and fetus genotype, as well as their interaction (significant if $P < 0.05$). Experiments were performed under the UK Regulation of Animals (Scientific Procedures) Act of 1986.

Results:

Female fetuses from α /+ dams were significantly lighter than those from WT dams (Fig. 1 A). There was an overall effect of maternal and fetal genotype to reduce fetal and LZ weight without a significant change in placental weight in females (Fig. 1 A-C).

Male fetuses from α /+ dams were also significantly lighter than those from WT dams, and in dams of each genotype, α /+ male fetuses were lighter than WT male littermates (Fig. 1 D). Placental and LZ weights from male fetuses in α /+ dams were greater than in WT dams, however the specific effect depended on fetal genotype (Fig. 1 E and F). Placental and LZ weights were less for male α /+ than WT littermates, an effect significant in α /+, but not WT dams (Fig. 1 E and F).

Overall, in females only, there was greater LZ complex IV oxidative phosphorylation in α /+ versus WT dams (Fig. 1 G). In males only, there were interactions between maternal and fetal genotype that determined LZ mitochondrial complex I+II and total oxidative phosphorylation capacities (Fig. 1 H and I). There was no effect of maternal or fetal genotype on other mitochondrial respiratory parameters assessed in the placental LZ.

Conclusion:

Both maternal and fetal α /+ genotype affect feto-placental growth and placental mitochondrial function in a sex-specific manner.

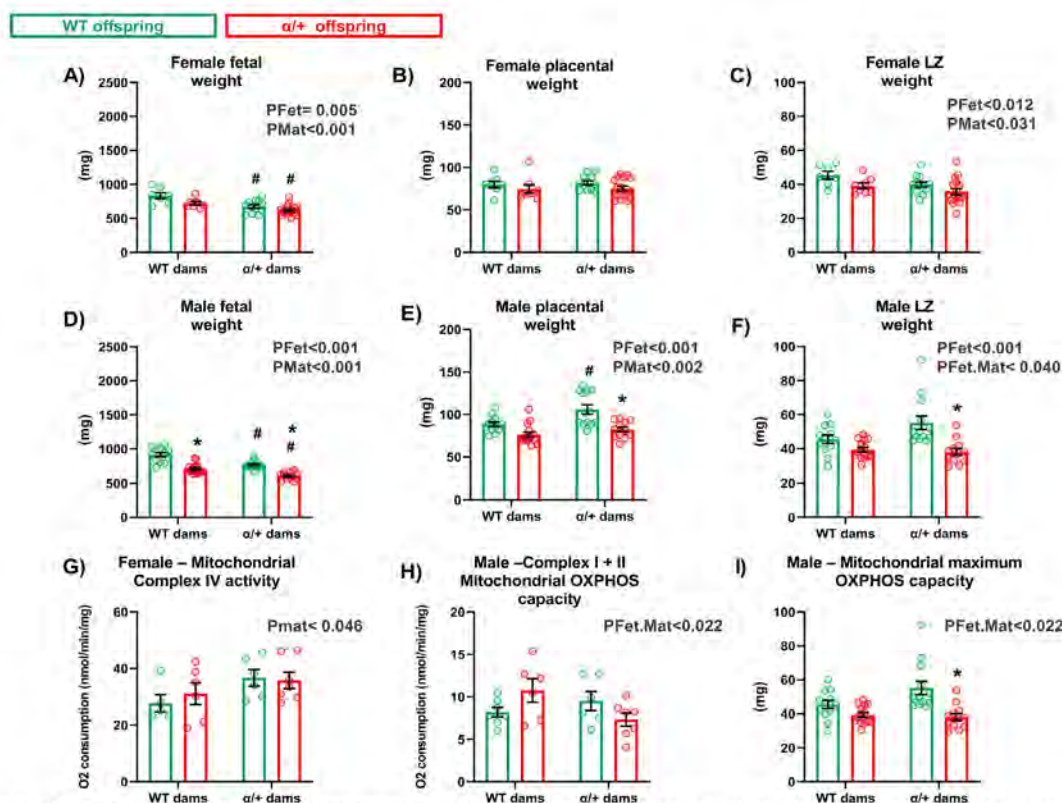


Figure 1. Female (A-C) and male (D-F) offspring fetoplacental growth. Mitochondrial respiration in female and male offspring (G-I). Statistical analysis was performed by using two-way ANOVA followed by Tukey's test to evaluate the effect of maternal (PMat) and fetus (PFet) genotype, as well as their interaction (PFet Mat) (n≥6, significant if P<0.05). *Significantly different to WT littermates (P < 0.05, pairwise comparison). # Significantly different to WT dams (P < 0.05, pairwise comparison). Experiments were performed under the UK Regulation of Animals (Scientific Procedures) Act of 1986. Abbreviations: F:p ratio (Fetal weight /placental weight); LZ (Labyrinth zone of the placenta); Oxphos (Oxidative phosphorylation).

Reference 1 :- Lopez-Tello, J., et al., *Fetal and trophoblast PI3K p110alpha have distinct roles in regulating resource supply to the growing fetus in mice*. Elife, 2019. **8**.

Reference 2 :- Sferruzzi-Perri, A.N., et al., *Maternal and fetal genomes interplay through phosphoinositol 3-kinase(PI3K)-p110alpha signaling to modify placental resource allocation*. Proc Natl Acad Sci U S A, 2016. **113**(40): p. 11255-11260.

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Acknowledgements :- This research was supported by the Lister Institute of Preventive Medicine.

PC099

In vivo body composition analysis - comparing data from Dual Energy X-ray Absorptiometry (DXA) and Bioelectrical Impedance Analysis (BIA)

Cíntia Ferreira-Pêgo¹, Rejane Giacomelli Tavares¹, Sofia Lopes², Tatiana Fontes², Luís Monteiro Rodrigues¹

Introduction: The non-invasive assessment of body composition is a valuable, informative tool for nutritional evaluation in a wide range of settings. Bioelectrical Impedance Analysis (BIA) technology has become very popular in recent years, recommended for this purpose primarily due to its ease of use and affordability. Nevertheless, many doubts remain regarding its physiological significance and data reliability. Dual-energy X-ray absorptiometry (DXA), on the other hand, is regarded as the gold standard for body composition evaluation, although (equipment) cost and time-consuming operation can limit its use in routine clinical practice or large epidemiological surveys.

Aims / Objectives: To examine and compare the total body composition in healthy university students using two different technologies - BIA and DXA.

Methods: This pilot cross-sectional study involved 25 individuals, 88% women (23 years old $\pm$ 6,39) and a mean BMI of 21.86 kg/m². Body composition was assessed using a DXA Lunar Prodigy Advance (General Electric Healthcare®) and a BIA (Tanita TBF 300®). Other descriptive variables were also collected by trained dietitians, including weight, height, abdominal circumference, smoking status, sleeping hours, intestinal and urinary rhythm, and physical activity practice. Procedures respected all the principles of good clinical practice for human studies research. Statistical analysis was performed using SPSS software, and all statistical tests (descriptive and regression analysis) were two-tailed and the significance level was set at $p < 0.05$.

Results and Discussion: A weak correlation of fat mass (FM) detected with DXA and BIA ($r = 0.8639$, $P < 0.01$) was found. (Fig.1). In absolute terms BIA underestimated FM by 15.5%, in comparison to DXA. In contrast, a higher concordance was observed ($r = 0.97$) for fat-free mass (FFM), however, these results were not statistically significant. Similar results were previously reported, indicating that a correlation between BIA and DXA is not always accompanied by sufficiently reliable limits of agreement between methods.

Conclusion: In the present experimental conditions, there is a lack of agreement between FM and FFM as assessed by BIA and DXA. More work is needed to know how to correlate data from one versus the other.

PC100

The role of hypoxia in mouse pancreatic carcinogenesis. Effect of limited oxygen availability on the effectiveness of anti-cancer therapy.

Daria Krzysztofik¹, Agnieszka Kusiak², Kinga Stopa¹, Paweł Ferdek², Ewa Werner¹, Karolina Hajduk¹, Jan Morys^{1,2}, Sylwester Mosiołek^{1,2}, Filip Łoziński^{1,2}, Monika Jakubowska¹

Pancreatic cancer affects as much as 400 000 people worldwide. This cancer is estimated to become the third most common type of cancer. Despite the development of the novel anti-cancer therapeutic strategies, survival rate of the patients did not increase significantly. Due to the high resistance of pancreatic cancer to chemotherapy and radiotherapy, or combination of these, pancreatic cancer remains one of the major unresolved health problems, and is characterized by one of the lowest of all cancers survival rates after diagnosis (1).

In this work, we focus mainly on the role of hypoxia (poor oxygenation) in the tumor microenvironment (the stroma). By forming the physical barrier between cancer cell-normal cell, stromal component limits the effectiveness of anti-cancer treatment. Activated pancreatic stellate cells (PSCs) have been implicated into the hypoxia-mediated production of a dense fibrous stroma (the matrix), that in pancreatic cancer can constitute up to 80% of the tumor mass (2-4).

In this study we used BALB/c nude mice as the hosts for the solid human pancreatic tumors. The mice were injected subcutaneously with the human pancreatic adenocarcinoma cells PANC-1 with or without the human pancreatic stellate cells (hPSC). The latter cells were cultured under normoxic (21% O₂) or hypoxic (0.5% O₂) conditions 24 h prior the injection. The tumors were treated with gemcitabine (GEM, 50 mg/kg, intraperitoneal injection) twice a week for two weeks, with or without Tarloxotinib, a hypoxia-activated pro-drug (GEM+TARL, 30 mg/kg, intraperitoneal injection); the control tumors received placebo (PL). Each experimental group consisted of 6-8 tumors. Tumor growth was monitored for 16 weeks (Figure 1): the mice were weighed and the tumor size was measured using calipers. After the experiment, the mice were humanely killed using CO₂, the tumors and organs were excised, formalin-fixed and paraffin-embedded for immunohistological analysis. The animal experiments were approved by the local ethical committee, licence 41/2019. The quantitative results were expressed as means ± sem. The statistical significance threshold was set at 0.05.

Analysis of the PANC-1 tumor host survival (Figure 1A) shows that the monotherapy with GEM (blue) is less effective than the combined therapy GEM+TARL (orange). In that contained the stromal component (Figure 1B and 1C), monotherapy with GEM (blue) seems to be more effective than in the PANC-1 group (see Figure 1A). However, the combination therapy (orange) in the normoxic group (Figure 1B) was ineffective and mouse hosts of these tumors did not survive to the experiment end. Also, the combination therapy (orange) was less effective than the monotherapy (blue) in the group wherein PSCs were pre-treated with low O₂ (Figure 1C). Immunohistological analysis of the tumor tissue revealed the increased level of the α SMA-positive cells in the tumors collected from the hosts of the best survival (Figure 1, blue). Taken together, our study explores the role of hypoxic stress in the pancreatic tumor growth and its response to the therapy.

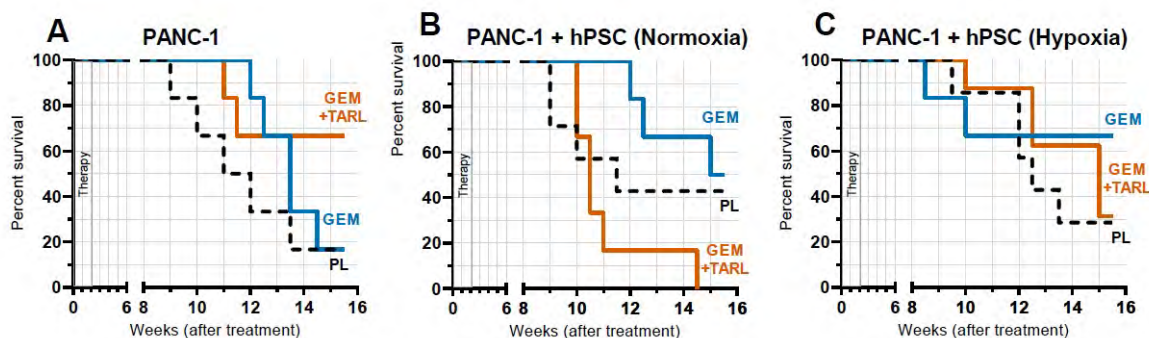


Figure 1. Kaplan-Meier survival curve of PANC-1 (A), PANC+hPSC Normoxia (B), PANC+hPSC Hypoxia (C) mouse tumor hosts. The mice were treated with Gemcitabine (GEM), Gemcitabine and Tarloxotinib (GEM+TARL) or received placebo (PL). Number of individuals: (A): PL n = 6; GEM n = 6; GEM+TARL n=6; (B): PL n = 7; GEM n = 6; GEM+TARL n=7; (C) PL n = 7; GEM n = 6; GEM+TARL n=8.

Reference 1 :- Li D et al (2004) 363,1049-1057

Reference 2 :- Erkan M et al (2012) Nat Rev Gastroenterol Hepatol 9, 454–467

Reference 3 :- Masammune A et al (2008) Am J Physiol Gastrointest Liver Physiol 295, G709–G717

Reference 4 :- Ferdek PE and Jakubowska MA (2017) Eur J Physiol 469(9), 1039–1050

Acknowledgements :- This work was supported by the HOMING Programme of the Foundation for Polish Science (Fundacja na rzecz Nauki Polskiej, FNP, No. HOMING/2017-3/23 to MJ, No. HOMING/2017-4/31 to PF) co-financed by the European Union under the European Regional Development Fund.

PC101

***Pleurotus tuber-regium* inclusion in diet ameliorates dyslipidemia in obese-Type 2 diabetic rats**

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Pleurotus tuber-regium (P.T) is an edible mushroom with abundant polysaccharides that has been used in traditional medicine to treat diabetes mellitus. This study investigated the hypoglycaemic potential and ameliorative activity of *Pleurotus tuber-regium* incorporated diet on diabetes induced dyslipidaemia. Thirty five (35) adult male rats were randomly assigned to seven groups; Normal control, diabetic control, obese control, obese diabetic control, 10% PT, 20% PT, and Drug control. Type II DM was induced by placing the animals on high fat diet and a single intraperitoneal injection of streptozotocin (50 mg/kg/BW). P.T was incorporated into the feed and given to the animals for two weeks daily after the confirmation of diabetes. Treatment of the obese diabetic rats with P.T supplemented diet caused a decrease in the blood glucose level compared to the control groups. Increased organo-somatic ratio of the kidney and heart were markedly ($p < 0.05$) reduced following treatment (20% P.T). Furthermore, cholesterol, triglycerides, LDL-C and VLDL-C levels were reduced

due to treatment accompanied by increased HDL-C in the liver. Histological evaluation of the liver, kidney, heart, and pancreas of the P.T treated groups were comparable to normal. Incorporation of *P. tuber-regium* in diets could be effective in reversing dyslipidemia in obese diabetic patients.

PC102

Nrf2 as a modulator of (patho)physiological calcium responses in murine pancreatic secretory cells - *ex vivo* studies.

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Nuclear factor erythroid 2-related factor (Nrf2) is a master regulator of redox homeostasis. Downstream targets of this transcription factor provide protection against oxidative damage to normal and cancer cells, and thus impact these cells in a multifaceted way. On the one hand, Nrf2 decreases the level of reactive oxygen species and prevents carcinogenic tissue transformation. On the other hand, Nrf2 mediates cellular detoxification of electrophiles and thus supports cancer progression by increasing resistance to therapy.

Pancreatic acinar cells (PACs), which produce and store digestive enzymes, are the main cellular components of the exocrine pancreas. Enzyme secretion is tightly controlled in these cells by intracellular calcium signals. Dysregulation of these signals may lead to premature enzyme activation, auto-digestion of the organ and sterile inflammation of the pancreas (pancreatitis) – a risk factor for pancreatic cancer development.

In the present study we aimed to investigate whether the lack of transcriptional activity of the Nrf2 protein affects calcium signals in pancreatic acinar cells. In order to address this question, we have compared calcium signals recorded in real time in PACs isolated from 8-week-old male wild type (WT; N = 3) or Nrf2 transcriptional knock-out mice (tKO; N = 3). Calcium responses were evoked by acetylcholine as a physiological stimulus, or pathophysiological agents, such as menadione which is an inducer of oxidative stress and bile acid salts, potent triggers of acute pancreatitis. Data were analysed using a Mann-Whitney U test and the significance threshold was set at $p = 0.05$.

Our results revealed that PACs isolated from the Nrf2 knock-out animals were characterized by a decreased calcium response to physiological stimuli. However, calcium signals in response to pathological cues were increased compared to the wild-type counterparts. This may point towards a lower capacity of the Nrf2 tKO pancreatic cells in dealing with oxidative stress.

Acknowledgements :- This work was supported by the HOMING Programme of the Foundation for Polish Science (Fundacja na rzecz Nauki Polskiej, FNP, No. HOMING/2017-3/23 to MJ, No. HOMING/2017-4/31 to PF) co-financed by the European Union under the European Regional Development Fund.

PC103

Lateral symmetry in the force contractile properties of gastrocnemius muscles of rats

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Introduction

It is usual in physiological research to assess the effects of some intervention on extremities (e.g., training programmes or injury recovery protocols) using one muscle for the intervention and its contralateral as control. For methodological and ethical reasons, the laboratory rat is used as animal model and an obvious question, which has been posed few times in the scientific literature, arises: could it be any structural, metabolic or functional difference between rat right and left muscles? In rats, gastrocnemius is one of the muscles most widely used in research because of its importance in locomotion and high relative leg mass. To the best of our knowledge, only the study by Guo and Zhou (2003) reported a laterality assessment on the metabolic profile of rat's gastrocnemius muscle. To deepen the knowledge on laterality in rats and to clarify the contralateral muscles interchangeability for experimental design, our study aimed to evaluate the force contractile properties between right and left rat's gastrocnemius muscle.

Material and methods

Muscle force properties were analysed in right and left gastrocnemius muscles from six male rats using ADInstruments (Oxford, UK) hardware and software. Gastrocnemius was isolated from surrounding tissues, the Achilles tendon attached to a force transducer (MLT 1030/D) and the muscle stimulated (Stimulus Isolator FE180) via its sciatic nerve. The following force parameters were registered with data acquisition hardware PowerLab/16SP and analysed with LabChart v7.3.7 software: peak (PF, mN/g) and tetanic forces (TF, mN/g) and contraction (CT) and half-relaxation times (HRT) in milliseconds (ms). Fatigue properties after low frequency (40 Hz) continuous stimulation were also assessed as a fatigue index (FI) that considered the force-time area to baseline after 2 min. At the end of the experiment, both gastrocnemius were excised and weighed to the nearest 0.01 g. Protocols were performed following the European Union and Spanish Law on Animal Protection and approved by the Local Bioethics Committee. Data were statistically analysed running paired *t*-Student tests (SigmaPlot 11, Systat Software, San Jose, US) and values expressed as mean $\pm$ standard deviation.

Results

No statistically significant differences ($P=0.266$) were found in gastrocnemius to body weight ratio (%) between right (6.31 ± 0.32) and left (6.41 ± 0.27) muscles. Force parameters normalized to gastrocnemius weight (in $\text{mN}\cdot\text{g}^{-1}$) did not show any significant difference between right (PF= 74.0 ± 13.4 , TF= 219.4 ± 13.0) and left (PF= 70.9 ± 10.7 , TF= 213.0 ± 18.0) legs with $P=0.623$ (PF) and $P=0.514$ (TF). Twitch time parameters (in ms) lacked also of significant differences between legs (CT: 43.4 ± 8.6 vs 45.0 ± 14.3 , $P=0.639$; HRT: 77.6 ± 15.0 vs 82.3 ± 25.3 , $P=0.475$). Finally, both muscles also showed similar ($P=0.718$) fatigue properties (in N·s) with FI= 16.5 ± 1.3 (right) and FI= 16.1 ± 2.0 (left).

Conclusion

Strength, muscle mass and fatigue and force contractile properties did not statistically differ between right and left gastrocnemius in rats. This absence of laterality at the functional level raises the possibility of using right and left gastrocnemius interchangeably for experimental designs where one muscle is used to analyse data after a physiological intervention and its contralateral muscle plays the control role, thus allowing unbiased paired comparisons to derive accurate conclusions.

Reference 1 :- Guo AK & Zhou L (2003) *Metabolism* 52, 1571-1575.

PC104

Prion protein protects the large-conductance calcium-activated potassium channel from the inhibitory effect of Tau oligomers

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Alzheimer's disease (AD) is the most common tauopathy, taking millions of lives every year. AD is characterized by the deposition of aggregates of hyperphosphorylated Tau protein and amyloid- β peptide ($A\beta$) in the brain. The oligomeric forms of Tau and $A\beta$ are considered to be the neurotoxic species responsible for neurodegenerative processes in AD. Previously, we demonstrated the inhibition of the large-conductance calcium-activated potassium channel (BK_{Ca}) of mitochondria by the aggregates and oligomers of $A\beta$. In this study we investigated the effect of Tau oligomers on the plasma membrane BK_{Ca} activity. Furthermore, since prion protein (PrP) interacts with Tau and the N-terminal fragment of PrP, called N1, can be neuroprotective in tauopathies, we decided to check whether N1 can also act at the level of BK_{Ca} .

To obtain the oligomers of Tau we used its aggregation-prone fragment (K18) carrying tauopathy-associated mutation – deletion of Lys280 (K18 Δ 280). Additionally, to induce oligomerization, K18 Δ 280 was phosphorylated by protein kinase A. The studies were carried out on a primary culture of rat hippocampal neurons. The preformed K18 Δ 280 oligomers were incubated in the presence or absence of N1 fragment of PrP (for 15 min) immediately prior applying. The activity of the neuronal plasma membrane BK_{Ca} was recorded using single-channel patch-clamp technique in both the „inside-” and „outside-out” modes. K18 Δ 280 oligomers were added directly to the recording

chamber. In all experiments, the oligomers were used at 10 pM - 100 nM concentrations. The research was performed in compliance with EU and national legal and ethical requirements: EU Directive 2010/63/EU, Act of 15 January 2015 of the Ministry of Science and Higher Education of Poland.

We found that, in the case of the „inside-out” patch-clamp configuration on -40mV, the open probability (P_o) of the BK_{Ca} was not significantly affected by the K18 Δ 280 oligomers alone or after their combination with N1. Contrariwise, performing the extracellular application of the oligomers („outside-out”), significant and concentration-dependent inhibition of BK_{Ca} was observed. For example, in the control conditions (the probe without the oligomers) on +40mV the P_o of the channel was equal to 0.36 ± 0.02 ($n=35$). After incubation with the highest concentration of the oligomers this indicator dropped to 0.27 ± 0.03 ($n=25$) which consisted $76 \pm 8\%$ of the control ($P=0.0083$). The P_o of BK_{Ca} was fully recovered after washing the oligomers out: 0.39 ± 0.05 ($n=17$), that is $114 \pm 13\%$ from the control, or $168 \pm 17\%$ from the 100 nM of K18 Δ 280 ($P=0.0005$). It is noteworthy that after incubation with N1, the oligomers lost their cytotoxicity, since they were not able to significantly inhibit BK_{Ca} .

Our results suggest that N1 can convert neurotoxic oligomers of Tau into a form which is not able to inhibit BK_{Ca} channel. This effect occurs only from the extracellular side, which implies specific interaction of the oligomers with the channel. It is important from a physiological point of view, since it sheds light on the possible neuroprotective mechanism of PrP action in AD and other tauopathies.

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Occurrence of mild cognitive impairment with hyperinsulinaemia in Africans with advanced type 2 diabetes

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There is paucity of information on the prevalence of mild cognitive impairment (MCI) among individuals with type 2 diabetes (T2D) in sub-Saharan Africa, including Nigeria. In addition, the role of hyperinsulinaemia in the development of MCI needs further investigation. This study sought to assess cognition and hyperinsulinaemia, with the associated characteristics in patients with advanced T2D. Ethical approval for the study was obtained from Kano State Ministry of Health; the study was conducted in accordance with the Helsinki Declaration. Cognition was assessed using Montreal cognitive assessment test (MoCA), while fasting plasma insulin was measured using an ELISA kit. Data was processed using IBM SPSS Statistics version 20.0. categorical and continuous variables were expressed as percentages and mean $\pm$ SD. Student's t-test was used to compare means while Pearson

correlation was computed for correlations between variables. Sixty one diabetic subjects and 32 non-diabetic controls, matched for age, gender and level of education were studied. The diabetics had MCI while the controls had normal cognitive function. About 88.5 % of the diabetic subjects had MCI, in contrast with only 50 % of the non-diabetic controls. The most significantly affected cognitive domains among the diabetics were executive function, naming, attention, abstraction and delayed recall. Among the diabetics, MCI correlated with age, weight and body mass index (BMI); and in addition, age and weight found to be significant predictors of MCI. Plasma insulin concentration among the diabetics ($16.24 \pm 13.5 \mu\text{IU/ml}$) was more than twice that of the controls ($7.59 \pm 2.9 \mu\text{IU/ml}$). Hyperinsulinaemia among the diabetics correlated with weight, BMI, blood pressure and fasting blood sugar (FBS). Glycaeted haemoglobin and FBS levels were higher among diabetics compared with the non-diabetics. In conclusion, Africans with advanced T2D show multi-domain MCI at high prevalence, coexisting with hyperinsulinaemia. Majority of the patients have diabetic complications and poor glycaemic control. Hyperinsulinaemia may play a complementary role in the pathophysiology of MCI in T2D.

Key words: Mild cognitive impairment (MCI), Insulin, Hyperinsulinaemia, Type 2 diabetes (T2D), Dementia, Africans

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