

PL01

The perils and pleasures of educating 21st century physiology undergraduates

J. Choate

Department of Physiology, Monash University, Melbourne, VIC, Australia

Over the past 10 years, many Australian universities have experienced increased student enrolments in Physiology subjects. Reasons for this increase are twofold. Firstly, changes in government funding to universities with the uncapping of student enrolments in undergraduate degree-programs provided financial incentives for Physiology Departments to increase their student numbers. Secondly, the funding of graduate medical degree programs in favour of undergraduate medical programs and the inclusion of Physiology as a prerequisite subject for these graduate medical degree programs and other allied health degree-programs provided a market for physiology graduates, and therefore a strong incentive for students to study Physiology (Choate & Long, 2019).

At Monash University, student enrolments in a foundational physiology subject increased from 150 in 2011 to 720 in 2019. This increase had significant consequences for the Physiology academics involved in teaching. Staff had to reconsider how to effectively deliver the Physiology curriculum to large student cohorts. Key changes included the development of virtual experiments and large-scale workshops. The virtual experiments supported, and in some cases replaced, live experimental practical laboratory classes. Analysis of student learning showed that the virtual experiments enhanced student understanding of the physiology concepts underlying the practical classes, and supported the development of research skills (Quiroja & Choate, 2017; Quiroja & Price, 2016). The introduction of large-scale workshops arose as a consequence of falling attendance at lectures. Video-recording and live streaming of lectures provided students with the option of studying lecture materials at a time and place of their own choosing. In response to the large decreases in lecture attendance, the number of lectures was reduced, or they were removed altogether. Lectures were replaced with large-scale active learning workshops (for up to 150 students) in a new Learning and Teaching Building designed for this purpose. Attendance and participation of students in these workshops has been significantly higher than lectures, which was not surprising, as the workshops were not recorded or live-streamed. To date there has been no significant impact of these workshops on student learning as measured by assessment performance. The workshops are, however, less cost-effective than lectures as they require a substantially higher staff to student ratio, necessitating the recruitment and training of additional teaching associates.

With large numbers of students studying Physiology in non-vocational undergraduate degree programs (i.e. Science, Biomedical Science), there has been a high level of student stress and anxiety about career options. This observation, and the recent government concern about graduates' employability skills, prompted

the introduction of careers education into the curriculum of the Biomedical Science degree-program (Choate, Green, Cran, Macaulay & Etheve, 2016; Choate & Long, 2019). An integrated team of careers educators and biomedical science academics designed and embedded a course-wide, assessable Career Development Program into the biomedical science curriculum. This program was found to improve students' awareness of careers options, enhance their careers confidence and increase their engagement with the University Careers Service (Choate et al., 2016). Importantly, the program enabled careers education on a large scale. Following this success, a new work integrated subject, involving industry-based placements, has been implemented and has further enhanced students' career awareness.

In summary, this education research highlights how a holistic approach to the student experience in physiology through on-line virtual experiments, hands-on active learning workshops and careers education can enhance student engagement and learning in large student cohorts.

Choate J, Green J, Cran S, Macaulay J, Etheve M (2016). Using a course wide professional development program to enhance undergraduate career development and employability. *International Journal of Innovation in Science and Mathematics Education*. 24(3):49-70.

Choate J, Long H (2019). Why Do Science Students Study Physiology? Career Priorities of 21st Century Physiology Undergraduates. *HAPS-Educator, Journal of the Human Anatomy and Physiology Society*. 23(1).

Quiroga M, Choate J (2017). Using an online simulation to prepare students for an enquiry-based laboratory class. *AuPS Proceedings*. 48:30P.

Quiroga M, Price N (2016). Simulated in vivo Electrophysiology Experiments Provide Previously Inaccessible Insights into Visual Physiology. *J Undergrad Neurosci Educ*. 15(1):A11-A17.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PL02

Hunger Games – The Control of Appetite

L. Heisler

The Rowett Institute, University of Aberdeen, Aberdeen, UK

Obesity has become one of the major health concerns of this century because of its prevalence and resistance to treatment. Specifically, one in four people in the UK are currently clinically obese. Significant progress has been made over the past decade in clarifying brain neurochemicals and regions regulating energy homeostasis. Among these is the key precursor polypeptide pro-opiomelanocortin (Pomc) within the homeostatic brain region the arcuate nucleus of the hypothalamus (ARC). The 5-hydroxytryptamine (5-HT, serotonin) 2C receptor (*Htr2c*; 5-HT_{2C}R) agonist lorcaserin (Eisai Inc) is a new medication for obesity treatment that was recently launched in the USA. However, mechanism through which lorcaserin's

therapeutic effects are achieved remain to be elucidated. Here we clarify that the target of lorcaserin is 5-HT_{2C}Rs influencing the activity of ARC Pomc. Specifically, here we demonstrate that preventing Pomc production in the ARC (*Pomc*^{NEO}) is sufficient to abolish lorcaserin's anorectic effects and that restoration of *Pomc* specifically within a subset of ARC neurons expressing 5-HT_{2C}Rs (*Pomc*^{Htr2c}) is sufficient to restore lorcaserin's therapeutic effect. These findings illustrate that lorcaserin achieves its therapeutic benefit via activation of ARC Pomc peptides. To further clarify the brain circuit mediating lorcaserin's appetite suppression, we next considered the receptors binding Pomc peptides. The melanocortin receptor most closely linked to appetite and body weight control is melanocortin4 receptor (Mc4r). Using a combination of viral and genetic technology, we reveal that lorcaserin suppresses appetite via action at *Pomc*^{Htr2c} signaling to Mc4rs. These findings reveal the mechanism underpinning the therapeutic benefit of the new obesity medication lorcaserin, via activation of a discrete melanocortin brain circuit.

Work was supported by the Wellcome Trust, BBSRC and MRC

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PL03

Allosteric modulation of glutamate receptors

S.F. Traynelis^{1,2}, R. Perszyk¹, S.A. Swanger³, C. Shelley⁴, A. Khatri¹, G. Fernandez-Cuervo^{1,5}, M.P. Epplin⁵, E. Garnier-Amblard⁵, H. Yuan^{1,2}, D.S. Menaldino⁵, D.C. Liotta⁵ and L.S. Liebeskind⁵

¹Pharmacology and Chemical Biology, Emory University School of Medicine, Atlanta, GA, USA, ²Center for Functional Evaluation of Rare Variants, Atlanta, GA, USA, ³Department of Biomedical Sciences and Pathobiology, Virginia Tech Carilion Research Institute, Roanoke, VA, USA, ⁴Department of Biology, University of the South, Sewanee, TN, USA and ⁵Chemistry, Emory University, Atlanta, GA, USA

NMDA receptors mediate a slow, Ca²⁺-permeable component of excitatory synaptic transmission in the central nervous system (CNS). These receptors are ligand-gated ion channels that are tetrameric assemblies of two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits. There are four different genes encoding GluN2 (*GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*), which are each expressed with a unique spatial profile throughout the CNS at different developmental stages. The different GluN2 subunits control the temporal signaling properties of the receptors, which likely allows different subunits to mediate different roles in development and circuit function. NMDA receptors require the binding of both glutamate and glycine in order to open the pore, which is triggered by protein rearrangements secondary to agonist-induced closure of a bi-lobed agonist binding domain that resembles a clamshell. We hypothesize that three key gating elements control opening of the ion-conducting pore following agonist binding. These elements include the 9

highly conserved residues (SYTANLAAF) that comprise the extracellular end of the M3 transmembrane helix, the short two turn helix that is parallel to the plane of the membrane and precedes the M1 transmembrane helix, and the short linker preceding the M4 transmembrane helix (Amin et al., 2018; Chen et al., 2017; Gibb et al., 2018, Yuan et al., 2014). A considerable amount of data implicates these regions in gating, including the observation that regions of the genes encoding these portions of the polypeptide chain are devoid of variation in the human population, yet are a locus for disease-causing mutations in patients with neurological disease (XiangWei et al., 2018). We have studied the functional consequences of these *de novo* mutations, which have diverse effects on the process of channel gating. Our results included the identification of a mutation in the pre-M1 helix that reduced single channel conductance (Ogden et al., 2017), which is rare for receptor mutations that lie outside of the pore-forming regions. These data suggest that the pre-M1 helix may influence the geometry and characteristics of the open pore. Consistent with this idea, we have identified several allosteric modulators that appear to act at the pre-M1 region and can also reduce single channel conductance, an effect not previously observed for exogenous modulators of NMDA receptor function. For example, the thienopyrimidone EU1622 series of positive allosteric modulators of NMDA receptor function (structures and synthetic methods available in Traynelis et al., US Patent Application WO2016081649A1, 2015) have structural determinants in the pre-M1 region. Members of this class can reduce single channel conductance while still potentiating the maximally effective current. Chord conductance levels of NMDA receptors on cultured cortical neurons changed from 52, 44 pS in vehicle (0.2% DMSO) to 42, 35, and 28 pS in 50 mM EU1622-14 (mean from 7 outside out patches recorded at -80 mV, SEM varied between 0.5-1.1 pS). Given that the pre-M1 region might influence the properties of the pore, we explored the mechanism underlying this effect, and discovered that one modulator that reduces conductance can also alter the relative permeability ratio for cations. We found that EU1622-14 reduced the relative permeability of Ca^{2+} to Na^{+} for recombinant GluN1/GluN2A and GluN1/GluN2B receptors expressed in HEK cells by more than 2-fold ($p < 0.05$ for both receptors, One-way ANOVA, post-hoc t-test of drug vs vehicle, Bonferroni correction for multiple comparisons, $F_{3,28} = 6.91$). This represents the first example to our knowledge of an exogenous drug-like allosteric modulator that can interact with the NMDAR protein complex to alter the relative permeability of ions, which has important implications. The result is perhaps intuitively understandable, as any change in the diameter or electrostatic properties of the pore that alters unitary conductance would be unlikely to do so in a manner that equally affects the permeability of ions with different diameters and distinct hydration shells. Rather, it seems likely that ions with unique atomic radii will have a different relative permeability when the pore diameter or electrostatic environment changes. These findings highlight important biophysical considerations about how the NMDA receptor tetrameric complex controls pore diameter and related properties. In addition, the precedent that Ca^{2+} permeability can be controlled pharmacologically creates a new potential therapeutic target (i.e. regulation of ionic selectivity) with intriguing possibilities, such as future development

of positive allosteric modulators of NMDA receptor function that do not elevate intracellular Ca^{2+} to potentially pathological levels.

Amin *et al.* (2018). *Nat Commun* 9, 3748.

Chen W *et al.* (2017). *Mol Pharmacol* 91, 317-330.

Gibb AJ *et al.* (2018). *J Physiol* 596, 4057-4089.

Yuan *et al.* (2014). *Nat Commun* 5, 3251.

XiangWei W *et al.* (2018). *Curr Opin Physiol* 2, 27-35.

Swanger SA *et al.* (2016). *Am J Hum Genet* 99, 1261-1280.

Ogden KK *et al.* (2017). *PLoS Genet* 13, e1006536.

All procedures involving the use of animals were approved by the Emory University Institutional Animal Care and Use Committee, and were performed in accordance with state and federal Animal Welfare Acts and the policies of the Public Health Service. The authors thank Kevin Ogden for help with analytical software development, and Pernille Buelow and Gary Bassell for providing cultured cortical neurons. This work was supported by the NINDS (R01NS065371, R01NS036654, SFT), NICHD (R01HD082373, HY), Citizens United for Research in Epilepsy (SAS) and the Emory University Research Committee (SAS). SFT is PI on a research grant from Allergan to Emory University School of Medicine, is a member of the SAB for Sage Therapeutics, is co-founder of NeurOp Inc, and receives royalties for software. DCL is a member of the Board of Directors for NeurOp Inc. DCL, DSM, EAG, GFC, LSL, MPE, SFT are co-inventors on Emory-owned Intellectual Property that includes allosteric modulators of NMDA receptor function. HY is PI on a research grant from Sage Therapeutics to Emory University School of Medicine.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PL04

Circuit solutions for programming actions

S. Arber^{1,2}

¹Biozentrum, University of Basel, Basel, Switzerland and ²Friedrich Miescher Institute, Basel, Switzerland

Movement is the behavioral output of the nervous system. Animals carry out an enormous repertoire of distinct actions, spanning from seemingly simple repetitive tasks like walking to more complex movements such as forelimb manipulation tasks. This lecture will focus on recent work elucidating the organization and function of neuronal circuits at the core of choosing, maintaining, adjusting and terminating distinct motor behaviors. It will show that dedicated circuit modules within different brainstem nuclei and their interactions in the motor system play key roles in action diversification.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PL05

From Retroviruses to Human Birth

R. Smith

Endocrinology, University of Newcastle, Newcastle, NSW, Australia

The emerging story of the physiology of human birth is full of unexpected twists and turns. As the morula diverges into the trophoctoderm and epiblast and forms the blastocyst, this critical developmental event sends these two sets of cells on two very different trajectories. The epiblast will form the fetus with every prospect of a future 90 years long. In contrast, the trophoctoderm will play a key role as a type of booster rocket for the fetus, but is doomed to be jettisoned after just 9 months. New data indicates that the placenta's short trajectory is initiated by chromosomal changes that allow expression of endogenous retroviruses. The formation of the human placenta requires the participation of the endogenous retrovirus coat protein, Syncytin 1. Syncytin 1 promotes the formation of the syncytiotrophoblast layer that is characteristic of the placenta. Syncytin 1 even has an immunosuppressive domain that may play a key role in the immune tolerance of the fetal allograft. The placenta then assumes the role of fetal guardian, immunological barrier, nutrient transporter, an agent of the father manipulating the metabolism and perhaps behaviour of the mother to improve the prospects for the fetus.

During early pregnancy trophoblast tissue secretes hCG that maintains ovarian progesterone production. Together, hCG and progesterone suppress uterine contractility, allowing the blastocyst to remain within the uterus and preparing the endometrium for implantation. Syncytin-mediated pathways promote cytotrophoblast fusion into syncytiotrophoblast and increase the production of a variety of placental hormones including corticotrophin releasing hormone (CRH). CRH is released into the maternal circulation and is one of the hormones promoting increased cAMP, protein kinase A activation and myometrial relaxation. Increased uterine relaxation is required as pregnancy progresses and the size of the fetus increases within this smooth muscular organ with a proclivity to contraction. The placenta assumes more of this relaxatory role by becoming the major source of progesterone production.

CRH is also released into the fetal circulation. Within the fetus, CRH stimulates fetal adrenal zone production of the steroid DHEAs. Fetal adrenal DHEA is a substrate for fetal liver 16 hydroxylase and the 16 hydroxylated steroid is converted in the placenta to estriol. Oestriol is therefore almost exclusively formed from fetal precursors. Meanwhile the placenta synthesises oestradiol from predominantly maternally derived DHEA. Why have two oestrogens produced in large amounts in this way? One potential explanation is that the oestriol-oestradiol occupancy of oestrogen

receptor alpha inhibits transactivation at oestrogen responsive genes. This mechanism may prevent synthesis of pro-contractile proteins such as connexin 43.

Late in pregnancy increasing CRH concentrations drive further increases in oestriol production. This allows formation of oestriol homodimers which cause transactivation of oestrogen responsive genes, leading to increased connexin 43 expression and consequent linkage of myometrial cells. The changes in the oestrogenic environment likely leads to use of an alternate promoter for the progesterone receptor gene leading to the formation of progesterone receptor A which blocks normal progesterone action. Progesterone action is also reduced by increased production of 20 alpha hydroxysteroid dehydrogenase. Changes in splicing of the oestrogen receptor alpha gene enhance the oestrogenic environment. Together these changes remove the relaxatory brake on the myometrium, and increase myometrial excitability by reducing potassium channels. The growing and maturing fetus participates in the onset of labour by creating the steroid precursor for placental oestriol leading to myometrial cell excitability and connectivity. Increased excitability and connectivity lead to increased synchrony of contractile behaviour of individual myometrial cells, increased intrauterine pressure, cervical dilatation and birth.

The fetus and placenta are interdependent in human pregnancy, a well-matched developmental program for the partners is essential for a successful outcome that sets the fetus on a trajectory for a healthy life, leaving a senescent placenta in its wake.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA001

The cardiotoxicity of phenanthreneC. Marris¹, S.N. Kompella¹, J.C. Hancox² and H. Shiels¹¹Cardiovascular Sciences, University of Manchester, Manchester, UK and ²School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK

The disease burden from ambient air pollution is becoming increasingly apparent. Despite the lung being the first site of exposure for air pollution, numerous epidemiological studies show strong correlations between air pollution and cardiovascular disease. Inhaled particulate matter (PM) can interact directly with airway epithelia but the surface chemicals on PM, like polyaromatic hydrocarbons (PAH), are also known to exert a cardiovascular response. The focus of our current work is phenanthrene (Phe), a small 3-ringed member of the PAH family which is ubiquitous in air and water fossil fuel-based pollution.

We have examined the mechanisms underlying the cardiotoxicity of Phe on the ion channels and intracellular Ca^{2+} cycling pathways in zebrafish ventricular myocytes. Data are presented as percentage mean $\pm$ SEM. Using whole-cell current clamp we find a significant shortening of action potential duration (APD) in presence of 10 μM Phe, with APD_{50} and APD_{90} shortening by approximately $49\% \pm 6.4$ and $41\% \pm 7.5$ of control values, respectively ($p < 0.05$, two-way ANOVA). This finding is in contrast to the prolongation of APD_{90} found in marine species at similar concentrations which was attributed to direct inhibition of *erg* (*Ether-à-go-go Related*) channel (Brette *et al*, 2017). However, during the current clamp experiments, subsequent application of 2 μM E-4031, a selective inhibitor of *erg* channel, after 10 μM Phe showed no further prolongation of APD_{90} indicating the block of *erg* channel. Whole-cell voltage clamp experiments confirmed inhibition of I_{Kr} tail-current following 10 μM Phe exposure (by $82\% \pm 2.6$, $p < 0.05$, unpaired t-test). Phe exposure also reduced the intracellular calcium transient amplitude in field stimulated zebrafish ventricular cardiomyocytes by 13 % ($p < 0.05$; paired t-test) and doubled the rate of transient decay ($p < 0.05$; paired t-test).

This work indicates the disruptive effect of Phe on the zebrafish myocardial function and also reveals how variable susceptibility of outward and inward currents to PAH-based pollution can affect the electrical and contractile properties of the vertebrate heart.

Brette, F., Shiels, H. A., Galli, G. L., Cros, C., Incardona, J. P., Scholz, N. L., and Block, B. A. (2017) A Novel Cardiotoxic Mechanism for a Pervasive Global Pollutant. *Scientific Reports* 7

Supported by British Heart Foundation Grant PG/17/77/33125.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA002

From car to coronary: inhaled particles and the cardiovascular system

M.R. Miller

Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

Over the last few decades it has become apparent that particles in air pollution have many detrimental effects in different organ systems. The cardiovascular effects of inhaled particles are especially important, given the high proportion of deaths attributed to air pollution that arise from cardiovascular causes.

Our group has shown that exposure to diesel exhaust particles, an especially rich source of nanoparticles in urban air pollution, has multiple detrimental actions throughout the cardiovascular system; promoting blood clotting, impairing vascular function and exacerbating stress on the heart. However, the biological processes underlying the pulmonary exposure of particles and that of the cardiovascular system have yet to be fully established.

This presentation describes a series of experimental studies in human subjects and preclinical models investigating whether nanoparticle translocation (the movement of particles from the lung into the circulation) could be an underlying pathway to account for the widespread cardiovascular actions of vehicle exhaust. Using gold nanoparticles as a model, we were able to demonstrate the presence of gold in the blood and urine within hours of inhalation in healthy volunteers. Furthermore, levels in the blood persisted for a number of months after the end of exposure. The translocation of gold nanoparticles was size-dependent, with smaller particles more readily entering the blood. Using a mouse model of atherosclerosis we demonstrated that translocated nanoparticles preferentially accumulated at areas of vascular disease. Finally, we demonstrated that inhaled particles reached areas of atherosclerosis in patients with a history of stroke.

Particle translocation represents a convincing explanation for the multiple detrimental effects of inhaled nanoparticles, which could lead to increases in cardiovascular morbidity and mortality. These findings emphasise the need to reduce emissions from traffic as an important means to reduce the health effects of air pollution.

This research was supported by Programme and Project Grants from the British Heart Foundation (RG/10/9/28286; PG/10/042/28388; PG/12/8/29371; SP/15/8/31575) and a Project Grant (202111) from the COLT Foundation, with additional support from the Dutch Ministry of Infrastructures and Environment (M/630196) and the UK Department of Health (PR-NT-0208-10025).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA003

The greenwashing of fuels

J. Bosson

Umeå University, Umeå, Sweden

Within the last decades the growing concern for anthropogenic pollution and its extensive effects have taken center stage influencing global technology, politics and business. After the Kyoto Protocol came about in 1997, the EU initially promoted diesel as the more environmentally friendly fuel, as carbon dioxide emissions tend to be reduced. Motorists were given incentives in order to buy more diesel cars, in the hopes that this would help to reduce greenhouse gas emissions. New research developments on diesel effects, emissions scandals, continued climate change advances and dependence on diminishing fossil fuel supplies have contributed to a shift in focus to renewable biofuels. “Green”, “eco-”, “bio-”, “sustainable”, “carbon-neutral” are just a few of the environmental buzzwords seen around us each day in order to now evoke a positive reaction. The most common biofuel within the EU is currently a biodiesel, RME (rapeseed oil methyl ester). It has been shown to emit fewer exhaust particles compared to standard petrodiesel and this purported “green fuel”, being of biological origin, is projected to be less harmful to human health. Whilst standard petrodiesel is now widely known to contribute to the onset and mortality of respiratory and cardiovascular diseases, the consequences to public health remain unexplored in regards to the majority of biofuels on the marketplace today.

In a series of studies performed by our group, healthy volunteers were exposed to filtered air, petrodiesel or biodiesel dilute exhaust in a controlled chamber or by specially-designed mask. Following exposures in the first two studies, numerous cardiovascular endpoints were assessed as well as characterization of the exhaust. In further studies autonomic nervous system function was evaluated utilizing microneurography.

Compared to petrodiesel, RME exhaust contained less elemental carbon, fewer polyaromatic hydrocarbons, lower levels of hydrocarbons and higher concentration of nitrogen oxides. RME also showed a shift in the particulate matter size distribution towards the ultrafine range and a significantly increased concentration of a range of metals relative to petrodiesel. These ultrafine particles have a greater surface area per mass and can access further into the respiratory tract making them potentially more damaging per mass than larger particles.

The collective results in these studies demonstrated that despite their varying composition and particle reactivity, RME exhaust exposure induced comparable adverse health effects as exposure to standard petrodiesel exhaust through similar underlying mechanisms.

Replacing petrodiesel with biodiesel may have certain advantageous ecological impacts, however, it would be well advocated that adverse health effects be

addressed alongside environmental concerns when new fuel policies are considered due to potential major public health impacts.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA004

Teaching physiology using human-patient simulators

R. Helyer

Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK

High-fidelity, manikin-based human patient simulators have been adopted as a mainstay of medical education. They are typically used in teaching symptom-based diagnosis, responses to situations such as trauma, and clinical and interprofessional skills. Many simulators are able to display data for physiological variables, used to report the 'physiology' or vital signs. In some, the physiological model is sufficiently complex to be considered integrated in terms of systems (typically cardio-respiratory), and responsive in terms of modelling autonomic control (such as the baroreflex) (Helyer & Dickens, 2016).

There are two opportunities, at least, presented to educators of physiology and biosciences. First, the investment in manikin-based simulation in medical schools, teaching hospitals, and university departments (often those teaching professional courses) can increase their accessibility to teachers of biosciences in general. Second, there is a commitment to take steps to ensure that students on professional courses have a firm grounding in bioscience throughout their education, both undergraduate and postgraduate.

Here at the University of Bristol we have been fortunate to be able to exploit both of these opportunities, and after over 10 years of utilising simulation in teaching physiology (Harris et al, 2011), it is now a core part of our teaching curriculum across both professional and biomedical sciences courses.

This presentation will discuss how we have developed simulation-based teaching that aims to enrich teaching of key fundamentals of physiology of the healthy human (and animal), and how more recently we have used case-based simulations to explore the depth of physiology that underlies disease. It will also open discussion around the areas of cost and effective use of resources, such as group size, and use in student project work (Gibb et al, 2018).

Gibb J, Vasudev A, Helyer, R. Simulation provides deep learning opportunities for medical students intercalating in the biosciences. *BMJ Simulation and Technology Enhanced Learning* Mar 2018, bmjstel-2018-000318; DOI: 10.1136/bmjstel-2018-000318 (epub ahead of print).

Harris JR, Helyer RJ, Lloyd E. Using high-fidelity human patient simulators to teach physiology. *Med Educ.* 2011 Nov;45(11):1159-60. doi: 10.1111/j.1365-2923.2011.04105.x.

Helyer R, Dickens P. Progress in the utilization of high-fidelity simulation in basic science education.

Adv Physiol Educ. 2016 Jun;40(2):143-4. doi: 10.1152/advan.00020.2016.

I would like to acknowledge our technical team at the Biomedical Sciences Simulation Centre, Mr David Morgan and Mr Alex Cowen.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA005

Technology-enhanced learning-by-doing: Inclusive experimental human physiology in Higher Education.

A. Rickard

School of Science, University of Greenwich, Chatham, UK

Human physiology is most powerfully demonstrated to undergraduate students with real-time observations on the body's physiological responses to experimental interventions. However, with the breadth of applications of physiology to subjects such as biomedical science, forensics, human nutrition, pharmaceutical science, and beyond, the skill-sets and previous laboratory experience of students undertaking experimental physiology modules are widely varied. This can result in apprehension and anxiety in students within the physiology laboratory and can result in students focussing on the methodology, rather than the physiology that the experiments intend to demonstrate. In addition, inevitably large class sizes with a broad international and multicultural diversity call for human physiology practicals to be re-designed to allow all students to be engaged in the learning-by-doing experiments. Undergraduate students today confidently use technology to inform their learning and understanding of the world around them. Importantly, this confidence in learning and exploration using technology is shared by all students, regardless of their discipline or cultural background. The additional support that technology-solutions can provide to students both inside, and outside of the laboratory can better prepare them to be able to use the contact-time most constructively.

Over the last 5 years, students working in interdisciplinary groups from across all life science disciplines offered at the University of Greenwich have been using ADInstruments' teaching hardware and cloud-based learning platform, Lt. The pre-laboratory interactive material provides students with an introduction to the relevant physiology concepts, and instructional videos remove the reliance on teaching staff to provide technical support in the laboratory. Laboratory sessions were aligned to occur on the same day as the relevant lecture, allowing students to engage with the physiology theory in practice, reinforcing their understanding of the concepts delivered in the classroom.

Since the introduction of the new experimental format, attendance at practical sessions increased, there was better engagement from students of all backgrounds, average whole-module marks increased for students across all disciplines, and the standard deviation of average marks reduced. Students also reported better satisfaction with the laboratory sessions, and the module as a whole.

With students at the heart of their learning as both subjects and observers, and the removal of anxiety over more novel practical techniques through the support in the cloud-based learning interface, the focus of these sessions shifted from instructor-led, to student-led. This not only engaged students in the physiology itself, but encouraged conversation between the inter-disciplinary working groups, where students gained deeper-learning, and an understanding of the relevance and application of physiology to their own field, and beyond.



Students undertaking a spirometry experiment in a physiology teaching practical.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA006

Teaching physiology practical exercises, education solutions at the University of Antwerp

M.J. Demolder

Physio-Pharmacology, University of Antwerp, Wilrijk, Belgium

Physiology practical exercises went through a transformation from a text book lab for a limited amount of students to a cloud based system with a large cohort of students, at the University of Antwerp.

21st century practical exercises and learning need to be flexible, adaptive and reflecting the state of the art. We changed our home build setups, transducers, software and data-acquisition into the hardware en software technology from ADInstruments (Dunedin, New-Zealand). Twenty 'Ultimate Teaching Systems' with multiple additional transducers were acquired. All practical exercise sessions

are held in a specially build room, which provides world-class teaching facilities. The use of this facility has significantly improved the student learning experience. Students participate in groups of two or three students on one setup, which results in a total capacity of 60 students.

Initially, practical exercises were performed on the LabTutor platform (ADInstruments). Subsequently a migration to LabTutor-Online and to a new cloud-based education platform, Lt-KuraCloud, was performed.

To a limited extent, due to the cost of a license, pre-practical, practical, and post-practical work will now be done through this online software, which one can access at the practical lab room, and at any time in other places in the university, at home, etc. Due to budgetary constrain and the culture of cheap education in Belgium we still use LabTutor.

Since the introduction in 2010 of this facility for undergraduate students of Pharmacology and Biomedical Sciences, the amount of participants has increased over three different faculties (Pharmaceutical-Biomedical-Veterinary Sciences, Medicine and Health Sciences, Science Faculty). Including additional undergraduates in Biochemistry, Biotechnology, Veterinary Sciences, Revalidation Sciences and Physiotherapy, Biology and Bio-Engineering, about 1000 students.

LabTutor and LabTutor-Online student and instructor feedback: Students greatly appreciate the full software support and short learning curve for the exercises, the online pre-lab and example data and the post-lab report facility and the link to patient scenarios. Experimenting on their own body, being able to prepare the lab online at home enhanced their understanding of the course. However, a mandatory prelab test is necessary. Former experiments on animals have been replaced by pre-recorded data, reducing the use of lab-animals to zero. Instructors appreciate the pre-lab and post-lab evaluation, the large amount of lab exercises with the possibilities to edit or make new exercises and the server facilities. Our facility received an excellent evaluation ('State of the Art') from the Belgian-Dutch Accreditation Commission. The physiology practical lab is a good promotion during the 'open campus days' for new students.

After the discontinuing of LabTutor-Online we introduced Lt-KuraCloud. We already experienced a positive evaluation from the students with the physiology simulation program PhysioEx (Pearson Education), which uses the same concept of immediate feedback with multiple choice questions, as in Lt-KuraCloud.

Students appreciate the blended learning with the theoretical courses and feedback features in Lt as a great add-on to the ex-cathedra courses. Engagement and learning outcomes increased. Instructors reacted positive on the analytics of the student work progress and the automatic grading facility. The fast and easy implementing of new lessons opens great opportunities.

Transforming education through cloud-based technologies in a large modern facility enabled us to improve and increase the capacity of our physiology practical experiments. The cost of the licenses is still an issue. However, a coalition of the 'willing professors' to change their courses to a blended learning environment, will take some time.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA007

Using invertebrate and plant model systems to teach physiology and experimental design in inquiry-based practical teaching

D.U. Silverthorn

Medical Education, University of Texas at Austin, Austin, TX, USA

Traditionally, physiology practicals were an opportunity for students to observe physiological principles in action while gaining laboratory skills. In recent decades however, the call to teach students how to think like scientists, coupled with growing resistance to the use of vertebrate animals in the classic experiments, has led to the development of inquiry-based practicals that rely on invertebrate and plant model systems. These model systems can be used to demonstrate most of the same physiological principles as the vertebrate models while simultaneously developing student appreciation for the conservation of fundamental mechanisms throughout the animal and plant kingdoms. By eliminating the use of vertebrate animals, we avoid ethical issues (although we still stress appropriate handling and anesthesia of the animals) and supply problems while enhancing the ability of students to run multiple replicates rather than being limited to an n of 1 in their experiments. In the guided inquiry format, students are given some parameters (e.g., design an experiment to show the influence of extracellular Ca^{2+} on smooth muscle contraction) and a list of available equipment and supplies. They research the literature, design a protocol, execute it, analyse the data, and communicate their findings orally or in writing (1). Invertebrate model systems can be used for many neurobiology experiments (2). Crawfish (*Procambarus clarkii*) hearts substitute for frog hearts. Rat intestinal or uterine smooth muscle is replaced by earthworm (*Lumbricus sp.*) crop-gizzard preparations. In these heart and smooth muscle experiments students can apply neurotransmitters and various pharmacological agents to elicit effects. Renal tubule transport is easily visualized in Malpighian tubule preparations from crickets (*Acheta domesticus*) or cockroaches (*Periplaneta americana* or other species) and uses many of the same transporters as vertebrate kidney (3). Plant material such as pumpkin (*Cucurbita sp.*), privet (*Ligustrum sp.*), and peas (*Pisum sp.*) can be used in experiments demonstrating osmolarity and tonicity, membrane transport, environmental effects on oxygen consumption, and enzyme activity (e.g., catalase). These non-vertebrate model systems have proved more acceptable to students. The organisms used are cultured for food or other commercial uses or can be bred in colonies within the laboratory. Anesthesia consists of cold or immersion in ethanol, which avoids the use of controlled substances. With the greater availability and lower cost of these model organisms, students are able to repeat their experiments enough times to do statistical

analysis of the data. Overall, the use of non-vertebrate model systems in physiology practical teaching has supported active learning of physiological concepts and development of scientific thought processes and skills.

McNeal AP, Silverthorn DU, Stratton DB. Involving students in experimental design: three approaches. *Adv Physiol Educ.* 1998 Dec; 20(1):S28-34. <https://doi.org/10.1152/advances.1998.275.6.S28>

Oakley B, Schafer R. *Experimental neurobiology: a laboratory manual*. Ann Arbor: University of Michigan Press; 1978.

Silverthorn DU. Active transport in insect Malpighian tubules. In: Goldman CA, editor. *Tested studies for laboratory teaching*, Volume 16. Proceedings of the 16th workshop/conference of the Association for Biology Laboratory Education (ABLE). 1995. pp. 141-154. Available from <http://www.ableweb.org/biologylabs/wp-content/uploads/volumes/vol-16/11-silverthorn.pdf>

Many of these experiments were developed in conjunction with a National Science Foundation Instrumentation and Laboratory Improvement Grant, USE-9251764.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA008

Why Sodium Channel Nav1.7 is required for Pain Sensation

J.N. Wood, J.J. Cox and J. Zhao

UCL, London, UK

Since the initial demonstration of an essential role for SCN9A, the gene that encodes sodium channel Nav1.7, in human pain (1), attention has focused on the role of the channel in nociception. The channel is expressed in peripheral sensory neurons, and the initial hope was that selective Nav1.7 blockers that were peripherally restricted might produce analgesia at the same level as that observed in the loss of function mutants. However, this hope has not been fulfilled and potent analgesics have proved elusive. Mechanistic studies have demonstrated that Nav1.7 has pleiotropic roles in pain pathways. The first evidence for this came from microarray analysis of Nav1.7 null mutant sensory neurons. Deletion of SCN9A in sensory neurons results in a complex range of transcriptional changes, including a dramatic increase in the expression of preproenkephalin mRNA. A major role for endogenous opioids in Nav1.7 null analgesia has been demonstrated in both mice and two human null mutants, as naloxone reverses much of the analgesia (2). However, enhanced opioid peptide levels alone do not account for this analgesic mechanism (3). Interestingly, opioid receptors themselves also seem to be potentiated, possibly as a result of altered intracellular sodium levels (4). Apart from a link to the opioid system Nav1.7 has a remarkable role as an integrator of small depolarisations that may contribute to its essential role in nociceptive signalling (5). In addition, neuropeptide release from sensory neurons has been shown to be abolished in the absence of Nav1.7 (2). More recently, a remarkable transfer of Nav1.7 from sensor

neurons to dorsal horn neurons has been observed. Sensory neuron-derived Nav1.7 seems to play a significant role in contributing to dorsal horn neuron excitability (6). Thus we have a remarkable range of actions of Nav1.7, many within the dorsal horn of the spinal cord, that are important for normal pain pathways.

What are the consequences of these mechanistic observations for an analgesic drug development? It is now clear that highly selective Nav1.7 antagonists are not analgesic. However, when combined with low dose opioids or enkephalinase inhibitors, useful analgesia can be achieved (7). This has potential utility in dealing with the disastrous opioid analgesia crisis in the United States. However, the contribution of potentiated opioid receptors, and the role of dorsal horn Nav1.7 channels may limit the synergistic approach of Nav1.7 inhibitors and opioid peptides. If so, gene therapy, silencing or deleting the SCN9A gene may be a necessary approach to exploit the full potential of Nav1.7 as an analgesic drug target.

Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, Karbani G, Jafri H, Mannan J, Raashid Y, Al-Gazali L, Hamamy H, Valente EM, Gorman S, Williams R, McHale DP, Wood JN, Gribble FM, Woods CG. An SCN9A channelopathy causes congenital inability to experience pain. *Nature*. 2006 Dec 14;444(7121):894-8. doi:10.1038/nature05413

Minett M.S., Pereira P, Sikandar S, Matsuyama A., Lolignier S., Alexandros H. Kanellopoulos AH, Mancini F, Iannetti G.D., Bogdanov Y., Santana-Varela S., Millet Q., Baskozos G., MacAllister R., Cox J.J., Zhao J. and Wood J.N. Endogenous opioids contribute to insensitivity to pain in humans and mice lacking sodium channel Na_v1.7 2015 *Nature Comms* Dec 4;6:8967. doi: 10.1038/ncomms9967

Pereira V, Millet Q, Aramburu J, Lopez-Rodriguez C, Gaveriaux-Ruff C, Wood JN. Analgesia linked to Nav1.7 loss of function requires μ - and δ -opioid receptors. *Wellcome Open Res*. 2018 Aug 16;3:101. doi: 10.12688/wellcomeopenres

Isensee J, Krahé L, Moeller K, Pereira V, Sexton JE, Sun X, Emery E, Wood JN, Hucho T Synergistic regulation of serotonin and opioid signaling contributes to pain insensitivity in Nav1.7 knockout mice. *Sci Signal*. 2017 Jan 10;10(461)

Branco T, Tozer A, Magnus CJ, Sugino K, Tanaka S, Lee AK, Wood JN, Sternson SM. Near-Perfect Synaptic Integration by Nav1.7 in Hypothalamic Neurons Regulates Body Weight. *Cell*. 2016 Jun 16;165(7):1749-61.

Sensory neuron-derived Nav1.7 contributes to dorsal horn neuron excitability

Sascha R.A. Alles, Filipe Nascimento, Rafael Lujan, Queensta Millet, Sonia Santana, Ali Bangash, James Cox, Marco Beato, Jing Zhao, John Wood bioRxiv 551747; doi: <https://doi.org/10.1101/55174>

Deuis JR, Dekan Z, Wingerd JS, Smith JJ, Munasinghe NR, Bhola RF, Imlach WL, Herzig V, Armstrong DA, Rosengren KJ, Bosmans F, Waxman SG, Dib-Hajj SD, Escoubas P, Minett MS, Christie MJ, King GF, Alewood PF, Lewis RJ, Wood JN, Vetter I. Pharmacological characterisation of the highly Na_v1.7 selective spider venom peptide Pn3a. *Sci Rep*. 2017 Jan 20;7:40883.

We thank the Wellcome Trust, MRC, EU and Versus Arthritis for support.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA009

Single-cell RNAseq reveals seven classes of colonic sensory neuron

J. Hockley¹, T. Taylor¹, G. Callejo¹, A.L. Wilbrey², A. Gutteridge², K. Bach¹, W.J. Winchester², D.C. Bulmer¹, G. McMurray² and E. Smith¹

¹Pharmacology, University of Cambridge, Cambridge, UK and ²Neuroscience and Pain Research Unit, Pfizer, Cambridge, UK

Integration of nutritional, microbial and inflammatory events along the gut-brain axis can alter bowel physiology and organism behaviour. Colonic sensory neurons activate reflex pathways and give rise to conscious sensation, but the diversity and division of function within these neurons is poorly understood. The identification of signalling pathways contributing to visceral sensation is constrained by a paucity of molecular markers. Here we address this by comprehensive transcriptomic profiling and unsupervised clustering of individual mouse colonic sensory neurons. Thoracolumbar (TL) and lumbosacral (LS) dorsal root ganglia were collected from 10 mice 3-10 days after the neuronal tracer Fast Blue was injected into the wall of the colon via laparotomy under inhalation anaesthesia (1.25 % isoflurane) following previously described protocols (Hockley et al, 2014). Fast Blue labelled mouse colonic sensory neurons were subsequently picked using an adapted patch-clamp rig and pulled glass pipettes, and single-cell RNA-sequencing (Picelli et al, 2013) was performed on cells isolated from both TL (n=159 neurons) and LS (n=155 neurons) dorsal root ganglia associated with lumbar splanchnic and pelvic spinal pathways, respectively. Transcriptomic profiling and unsupervised clustering of 314 colonic sensory neurons revealed seven neuronal subtypes using Single-cell Consensus Clustering (SC3, Kiselev et al, 2017). The subtypes were validated by performing single-cell qRT-PCR on a second cohort of 168 colonic sensory neurons against a panel of 7 subtype-specific marker genes (*Mrgprd*, *Cbln2*, *Fam19a1*, *Smr2*, *Trpa1*, *Hpse* and *Ntm*). We also used immunohistochemistry (IHC) against a panel of markers including TrkC, Gfra3 and Spp1 to validate the spinal segmental patterning observed between TL and LS neurons. Of the seven subtypes identified, five neuronal subtypes accounted for 99% of TL neurons, with LS neurons almost exclusively populating the remaining two subtypes. We use Ca²⁺-imaging of mouse colonic sensory neurons combined with post-functional single-cell qRT-PCR to demonstrate subtype-selective differential agonist activation (e.g. 10 of 125 TL neurons responded to 5-HT₄ receptor agonist BIMU8 but no LS neurons (0 of 42) matching the expression profile of 5-HT₄ within TL only subtypes). These results provide a framework to the molecular interrogation of colonic sensory innervation in health and disease and also an opportunity to identify novel targets for future drug development.

Hockley JR et al. (2014). *Pain* 155, 1962-75.

Picelli S et al. (2013). *Nat Protoc* 9, 171-181.

Kiselev VY et al. (2017). *Nat Methods* 14, 483-6.

This work is supported by a Rosetrees Postdoctoral grant (A1296) and BBSRC grant (BB/R006210/1) awarded to J.R.F.H and E.S.J.S, Medical Research Council Grant (G0900907) to DCB and Versus Arthritis Research grant to GC and EstJS (RG 20930).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA010

Finding new analgesic targets – the long way round.

L.F. Donaldson

University of Nottingham, Nottingham, UK

Vascular endothelial growth factor-A (VEGF-A) is known to promote angiogenesis in solid tumours, and in inflammatory conditions such as arthritis. It is now recognised that VEGF-A is also directly implicated in pain, particularly neuropathic pain such as traumatic, diabetic and chemotherapeutic neuropathy. VEGF-A is alternatively spliced to produce two different families of VEGF proteins – VEGF-A_{xxx}a and VEGF-A_{xxx}b, where xxx denotes the number of amino acids. These differ only in the terminal 6 amino acids, but this difference results in distinct receptor interaction and function. In our studies of the different actions of distinct VEGF-A splice variants, we identified contributions of VEGF-A to nociceptive processes, both peripherally and centrally. Targetting VEGF receptors has potential serious adverse effects on the vasculature, so if a problematic route for development of analgesic drugs based on VEGF-A actions. Taking a step back, and looking at the control of the splicing of VEGF-A, rather than the downstream proteins themselves, we are investigating the possibility of using novel inhibitors of splicing kinases to reverse changes in alternative RNA splicing found in inflammation, cell damage and pain.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA011

Optimizing Exercise Prescription for Pulmonary Hypertension: Insight Gained from Acute and Chronic Exercise Studies in Rodent Models and in Patients

M. Brown

Rehabilitation Medicine, University of Washington, Seattle, WA, USA

Pulmonary arterial hypertension (PAH), a Group 1 pulmonary hypertension (PH), is a disease of progressive pulmonary vascular remodeling and dysfunction for which there is no cure and only minimally effective treatment. Though traditionally discouraged for PAH, emerging evidence suggests exercise as a potential therapeutic.

However, current training approaches generally fail to lower elevated pulmonary pressure, or mitigate right ventricular maladaptive hypertrophy, a major determinant of survival in PAH. The clinical and animal studies of the Brown Laboratory have been directed towards defining the physiological and biochemical consequences and functional outcomes following acute and chronic exercise in PH, to permit much needed optimization of exercise prescription for patients. This seminar will discuss studies testing the hypothesis of recent work- that optimal training outcomes for PH require exercise parameters that strongly stimulate the pulmonary vascular endothelium, and maximize favorable cardiac and skeletal muscle adaptations (e.g. metabolic), while reducing risk for exercise cardiac wall stress-induced acceleration of right heart dysfunction. We will present evidence in a Sprague-Dawley rat model suggesting superior benefit of high intensity interval training (HIIT) for the treatment of PAH, but with a caveat that responses to HIIT and other types of exercise in experimental PH are phenotype- and severity- dependent. We will discuss what we may be able to glean about potentially unsafe exercise training approaches from alarming exercise hemodynamics, mortality, and RV maladaptation in PH rats. Data from two novel in-vivo methodologies supporting the study of exercise responses in experimentally-induced PH will also be presented- implantable telemetry recording of pulmonary pressures during treadmill running, and assessment of skeletal muscle blood flow during treadmill running using fluorescent microspheres. Finally, we will look at recent clinical data piloting the use of an unsupervised exercise intervention for patients with PAH and discuss important remaining exercise prescription knowledge gaps gleaned from animal and clinical optimization studies.

The experimental protocols for studies presented from the Brown Lab were approved by the Institutional Animal Care and Use Committee of Indiana University, which is in compliance with National Institutes of Health guidelines. All animals received care in compliance with the Guide for the Care and Use of Laboratory Animals 8th edition, Copyright 2011 by the National Academy of Sciences. For animal surgeries, the anaesthetic used was inhaled isoflurane (2.5%). Prior to tissue harvest, rats of these studies were killed under general anaesthesia (inhaled isoflurane (5%)) via exsanguination.

Galie, N., et al. (2016). "2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT)." *Eur Heart J* 37(1): 67-119.

Brown MB, Neves E, Long G, Novack R, Graber J, Gladish B, Owens M, Wiseman A, Fisher A, Presson R, Petrache I, Kline J, Lahm T. High Intensity Interval Training but not Continuous Training Reverses Right Ventricular Remodeling and Dysfunction in a Rat Model of Pulmonary Arterial Hypertension; *Am J Physiol Reg Int Comp Physiol*. 2017 Feb 1;312(2): R197-R210 doi:10.1152/ajpregu.00358.2016.

Brown MB, Kempf A, Collins C, Long G, Gupta S, Hellman Y, Wong V, Farber M, Lahm T. A Prescribed Walking Regimen plus Arginine Supplementation Improves Function and Quality of Life for Patients with Pulmonary Arterial Hypertension: A Pilot Study. *Pulmonary Circulation* 2017; 8(1) 1–12. DOI: 10.1177/2045893217743966

Neves E, Brown MB, Zaretskaia M, Rezania S, Goodwill A, McCarthy B, Persohn S, Territo P, Kline J. Chronic embolic pulmonary hypertension caused by pulmonary embolism and vascular endothelial growth factor inhibition; *Am J Pathol*. 2017 Apr;187(4):700-712. PMID: 28183533

Lahm T, Frump A, Albrecht A, Fisher A, Cook T, Jones T, Yakubov B, Whitson J, Fuchs R, Liu A, Chesler N, Brown MB. 17 β -Estradiol Mediates Superior Adaptation Of Right Ventricular Function To Acute Strenuous Exercise In Female Rats With Severe Pulmonary Hypertension, *Am J Physiol Lung Cell Mol Physiol*. 2016 Aug 1;311(2):L375-88. doi: 10.1152/ajplung.00132.2016.

Brown MB, Chingombe TJ, Zinn AB, Reddy JG, Novack RA, Cooney SA, Fisher AJ, Presson RG, Lahm T, Petrache I. Novel assessment of hemodynamic kinetics with acute exercise in a rat model of pulmonary arterial hypertension. *Exp. Physiol*. 2015 Apr 13;100(6):742-754, doi: 10.1113/EP085182.

Benjamin N, Marra AM, Eichstaedt C, Grünig E. Exercise Training and Rehabilitation in Pulmonary Hypertension. *Heart Fail Clin*. 2018 Jul;14(3):425-430. doi: 10.1016/j.hfc.2018.03.008.

Funding:

NIH NHLBI R-15

American Heart Association, Midwest Affiliate - Scientist Development Grant

Pulmonary Hypertension Association/American Thoracic Society - 'Proof of Concept' Grant

Indiana Clinical Translational Science Institute - CTR Grant

Collaborators:

Irina Petrache, MD

Gary Long, MS (PhD student)

Tim Lahm, MD and Andrea Frump, PhD

Jeff Kline, MD and Evandro Neves, PhD

Robert Presson, MD

Mark Farber, MD

Dan Rusyniak, MD

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA012

Effects of voluntary exercise on cardiac remodelling and vulnerability to arrhythmia in rats with pulmonary hypertension

E. Pervolaraki, E. White and A. Benson

University of Leeds, Leeds, UK

Motivation: Right heart failure, contractile dysfunction and arrhythmia are associated with pulmonary hypertension (PH) induced in rats by monocrotaline (1-3). Voluntary wheel running can improve cardiac function and delay the onset of right heart failure in these animals (3). Although the mechanisms associated with this benefit are unknown, amelioration of cardiac electrophysiological and structural remodelling may play a part (1-3). Understanding the mechanisms by which exercise acts on PH may potentially reveal new therapeutic pathways for treatment. **Methods:** Male Wistar rats (180-190 g) were injected with 60 mg/kg monocrotaline to induce PH, or an equivalent volume of saline (control; CON). Animals were randomly assigned free access to a running wheel (exercise; EX) or not (sedentary; SED). PH-SED ($n = 12$) were sacrificed upon external signs of heart failure; CON-SED ($n = 6$), CON-EX ($n = 11$) and PH-EX ($n = 6$) animals were sacrificed on matched days, unless heart failure developed earlier in PH-EX animals. Isolated hearts were Langendorff perfused, then action potentials and calcium transients were optically mapped with RH237 and Rhod2 respectively. Hearts were then fixed in 5% paraformaldehyde, and imaged using diffusion tensor MRI in a Bruker 9.4 T scanner at 200 μm isotropic resolution to quantify cardiac geometry and myocyte orientations. Animal-specific computational models based on these experimental data were used to assess vulnerability to arrhythmia (1,2,4).

Results: Mean daily voluntary running distance was not significantly different between CON-EX and PH-EX (mean $\pm$ SEM: 3.1 ± 0.6 vs. 4.4 ± 1.1 km, $p = 0.29$, t -test). Voluntary exercise prolongs survival in PH: no PH-EX animals developed heart failure signs on the day of such signs in PH-SED animals (21 ± 0.26 days). PH significantly increased right ventricular action potential duration (APD_{90}) at 5 Hz pacing (CON 63.3 ± 4.3 ms, PH 92.3 ± 4.6 ms, $p = 0.0003$, two-way ANOVA), but exercise had no effect (SED 77.1 ± 7.7 ms, EX 78.4 ± 5.6 ms, $p = 0.52$). There were no PH or exercise effects on calcium transient duration. Myocyte disarray, quantified by R^2 from a linear fit to transmural myocyte elevation angles, was increased by PH in the right ventricular wall (CON 0.80 ± 0.05 , PH 0.65 ± 0.02 , $p = 0.01$) but was not significantly affected by exercise (SED 0.75 ± 0.04 , EX 0.73 ± 0.05 , $p = 0.74$). Exercise improved myocyte disarray in the left ventricular wall (SED 0.85 ± 0.02 , EX 0.89 ± 0.05 , $p = 0.04$). Computational simulations show that structural (myocyte orientation) remodelling underpins instability of re-entrant arrhythmias to a significantly greater extent than electrophysiological (APD_{90}) remodelling in PH ($p = 0.004$, one-way ANOVA).

Conclusion: These data suggest that exercise may be a suitable therapeutic intervention to prevent right heart failure and arrhythmia in PH.

Benoist *et al.*, Am. J. Physiol. 302:H2381-95;2012.

Benoist *et al.*, Prog. Biophys. Mol. Biol. 115:162-72;2014.

Natali *et al.*, Am. J. Physiol. 309:H421-4;2015.

Benson *et al.*, Interface Focus 1:101-116;2011.

Supported by a British Heart Foundation project grant to APB and EW (PG/16/74/32374).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA013

Pulmonary pressures during exercise: Comparing the healthy older adult to the pulmonary hypertension patient

K. Coffman^{1,2} and B. Johnson²

¹Exercise Science, University of Puget Sound, Tacoma, WA, USA and ²Cardiovascular Diseases, Mayo Clinic, Rochester, MN, USA

In pulmonary hypertension (PH), pulmonary vascular remodeling and excessive vasoconstriction leads to an increase in pulmonary vascular resistance, ultimately leading to a deleterious rise in pulmonary pressures. In heart failure (HF) patients with combined pre- and post-capillary PH, functional outcomes of gas exchange are correlated with this rise in pulmonary pressures. At rest, the transpulmonary gradient (i.e., pulmonary arterial-venous pressure gradient) is negatively correlated with pulmonary arterial (PaO₂) and venous (PvO₂) partial pressures of oxygen, as well as arterial (SaO₂) and venous (SvO₂) oxygen saturation [1]. This negative impact of increasing pulmonary pressures on gas exchange persists during submaximal exercise, where the diastolic pressure difference is positively correlated to dead space ventilation (V_D/V_T) and negatively correlated with end-tidal partial pressure of carbon dioxide (P_{ET}CO₂) [2].

Older, otherwise healthy adults also experience remodeling of the pulmonary vasculature, leading to a subsequent increase in pulmonary pressures, particularly during exercise [3, 4]. Recent work suggests that an increase in basal pulmonary smooth muscle tone may also be present in the healthy older adult [5]. In concert, these age-dependent alterations reveal several similarities between the healthy older adult and the PH patient. Nevertheless, while individuals with diagnosed PH are negatively impacted by a rise in pulmonary pressures, this may not be the case in older individuals, for whom a modest increase in pulmonary pressures is often asymptomatic.

Previous work aimed at understanding the role of pulmonary pressures during exercise in healthy older adults (>65 years) demonstrated that reducing the age-related

increase in pulmonary pressures via a pharmacological intervention actually worsened lung diffusing capacity (DLCO), a critical functional gas exchange outcome [6]. In this study, 9 younger and 9 older healthy individuals completed an incremental cycling exercise protocol before and after 100 mg sildenafil. As expected, prior to sildenafil administration older individuals demonstrated greater pulmonary pressures and lower DLCO for a given cardiac output in comparison to their younger counterparts. Interestingly, however, the reduction in pulmonary pressures elicited by pharmacological vasodilation actually reduced DLCO during exercise, and this reduction became significantly greater as exercise intensity increased. Specifically, on average among all subjects (younger and older combined), pulmonary artery pressure was reduced by 5 mmHg, venous pressure by 2 mmHg, and thus the transpulmonary gradient fell by 3 mmHg following sildenafil administration (all $p < 0.001$). This reduction in the driving pressure through the pulmonary vasculature led to a fall in DLCO of 2 ml/min/mmHg at a cardiac output of 10 L/min and a fall of 4 ml/min/mmHg at a cardiac output of 15 L/min ($p < 0.001$). In other words, the loss in forward driving pressure elicited by the reduction in the transpulmonary gradient hindered effective ventilation and perfusion matching, causing a reduction in gas diffusion within the lungs. Therefore, these data may suggest that an increase in pulmonary pressure is necessary and advantageous in the older adult, particularly during exercise. Specifically, a rise in pulmonary pressures may occur to overcome the age-dependent increase in pulmonary vascular resistance, and thus act to maintain adequate perfusion of the pulmonary vasculature in the healthy older adult.

In summary, pulmonary pressures are increased not only in the PH patient, but also in the healthy older adult during exercise. However, while the PH patient experiences detrimental impacts on various markers of cardiopulmonary function, there is evidence to suggest that the healthy older adult may require an increase in pulmonary pressures – albeit to a lesser extent – to maintain cardiopulmonary function during exercise. Thus, in terms of pulmonary gas exchange, pulmonary hypertension should be thought of as a continuum, where mild pulmonary hypertension can be functionally advantageous.

Taylor, B.J., et al. (2013). *J Card Fail*, 19(1), 50-9.

Taylor, B.J., et al. (2015). *J Card Fail*, 21(8), 647-55.

Kovacs, G., et al. (2009). *The European respiratory journal*, 34(4), 888-94.

van Empel, V.P., et al. (2014). *The American journal of cardiology*, 114(1), 131-5.

Coffman, K.E., et al. (2018). *Physiol Rep*, 6(17), e13834.

Coffman, K.E., et al. (2018). *Physiol Rep*, 6(2).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA014

Using exercise testing in the evaluation of pulmonary hypertension

L. Howard^{1,2}

¹National Pulmonary Hypertension Service, Imperial College Healthcare NHS Trust, London, UK and ²National Heart & Lung Institute, Imperial College London, London, UK

Pulmonary Hypertension (PH) is a condition characterised by increased pulmonary vascular resistance due to pulmonary vasoconstriction and remodelling leading to an increase in afterload on the right ventricle. This manifests as impairment of cardiac output on exercise and as the disease progresses this results in right heart failure and death. As such a cardinal feature of PH is exercise intolerance, usually reported as breathlessness, but presentation is usually relatively late in the overall disease process, by the time pulmonary vascular resistance has increased several fold above normal values. The standard investigations such as lung function and six-minute walk testing may be normal, and exercise testing can be used in breathless patients to point towards a diagnosis of PH which is confirmed by right heart catheterisation. In some cases, resting catheterisation may be relatively normal and stressing the circulation with fluid challenge or exercise may be required to unmask more subtle changes in pulmonary vascular resistance or left ventricular compliance. Once diagnosed, exercise physiology is invaluable in assessing response to therapy and determining therapeutic strategies.

Kovacs et al. *Eur Respir J*. 2018 Jan 18;51(1). pii: 1750578. doi: 10.1183/13993003.50578-2017

McLaughlin et al. *J Am Coll Cardiol*. 2013 Dec 24;62(25 Suppl):D73-81

Galie, Humbert et al. *Eur Respir J*. 2015 Oct;46(4):903-75

Arena et al. *J Heart Lung Transplant*. 2010 Feb;29(2):159-73

Badagliacca et al. *Chest*. 2016 Dec;150(6):1313-1322

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA015

Skeletal muscle redox signalling in rheumatoid arthritis

M. Steinz, M. Kalakoutis, T. Mader, A. Boller and J. Lanner

Physiology & Pharmacology, Karolinska Institutet, Solna, Sweden

Skeletal muscle weakness debilitates persons with rheumatoid arthritis (RA) and contributes to decreased quality of life for the afflicted patients. Oxidative stress is thought to be part of the pathophysiology of RA and we recently mapped specific oxidative posttranslational modifications (oxPTMs) on muscle actin (α -actin) that promote muscle weakness in mice and patients with RA. Specifically,

we found oxPTMs 3-nitrotyrosine (3-NT) and malondialdehyde (MDA) adducts in three distinct regions of α -actin from mice and patients with RA. 3-NT and MDA are oxPTMs induced by free radical species superoxide (O_2^-) and peroxynitrite ($ONOO^-$). Up until now, the intramuscular redox signalling in RA, including the interplay between sources of $O_2^-/ONOO^-$ and free radical scavengers is still unclear. Here we aim to elucidate the intramuscular redox signalling that contributes to skeletal muscle weakness in inflammatory conditions.

Gene expression analysis by qRT-PCR showed a significant lower gene expression of ROS scavengers, including NQO1, catalase (CAT), SOD2 and GPx1 ($n=6$; $p<0.05$; two-tailed t-test) in skeletal muscle of mice with complete freund's adjuvant (CFA)-induced arthritis than in healthy controls (Ctrl). SOD2/CAT scavenge O_2^- that can be produced by the mitochondria. Immunofluorescence staining shows a distinct striated CAT pattern every $1.83\ \mu m$ and muscle fibres from mice with CFA-induced arthritis show an increased band width of the CAT protein (Ctrl= $0.69 \pm 0.04\ \mu m$; CFA= $0.87 \pm 0.05\ \mu m$; $n=20$; $p<0.05$; two-tailed t-test). Experiments are now being performed with genetically-encoded redox-sensitive GFP probes specifically targeting mitochondria in order to quantify mitochondrial free radical production over time in muscle of mice with arthritis and healthy controls. Moreover, intraperitoneal injection of the CAT/ SOD2 mimetic EUK-134 restored muscle force in mice with arthritis to the level of healthy control mice (Ctrl= $477 \pm 14\ kN/m^2$; CFA $389 \pm 26\ kN/m^2$; CFA_{EUK-134} $520 \pm 25\ kN/m^2$, mean $\pm$ SEM; $n=5-6$), which is in accordance with our previously published data from rats with arthritis.

In summary, we show altered gene expression and localisation of ROS scavengers in muscles from mice with arthritis. In addition, treatment with targeted antioxidant EUK-134 improved muscle force in these mice. This implies that the ROS scavenging capacity is altered which can contribute to oxidative stress and muscle weakness in RA. All animal and human experiments were approved by the Swedish national and local ethical boards. Arthritis in mice was induced under anaesthetic conditions (2.5% induction - 4% maintenance).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA016

Role of redox signaling in skeletal muscle adaptations to training

M. Gomez-Cabrera, C. Arc-Chagnaud, A. Salvador-Pascual, A. de la Rosa, F. Millan, G. Olaso-Gonzalez, A. Carretero, A. Correias and J. Vina

Physiology, University of Valencia, Valencia, Spain

Physical exercise increases the cellular production of reactive oxygen species (ROS) in muscle, liver, and other organs. Originally, ROS were considered as detrimental and thus as a likely cause of cell damage associated with exhaustion. In the last decade, evidence showing that ROS act as signals has been gathered and thus the

idea that antioxidant supplementation in exercise is always recommendable has proved incorrect (1). In fact, we proposed that exercise itself can be considered as an antioxidant because training increases the expression of classical antioxidant enzymes such as superoxide dismutase and glutathione peroxidase and, in general, lowering the endogenous antioxidant enzymes by administration of antioxidant supplements may not be a good strategy when training (2).

Antioxidant enzymes are not the only ones to be activated by training. Mitochondriogenesis is an important process activated in exercise (3). Many redox-sensitive enzymes are involved in this process. Important signaling molecules like MAP Kinases, NF- κ B, PGC-1 α , p53, Heat Shock Factor, and others modulate muscle adaptation to exercise. Interventions aimed at modifying the production of ROS in exercise must be performed with care as they may be detrimental in that they may lower useful adaptations to exercise (4).

1. M. C. Gomez-Cabrera *et al.*, *J Physiol*, (2005).

2. M. C. Gomez-Cabrera, E. Domenech, J. Vina, *Free Radic Biol Med* 44, 126 (2008).

3. M. C. Gomez-Cabrera *et al.*, *Am J Clin Nutr* 87, 142 (2008).

4. M. C. Gomez-Cabrera, A. Salvador-Pascual, H. Cabo, B. Ferrando, J. Vina, *Free Radic Biol Med* 86, 37 (2015).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA017

The Smokefreebrain study: A multidisciplinary examination of e-cigarette toxicity

T. Marczylo^{1,2}

¹Toxicology, Public Health England, Didcot, UK and ²PHE & King's College London, Health Impacts of Environmental Hazards HPRU, London, UK

Electronic cigarette (EC) use for smoking cessation and harm reduction has divided opinions in scientific and smoking cessation communities. Opinions are diverse from EC being the answer to the global tobacco epidemic to EC being a major public health concern both in their own right and as a gateway into smoking. Many of the concerns around EC use focus upon addiction and potential health effects from long-term exposures to EC aerosol especially upon the respiratory system. The smokefreebrain study is a H2020-funded project which includes an examination of EC to investigate some key questions with respect to toxicity including toxicant exposures and formation of carcinogens, effects upon markers of inflammation and effects upon enzymes involved in the bioactivation of environmental procarcinogens. EC aerosol was generated using an Innokin Endura T18E vape pen through a commercially available EC aerosol generating system (e-aerosols, NY) employing the CORESTA EC puff profile (55 ml puff volume, 3 s puff duration, 30 s puff interval, and a square wave puff profile. In vitro studies employed human

pulmonary hBEC-3KT cells exposed to aerosol generated from differently flavoured e-liquids either with or without nicotine in an air-liquid interface system. Endpoints focussed upon cell viability, and markers of inflammation and oxidative stress. Male AJ mice (n=9) were exposed to aerosol from tobacco-flavoured e-liquid, (0, 6 mg/ml and 18 mg/ml nicotine; 50:50 PG:VG) or air-only controls for 14 consecutive days by the nose only route. Primary endpoints in this hazard identification study included whole body and organ weights, carcinogen biomarker studies, changes to nicotinic acetylcholinesterase receptors in brain, and targeted metabolomics. Finally, a longitudinal study of heavy smokers (n=55) switching to EC only use was conducted with the aim of monitoring toxicant exposures, including nicotine, through urinary and salivary biomarkers together with psychometric measures (nicotine dependence, nicotine withdrawal, anxiety, urges to smoke/vape etc). In addition, a subset of participants (n=13) had their brain electrical activity measured using electroencephalography, before and 3 weeks after the transition to EC-only use. Finally, participants that completed the 28-day intervention (n=31) were followed up at 6 and 12 months and smoking status determined. Here we will present the findings of these studies and discuss the possible consequences for human health.

Many thanks to Nathan Goldsmith and Elizabeth Zhuikova, PhD students at PHE and St George's University of London, Elizabeth Macauley, Philippa Durrant, Harry Goss and especially Alexis Bailey at St George's University of London, Andrea Ioannides and Lichan Liu at AAI Scientific Cultural Services for their expertise in EEG data analysis and Emma Marczylo, Matthew Wright and Adam Laycock at PHE, Chilton for their experience and skill. Thanks also to EU H2020 programme and NIHR for funding this work.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA018

Physiologic effects of e-cigarettes on the cardiopulmonary system

L.E. Crotty Alexander^{1,2}

¹*Medicine, University of California San Diego, San Diego, CA, USA and* ²*Medicine, Veterans Affairs San Diego Healthcare System, San Diego, CA, USA*

Electronic (e)-cigarettes have become immensely popular across the globe, but little is yet known about the impact of inhaling e-cigarette vapor on a daily basis. Conventional cigarettes are known to cause cardiopulmonary disease, with associated changes in heart rate, heart rate variability, blood pressure, ventricular function, airways resistance, airway reactivity and lung compliance. Many of these changes occur after decades of cigarette smoking, thus we may not know whether e-cigarettes will cause physiologic changes in humans for years to come. Therefore, we created a mouse model of e-cigarette vaping, and have exposed two different,

genetically disparate, mouse strains to multiple types of e-cigarettes - including vape pens, Mods and JUULs - over 1-6 months to assess the impact of e-cigarette vapor inhalation on cardiopulmonary health. We have determined that inhalation of e-cigarette vapor on a daily basis is not benign. E-cigarette exposed mice develop cardiac fibrosis, a clear indication that myocardium is being damaged during e-cigarette use. Some mice develop airway reactivity and emphysema in a strain-specific manner, and this is also tied to specific types of e-cigarettes and the chemical composition of the e-cigarette vapor. There is much more work to be done to define the causal pathways behind e-cigarette effects on the mammalian system, which will hopefully lead to the development of safer devices.

Crotty Alexander LE, et al. (2018) *AJP, Reg Int Comp Physiology*. 314(6):R834-847

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA019

The Effect of E-cigarettes on Susceptibility to Pneumococcal Infection

L. Miyashita and J. Grigg

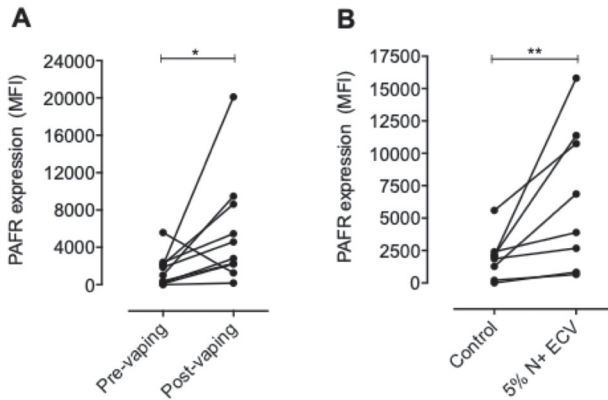
Genomics and Child Health, Queen Mary University of London, London, UK

E-cigarette use is rapidly increasing worldwide, yet there is limited research into the effects of vaping on susceptibility to respiratory infection. Evidence shows that vulnerability to invasive pneumococcal disease is associated with exposure to tobacco smoke^[1]. Bacterial adherence to host cells is key to colonisation and infection. A mechanism by which pneumococci adhere to airway cells is by co-opting host expressed platelet-activating factor receptor (PAFR). We previously reported that PAFR expression is increased by cigarette smoke^[1]. In this study, we sought to determine if nasal PAFR expression is increased with E-cigarette use *in vivo*.

Nasal biopsies were taken from E-cigarette users (N=10) before and 1 hour after an observed 5-minute vaping session. All individuals used their regular brand of E-cigarette and E-liquid. Nasal cells taken before the vaping session (N=8) were also exposed to 24mg/ml nicotine (N+) E-cigarette vapour (ECV) *in vitro* for 2.5 hours. Nasal epithelial PAFR expression was subsequently assessed by flow cytometry.

A significant increase in nasal PAFR expression was observed in participants post E-cigarette use ($p < 0.05$) (figure 1a). A significant increase in PAFR expression was also observed in nasal cells from participants that were exposed to 24mg/ml nicotine ECV *in vitro* ($p < 0.01$) (figure 1b). Data are expressed as median fluorescence intensity (MFI) adjusting for isotypic control and analysed by paired t-test.

This study provides preliminary evidence that nasal epithelial PAFR expression, as with conventional tobacco, is increased by E-cigarette vapour. *In vitro* studies suggest that this is in part due to nicotine. We speculate that increased PAFR expression in E-cigarette exposed airway cells increases vulnerability to pneumococcal infection.



Grigg, J., et al., Cigarette smoke and platelet-activating factor receptor dependent adhesion of *Streptococcus pneumoniae* to lower airway cells. *Thorax*, 2012. 67(10): p. 908-13.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA020

What you don't know can't hurt you? The effects of E-cigarettes on the human lung

R. Tarran

Cell Biology & Physiology, University of North Carolina, Chapel Hill, NC, USA

Unlike conventional tobacco products, e-cigarette use (i.e. vaping) does not involve tobacco combustion, suggesting that the potential for harm may be reduced. E-cigarette liquids contain a propylene glycol/vegetable glycerin (PG/VG) base, nicotine and flavorings. However, the long-term health effects of exposing human lungs to vaped e-liquids are largely unknown. We performed research bronchoscopies on healthy non-smokers, smokers and vapers to obtain bronchial epithelia and bronchoalveolar lavage (BAL) under UNC ethics board-approved conditions. We also exposed primary bronchial epithelial cultures and immune cells to e-cigarette components *in vitro*. Bronchoscopy revealed visually abnormal airways in chronic vapers and we identified ~300 proteins that were differentially expressed in smokers and vapers, with only 79 proteins being commonly altered in both groups. The mucin MUC5AC, and VAMP8, which facilitates mucin secretion, were upregulated in both vapers and smokers. Exposure of epithelia to aerosolized PG/VG, but not nicotine, was sufficient to increase MUC5AC abundance *in vitro*. Excessive proteolysis occurs with chronic tobacco use and is causative for bronchiectasis and emphysema. Neutrophil elastase, MMP-2 and MMP-9 activities and protein levels were equally elevated in both vapers and smokers BAL relative to non-smokers. We also found that exposure of neutrophils and macrophages to

nicotine *ex vivo* caused dose-dependent increases in protease release. We conclude that vaping induces nicotine-dependent and independent effects on the lung, alters both epithelia and immune cells and disrupts the protease-antiprotease balance, which may place vapers at risk of developing chronic lung disease. These data indicate that vaping may not be safer than tobacco smoking.

Funded by the NIH and the FDA.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA021

Current state and outstanding mechanisms in normal arterial physiology – lessons from implantable cardiac devices

E.J. Stöhr^{1,2}, H. Rosenblum², M. Kanwar³, W.K. Cornwell⁴, J.R. Cockcroft¹ and B.J. McDonnell¹

¹Cardiovascular Physiology & Health, Cardiff Metropolitan University, Cardiff, UK, ²Columbia University Irving Medical Center, New York City, NY, USA, ³Allegheny Health Network, Pittsburgh, PA, USA and ⁴University of Colorado Anschutz Medical Campus, Aurora, CO, USA

For centuries, scientists have studied the circulation. Today, our knowledge spans all aspects of the circulation, from whole-systems biology as well as molecular and cellular aspects of the circulation. This present knowledge has been generated from basic science discoveries (including the seminal identification of the role of the endothelium) as well as from healthy and diseased humans such as hypertensive patients. However, recent medical therapies have created the possibility to study the human circulation under conditions that were never possible before. This first talk of the symposium entitled “Bionic Women and Men” will provide a 5-min overview of the most fundamental aspects of the circulation (flow, blood pressure, sympathetic activity, endothelial function, pulsatility) before introducing the “Bionic Women and Men”: Heart failure patients who have had a left ventricular assist device (LVAD) implanted into their hearts. Since LVADs produce continuous-flow, these humans live without a pulse. Consequently, all the normal fundamental circulatory variables (flow, blood pressure, sympathetic activity, endothelial function, pulsatility) are drastically altered in these humans. The resultant effects on the circulation, and the response of the circulation to such altered haemodynamics, are only beginning to be uncovered. This talk will highlight the unique haemodynamics of these patients, indicate the current knowledge and propose new hypotheses for future investigation.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 705219 and from the Lisa and Mark Schwartz Program to Reverse Heart Failure at New York-Presbyterian Hospital/Columbia University.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA022

Increased Aortic Stiffness in Continuous-Flow Left Ventricular Assist Device Patients is Associated with Higher Rates of Adverse Events

H.R. Rosenblum¹, E.J. Stöhr^{1,2}, W.K. Cornwell³, M. Kanwar⁴, J.R. Cockcroft² and B.J. McDonnell²

¹Cardiology, Columbia University Medical Center/New York Presbyterian Hospital, New York, NY, USA, ²Cardiff Metropolitan University, Cardiff, UK, ³University of Colorado Anschutz Medical Campus, Aurora, CO, USA and ⁴Cardiovascular Medicine, Allegheny General Hospital, Pittsburgh, PA, USA

In the general population, increased aortic stiffness, a measure of larger artery distensibility, is associated with an increased risk of cardiovascular events. It has been previously demonstrated that continuous-flow left ventricular assist device (CF-LVAD) patients have increased aortic stiffness, perhaps related to abnormal arterial blood flow patterns. In an investigational study we aimed to determine whether increased aortic stiffness in CF-LVAD patients is 1) associated with LVAD parameters, and 2) associated with adverse events, including stroke, gastrointestinal bleed (GIB) and pump thrombosis (PT). We studied 40 HeartMate II (HMII) pts (51 ± 8 yrs, 25% ischemic, 32% F), implanted between 1/2011 to 9/2017, with subsequent orthotopic heart transplant. Two dimensional transthoracic echocardiograms obtained prior to HMII placement and immediately after heart transplant were analyzed for aortic stiffness. A composite outcome of GIB, stroke, and PT was compared between patients who had increased vs. reduced aortic stiffness following LVAD support. Overall, aortic stiffness increased significantly following HMII support (AS: 10.6 to 9.6, $p=0.06$; DC: 4.8 to 4.1, $p<0.0001$; b-SI: 5.8 to 8.2, $p=0.04$), however a number of patients actually had decreased aortic stiffness. Patients with increased ($N=24$) compared to decreased aortic stiffness had significantly higher rates of the composite end-point of adverse events (58% vs 12%, OR 9.8 (1.81-53.07), $p<0.01$). Additionally, patients with increased aortic stiffness had a non-significant trend towards longer duration of time on CF-LVAD support (627+572 vs 349+216 days, $p=0.064$), higher LVAD speed (9124 vs 8973 rpm, $p=0.087$) and less frequent use of ACE or ARB therapy (29 vs 56%, $p=0.087$). In conclusion, increased aortic stiffness in HMII patients is associated with significantly higher rates of adverse events while on CF-LVAD support. Further studies are

warranted to determine the causality between aortic stiffness and adverse events, as well as potential modulators of aortic stiffness in CF-LVAD patients.

Change in aortic stiffness and adverse events

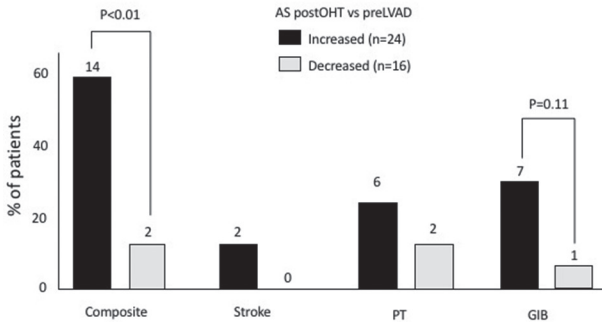


Figure 1. Increased compared to decreased aortic stiffness is associated with an increased rate of adverse events while on CF-LVAD support.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 705219 and from the Lisa and

Mark Schwartz Program to Reverse Heart Failure at New York-Presbyterian Hospital/ Columbia University

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA023

Right ventricular function in patients with pulmonary hypertension - The devil is in the details

M. Kanwar¹, H. Rosenblum², E.J. Stöhr³, W.K. Cornwell⁴, J.R. Cockcroft³ and B.J. McDonnell³

¹Cardiovascular Medicine, Allegheny General Hospital, Pittsburgh, United States
²Minor Outlying Islands, ³Columbia University Irving Medical Center, New York, NY, USA, ³Cardiff Metropolitan University, Cardiff, UK and ⁴University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Right ventricular failure (RVF) remains a common complication in patients with pulmonary hypertension (PH), regardless of the etiology of elevated pulmonary pressures. Normally, the RV stroke work, which is 1/6th compared to that of the LV, maintains forward momentum of blood flow into highly compliant, low resistant

pulmonary circulation. Therefore, the RV flow is characterized by low peak systolic pressure in absence of isovolumetric phases of contraction and relaxation during systole and diastole. Unfortunately, patients with pulmonary arterial hypertension (PAH) or left heart disease commonly develop secondary RVF. Mechanisms of RVF include: contractile failure secondary to myocardial ischemia, volume overload (preload) as a result of right-sided valvular insufficiency, displacement of the ventricular septum towards the LV and increase in RV afterload. Close monitoring of pulmonary artery pressure and RV function is necessary to appropriately guide therapy and predict survival. The development of ambulatory implantable hemodynamic monitors such as CardioMEMS provides clinicians with remote access to daily cardiovascular measurements to monitor progression, guide therapy, and detect or prevent early decompensation. They do so by allowing clinicians to review the RV functional response at rest and with exertion, in response to therapy. CardioMEMS sensors are implanted in the pulmonary artery and calibrated with pulmonary pressure and cardiac output, based on the PAP waveform, heart rate, and a reference cardiac output measured at implant.

We will review the pathophysiology of RVF failure in left heart disease and PAH and discuss the role of implantable monitors in guiding positive remodeling of the RV in these disease conditions.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 705219 and from the Lisa and Mark Schwartz Program to Reverse Heart Failure at New York-Presbyterian Hospital/Columbia University. In addition, the work performed at Allegheny General Hospital was supported by a NHLBI VITA Contract HHSN268201400008C.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA024

Cardiovascular, Cerebrovascular and Exercise Pressor Reflexes in the Bionic Man.

W.K. Cornwell¹, H.R. Rosenblum², M. Kanwar³, J.R. Cockcroft⁴, B.J. McDonnell⁵ and E.J. Stöhr⁴

¹Medicine-Cardiology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA, ²Columbia University Irving Medical Center, New York City, NY, USA, ³Allegheny Health Network, Pittsburgh, PA, USA, ⁴Cardiovascular Physiology and Health, Cardiff Metropolitan University, Cardiff, UK and ⁵Cardiovascular Physiology and Health, Cardiff Metropolitan University, Cardiff, UK

Left ventricular assist devices (LVADs) are an increasingly utilized strategy for management of patients with advanced heart failure with reduced ejection fraction (HFrEF). While these devices improve survival, there are a number of complications associated with LVADs in the human body, such as hypertension and stroke, as

well as persistent HF-related symptoms and impaired functional capacity despite normalization of a resting cardiac output. These devices provide “continuous-flow” circulatory support, meaning that pulsatility is markedly diminished and/or entirely absent. Further, current-generation devices are denervated machines, and in contrast to the normal heart, they do not participate in normal cardiovascular reflexes and there is no biofeedback loop to regulate flow. Emerging evidence suggests that these abnormalities are a major factor that predispose these patients to adverse outcomes and/or impairments in quality-of-life and functional capacity. The purpose of this discussion will be to provide an overview of the impact of chronic exposure to a nonphysiologic/diminished pulse on normal cardiovascular and cerebrovascular reflexes, and discuss the effect(s) of a denervated machine on the cardiovascular response to exercise. More specifically, emphasis will be placed on cerebral autoregulatory processes and the baroreceptor reflex pathway, as well as autonomic tone and sympathetic nerve activation. Novel insights into left and right ventricular function during exercise will be reviewed, based on emerging data derived from invasive cardiopulmonary exercise testing using traditional Swan-Ganz catheters, as well as high-fidelity conductance catheters to generate real-time pressure-volume loops.

Dr. Cornwell is supported by an NIH/NHLBI Mentored Patient-Oriented Research Career Development Award (#1K23HL132048-01), the Susie and Kurt Lochmiller Distinguished Heart Transplant Fund, and the Clinical Translational Science Institute at the University of Colorado Anschutz Medical Campus.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA025

The coronary microcirculation in advancing age: does aging set up the development of heart failure

A.J. LeBlanc

Physiology, University of Louisville, Louisville, KY, USA

If a coronary vascular network is restricted in its ability to carry more BF when stimulated, the distal cardiac tissue it supplies can become hypoxic immediately. The sheer area that the small resistance vessels cover in the myocardium warrants particular attention as a means to influence minute to minute oxygen exchange and impact BF distribution. This perfusion fine tuning is accomplished primarily through vasodilation of the coronary resistance arterioles, but the mechanism that achieves this effect changes significantly as aging occurs and as comorbidities arise. Not surprisingly, advancing age is associated with the increased generation and presence of reactive oxygen species (ROS) in nearly all tissues. This presentation will reconcile how ROS can be successfully utilized during increased metabolic demand as a substrate to mediate vasoreactivity in the coronary microcirculation in young

animal models, and how this signaling changes as “normal” aging progresses, thereby setting a stage for the development of heart failure.

Funding for this presentation was provided by NIH R01 AG053585 and the Gheen’s Foundation to AJL.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA026

Is heart failure a coronary microvascular disorder?

V. Ohanyan, T. Hakobyan, L. Shockling, T. Gyulkhasyan, M. Enrick, C.L. Kolz and W.M. Chilian

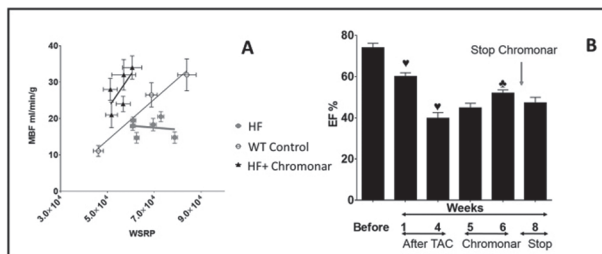
IMS, Northeast Ohio Medical University, Rootstown, OH, USA

Heart failure (HF) is the leading cause of death in the US and worldwide. Current therapies have produced some measure of success in patient outcomes, but they only slow the progression of the disease. We speculate that if the correct “lesion” was targeted by the therapy, the progression could be stopped and perhaps, reversed. Coronary microvascular disease is associated with heart failure, but whether dysfunction of the coronary microcirculation causes the disease is unknown. Thus, we hypothesize the cause of heart failure is insufficient myocardial blood flow (MBF) that fails to meet the needs of the heart. The flow insufficiency produces minute areas of ischemia, resulting myocyte loss, and fibrosis with the accumulation of these events eventually leading to failure. To test this, we determined the relationship between MBF and cardiac work (wall stress-rate product [WSRP]). We examined 3 groups of animals: Group 1: control C57Bl6/J mice (CTRL); Group 2: mice with transaortic constriction (TAC) to produce HF; and Group 3: HF mice treated with the coronary vasodilator, Chromonar (4 weeks of treatment). This latter treatment was devised to show proof of principle, i.e., if flow were increased in the failing heart, would the failure be reversed?

Transthoracic echocardiography was used for weekly cardiac function measurements. MBF and WSRP were measured during norepinephrine-induced increases in WSRP in anesthetized mice to create the relationship between MBF and cardiac work. The figure (Panel A) shows the relationship between WSRP and MBF in the 3 groups. Note the relationship between WSRP and MBF is uncoupled in HF, i.e., when work increases MBF does not. Moreover, Chromonar recoupled work with MBF and increased MBF (increased MBF per unit of work). These changes in flow paralleled cardiac function in the HF and HF+Chromonar groups. After TAC cardiac function decreased over 13 weeks: ejection fraction (EF) of about 40% in both groups (panel B). Chromonar treatment increased EF to 67±3%; whereas in untreated mice EF remained low. Based on these findings, we propose that a cause of HF is inadequate MBF to meet the metabolic demands of the working heart.

Pharmacological coronary vasodilation with Chromonar to increase MBF in HF can reverse the functional decline and improve cardiac function.

Cardiac function and myocardial blood flow after Chromonar treatment



WSRP –Wall stress rate product was calculated as MAP x HR x Radius/Wall thickness

NIH/NHLBI

AHA

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA027

Coronary Microcirculation in Left and Right Heart Failure

D. Merkus, O. Sorop, J. van de Wouw, J.J. Steenhorst, Z. Cai and D. Duncker

Cardiology, Erasmus MC, Rotterdam, Netherlands

Coronary microvascular function and cardiac function are closely related in that proper cardiac function requires adequate oxygen delivery through the coronary microvasculature. Insufficient oxygen supply contributes to development and progression of heart failure. Furthermore, endothelium-derived factors directly modulate cardiac function, and hence endothelial dysfunction may directly impact cardiac function. Risk factors like (pulmonary) hypertension, diabetes and/or hypercholesterolemia directly affect the coronary microvasculature. Evidence is accumulating that the myocardial oxygen balance is disturbed in the failing heart due to increased extravascular compressive forces and coronary microvascular dysfunction. Importantly, coronary microvascular dysfunction is associated with adverse cardiovascular events. Since alterations in microvascular as well as cardiac function are first revealed during increased physical activity, exercise testing provides a valuable method to interrogate coronary microvascular function. Identification of abnormal microvascular responses, i.e. alterations in neurohumoral influences, endothelial dysfunction and oxidative stress, may provide novel targets

for therapeutic interventions that improve microvascular function and thereby delay progression of ventricular dysfunction to overt heart failure.

Support of CVON-PHAEDRA and CVON-RECONNECT is gratefully acknowledged

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA028

Mechanisms of increased vulnerability to oxygen and glucose deprivation in astroglia carrying Alzheimer's disease mutations

D. Martin-de-Saavedra¹, E. Navarro², A. Moreno-Ortega², M. Cuhna³, I. Buendia², P. Hernansanz-Agustin⁴, R. Leon², M. Cano-Abad², A. Martinez-Ruiz⁴, R. Martinez-Murillo⁵, M. Duchen⁶ and M. Lopez²

¹Physiology, Northwestern University, Chicago, IL, USA, ²Universidad Autonoma de Madrid, Madrid, Spain, ³Universidade Federal de Santa Catarina, Florianopolis, Brazil, ⁴Instituto de Investigacion Sanitaria Princesa, Madrid, Spain, ⁵Instituto Cajal - CSIC, Madrid, Spain and ⁶University College London, London, UK

Growing evidence suggests a close relationship between Alzheimer's Disease (AD) and cerebral hypoxia. Astrocytes play a key role in brain homeostasis and disease states, while some of the earliest changes in AD occur in astrocytes. We have therefore investigated whether mutations associated with AD increase astrocyte vulnerability to ischemia. Two astrogloma cell lines derived from APP_{SWE} / PS1A246E (APP, amyloid precursor protein; PS1, presenilin 1) transgenic mice and controls from normal mice were subjected to oxygen and glucose deprivation (OGD), an in vitro model of ischemia. Cell death was increased in the APP_{SWE} / PS1A246E line compared to the control. Increasing extracellular calcium concentration ([Ca²⁺]) exacerbated cell death in the mutant but not in the control cells. In order to explore cellular Ca²⁺ homeostasis, the cells were challenged with ATP or thapsigargin and [Ca²⁺] was measured by fluorescence microscopy. Changes in cytosolic Ca²⁺ concentration ([Ca²⁺]_c) were potentiated in the APP_{SWE} / PS1A246E transgenic line. Mitochondrial function was also altered in the APP_{SWE} / PS1A246E astrogloma cells; mitochondrial membrane potential and production of reactive oxygen species were increased, while mitochondrial basal respiratory rate and ATP production were decreased compared to control astrogloma cells. These results suggest that AD mutations in astrocytes make them more sensitive to ischemia; Ca²⁺ dysregulation and mitochondrial dysfunction may contribute to this increased vulnerability. Our results also highlight the role of astrocyte dyshomeostasis in the pathophysiology of neurodegenerative brain disorders.

This work was supported by the Spanish Government (partially funded by the European Union ERDF/FEDER) Ref. SAF2015-63935R to M.G.L.; Ref. SAF2015-65586 to R.M.M; Ref. PI15/00107 to A.M.R.; FPU/contract Ref. AP2009/0343 to A.J.M.O., the Spanish Ministry of Health (Instituto de Salud Carlos III) Ref. PI14/00372 to R.L and a grant from the Fundacion Domingo Martinez (Spain) to A.M.R. and M.G.L. R.L. thanks the Instituto de Salud Carlos III and the European Regional Development s funds (FEDER) for a research contract under the Miguel Servet II Program (CPII16/00014). We also thank the Fundacion Teofilo Hernando for its continuous support.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA029

Ischaemic preconditioning of primary rat neural cultures induces tolerance to subsequent ischaemic insult (oxygen-glucose deprivation)

A. Singh¹, R. Chen¹ and S. Jenkins²

¹*School of Pharmacy, Keele University, Newcastle-under-lyme, UK and* ²*School of medicine, Keele University, Newcastle-under-lyme, UK*

Despite accounting for 6 million deaths every year worldwide[1], ischaemic stroke treatment remains inadequate. Prior exposure to a sub-lethal ischaemic event (ischaemic preconditioning, IPC) reduces the damage caused by a subsequent ischaemic insult[2]. This protection involves hypoxia-inducible factor-1 α (HIF1 α), but the precise molecular mechanisms are unclear[3]. We are developing *in-vitro* models to elucidate the mechanisms of IPC-induced tolerance to ischaemic stroke. Model1: Primary cultures (embryonic day 17-18 rat cortices; serum-free media) predominantly containing neurons (~75% Tuj1⁺) were subject to IPC [2 or 4 hour oxygen-glucose deprivation (OGD); 0.3% O₂] then 'reperfusion' (restored glucose and oxygen, 21% O₂; 24 h), followed by 6 hour OGD (0.3% O₂).

There was no cytotoxicity of 2 h and 4 h OGD, with or without reperfusion, validating these as sub-lethal IPC protocols. In contrast, 6 h OGD showed elevated LDH (27 $\pm$ 5% v 5 $\pm$ 2% control), reduced MTT (57 $\pm$ 4% of control), and fewer Tuj1⁺ cells (~50% of control). These effects were exacerbated with 24 hour reperfusion (LDH: 43 $\pm$ 5% v 5 $\pm$ 1%; MTT: 27 $\pm$ 4%; ~20% Tuj1⁺), simulating ischaemic injury.

HIF1 α was stabilised and a group of hypoxia genes (*Glut1*, *Vegf*, *Bnip3*, and *Phd2*) were upregulated by OGD for either 2, 4 or 6 hours.

Preconditioning the neurons with 2 or 4 hours OGD followed by reperfusion, significantly improved neuron's viability after 6 hours OGD (2 h – LDH[SJ1]: 20 $\pm$ 2%; [SJ2] MTT: 73 $\pm$ 2% of control; 4 h – LDH: 20 $\pm$ 2%; MTT: 81 $\pm$ 4%), compared to non-PC controls (6 h OGD insult – LDH: 35 $\pm$ 2%; MTT: 52 $\pm$ 3%).

Model2: High purity primary astrocyte cultures (postnatal day 1-3 rat cortices; ~98% GFAP⁺) showed no cytotoxicity for up to 6 h in OGD (0.3% O₂). Prolonged OGD

produced elevated LDH (24 h: $36 \pm 2\%$, 48 h: $66 \pm 3\%$ v $7 \pm 3\%$ control), morphological changes (i.e. dystrophic astrocytes with extended processes), increased intensity of GFAP immunostaining per cell, and reduced cell numbers.

In conclusion, our data demonstrate that astrocytes resist OGD-induced degeneration for a longer period than neurons. This OGD-induced neuronal damage was reduced by IPC, and deducing the mechanisms underpinning this may offer therapeutic insights for treating ischaemic stroke.

Mozaffarian, D., et al., 2016. Executive Summary: Heart Disease and Stroke Statistics—2016 Update: A Report from the American Heart Association. *Circulation*, 133(4), pp. 447-454.

Vetrovoy, O., et al., 2017. Cerebral mechanisms of hypoxic/ischemic postconditioning. *Biochemistry (Moscow)*, 82(3), pp. 392-400.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA030

Stem cell-based modelling of brown adipogenesis

K. Velickovic¹, A. Lugo Leija¹, H. Sacks², M. Symonds¹ and V. Sottile¹

¹University of Nottingham, Nottingham, UK and ²University of California, Los Angeles, CA, USA

Adipocytes are functional cells specialised in the storing and control of energy stores. While the majority of adipocytes belongs to the unilocular white lineage responsible for lipid storage, a subset of adipogenic cells known as brown adipocytes are able to dissipate energy through thermogenesis. This thermogenic capacity relies on the brown-specific expression of the mitochondrial uncoupling protein UCP1. *In vivo*, cold exposure is the main stimulus to activate UCP1, known for mediating heat production in brown and beige adipocytes, however the physiological factors favouring thermogenic brown adipogenic lineages *in vitro* remain largely elusive.

In order to identify key biological stimuli involved in adipogenic browning, we have developed an *in vitro* culture system to induce an adipogenic response using marrow-derived stem cells (MSCs) exposed to defined culture conditions. Under specific treatment, including exposure to reduced temperature during differentiation, MSCs can form adipocytes sharing metabolic, protein and molecular features of brown adipocytes. Incubation at 32°C instead of 37°C over the course of the culture treatment was seen to promote brown adipogenesis, with increased adipocyte response and upregulation of adipogenic and thermogenic factors including UCP1. Treated cultures generated multilocular adipocytes exhibiting a progressive increase in the number and size of intracellular lipid droplets within a week. Ultrastructural analysis indicated that alongside increased intracellular lipid inclusions, treated cells showed number of mitochondria with altered morphology. Quantitative metabolic measurements confirmed that organelle adaptation was

accompanied by enhanced basal respiration, increased glycolysis and oxidative phosphorylation, indicating metabolically active cells consistent with a thermogenic phenotype.

These latest results demonstrate the usefulness of stem cell cultures available from accessible tissue for the study of adipocyte formation, providing a unique model to measure cellular and metabolic adaptation to pharmacological and environmental stimuli. This *in vitro* model represents a new tool to identify pro-browning factors at the cellular level, and elucidate the mechanism of metabolic adaptation relevant to the browning process.

Velickovic K, et al. (2018)*Sci Rep.* 8(1):4974.

This work was funded by the EU-CASCADE fellowship scheme funded by the EU's 7th FP PCOFUND-GA-2012-600181, CONAcYT, Mexico and support from The Cardiometabolic Disease Research Foundation (Los Angeles, USA). This work was supported by the Biotechnology and Biological Sciences Research Council [grant number BB/L013827/1] (Multidisciplinary Super Resolution Microscopy Facility).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA031

Elucidation of the Roles of Brown and BRITE fat genes

M. Christian

Warwick Medical School, University of Warwick, Coventry, UK

Adipose tissues constitute a remarkably dynamic organ that is responsive to a range of stimuli from nutrients and hormones to external temperature. Brown and white adipose tissues (BAT and WAT) differ in their appearance and function. Whereas WAT is the primary site for lipid storage, BAT generates heat by oxidation of fatty acids to maintain body temperature. An additional adipocyte termed BRITE (brown-in-white or beige) is brown-like and found in WAT following beta adrenergic activation or cold exposure. White adipocytes contain a single lipid droplet (LD) occupying most of the cytoplasm (unilocular), while brown adipocytes are filled with many smaller LDs (multilocular) and contain many mitochondria. LD-associated proteins regulate the storage and release of lipids from LDs and are more highly expressed in BAT. We determined the differential gene expression profile of BAT and WAT depots and investigated the roles of key brown/BRITE adipocyte-enriched factors in lipid droplet (LD) biology and cell signalling. CIDEA is a marker of BAT and it coats the surface of LDs facilitating their enlargement. We have defined its mechanism of action and the discrete protein regions involved in: LD targeting, dimerization, LD clustering and lipid transfer. Furthermore, we identified an amphipathic helix which through binding of phosphatidic acid is required for LD enlargement. The physiological process of CIDE-dependent LD enlargement

is essential in adipocyte differentiation as well as serving to facilitate the tight coupling of lipolysis and lipogenesis in activated brown fat.

As with CIDEA, the G protein-coupled receptor Gpr120 is more highly expressed in BAT compared to WAT and is upregulated following cold exposure. The selective GPR120 agonist TUG-891 increases fat oxidation and reduces body weight and fat mass in mice. These effects coincide with decreased brown adipocyte lipid content and increased nutrient uptake by BAT. TUG-891 treatment of brown adipocytes in vitro induced O₂ consumption, through GPR120-dependent and -independent mechanisms. TUG-891-activated GPR120 induced intracellular Ca²⁺ release, mitochondrial depolarization, and mitochondrial fission. GPR120 mediates anti-inflammatory effects and cross-talk between signalling pathways can modulate BAT function. Focusing on inflammatory pathways we found that treatment of brown adipocytes with lipopolysaccharide to activate TLR4 receptors profoundly affected adipocyte biology. The treatment stimulated the secretion of a wide range of cytokines and enhanced lipid storage. Analysis of transcriptional changes revealed upregulation of inflammatory mediators and downregulation of brown-fat associated genes coincident with reduced O₂ consumption. The activation of brown adipocytes with the GPR120 agonist TUG-891 is a promising strategy to increase lipid combustion and reduce obesity. Future strategies to activate or repress signaling pathways in BAT hold promise for new obesity treatments.

Schilperoort M, van Dam AD, Hoeke G, Shabalina IG, Okolo A, Hanyaloglu AC, Dib LH, Mol IM, Caengprasath N, Chan YW, Damak S, Miller AR, Coskun T, Shimpukade B, Ulven T, Kooijman S, Rensen PCN, Christian M. The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat. *EMBO Mol Med*. 2018. pii: e8047. doi: 10.15252/emmm.201708047. PMID: 29343498.

Barnea D, Planas-Iglesias J, Gaspar ML, Mohammadyani D, Prasanna S, Dormann D, Han GS, Jesch SA, Carman GM, Kagan V, Parker MG, Ktistakis NT, Dixon AM, Klein-Seetharaman J, Henry S, Christian M. The brown adipocyte protein CIDEA promotes lipid droplet fusion via a phosphatidic acid-binding amphipathic helix. *Elife*. 2015. doi: 10.7554/eLife.07485. PMID: 26609809

Rosell M, Kaforou M, Frontini A, Okolo A, Chan YW, Nikolopoulou E, Millership S, Fenech ME, MacIntyre D, Turner JO, Moore JD, Blackburn E, Gullick WJ, Cinti S, Montana G, Parker MG, Christian M. Brown and white adipose tissues. Intrinsic differences in gene expression and response to cold exposure in mice. *Am J Physiol Endocrinol Metab*. 2014. 306(8):E945-964. PMID: 24549398.

Christian M. Transcriptional fingerprinting of “browning” white fat identifies NRG4 as a novel adipokine. *Adipocyte*. 2014; 4(1):50-54; PMID: 26167402.

Yu J; Zhang S, Cui L, Wang W, Na H; Zhu X, Li L, Xu G, Yang F, Christian M, Liu P. Lipid droplet remodeling and interaction with mitochondria in mouse brown adipose tissue during cold treatment. *Biochim Biophys Acta*. 2015;1853(5):918-928. PMID: 25655664.

BBSRC grants BB/P008879/1 and BB/H020233/1.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA032

The gut hormone secretin triggers a gut-brown fat–brain-axis in the control of food intake

M. Klingenspor^{1,2,3}, Y. Li^{1,2}, K. Schnabl^{1,2}, S. Gabler¹, M. Willershäuser¹, A. Bast-Habersbrunner¹, T. Fromme^{1,2,3} and F. Bolze¹

¹TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany, ²Else Kröner-Fresenius-Centre for Nutritional Medicine, Technical University of Munich, Freising, Germany and ³Institute for Food & Health, Technical University of Munich, Freising, Germany

Brown adipocyte mitochondria, equipped with UCP1, uncouple respiration from ATP synthesis by collapsing proton-motive force and thereby directly dissipate the chemical energy of fatty acids and glucose as heat. This energy wasting mechanism is constitutively suppressed by purine nucleotide binding to UCP1, but activated by free fatty acids. The canonical pathway stimulating brown fat thermogenesis requires activation of beta-adrenergic receptor signaling in brown adipocytes by the release of norepinephrine from sympathetic nerves innervating brown fat. Mimicking this pathway by a single injection of the beta-3-adrenergic receptor ligand CL316,243 not only activates brown fat thermogenesis, but also results in a pronounced inhibition of 24h food intake (1). Conversely, brown fat thermogenesis is activated during a meal (2). The role of meal-associated thermogenesis in brown fat and the underlying molecular mediator has been elusive (3). According to the thermoregulatory feeding hypothesis (4), we propose that brown fat thermogenesis plays a so far unappreciated role in the regulation of food intake (3). Ultradian cycles of brown fat activation may control meal initiation and termination. However, a major contribution of the sympathetic innervation as a mediator of such meal-associated cycles of thermogenesis is unlikely. Only carbohydrate rich meals activate the sympathetic nervous system, but all macronutrients elicit meal-associated thermogenesis. Beta-blockade with propranolol does not affect food intake, and propranolol treatment before a carbohydrate-rich meal does not attenuate meal-associated thermogenesis. Based on these observations, we screened for meal-associated signals derived from the gut that potentially activate oxygen consumption in brown adipocytes (3,5).

Our transcriptome analysis in murine brown fat revealed a number of G_s-protein coupled receptors, of which the secretin receptor showed relatively abundant expression (3,5). In microplate respirometry assays of primary brown adipocytes, configured to quantify UCP1-dependent uncoupled respiration, the gut hormone secretin increased oxygen consumption to a comparable level as the beta-adrenergic receptor agonist isoproterenol. High respiration rates induced by secretin, alike isoproterenol, were dependent on cAMP-PKA mediated activation of lipolysis, and absent in brown adipocytes from *Ucp1*-KO mice. siRNA mediated knockdown of the secretin receptor diminished secretin-induced respiration. Thus, the gut hormone secretin is a novel non-adrenergic activator of UCP1-dependent

thermogenesis in brown adipocytes. In vivo experiments with mice demonstrated that a single injection of secretin increases whole body resting metabolic rate and inhibits food intake in wildtype, but not in *Ucp1*-KO mice. Notably, secretin treatment decreased *AgRP* mRNA, and increased *Pomc* and *Trpv1* mRNA levels in the hypothalamus of wildtype, but not *Ucp1*-KO mice. Notably, the meal-associated rise in brown fat temperature was regularly followed by termination of feeding. Comparing feeding behavior in wildtype and *Ucp1*-KO mice, the latter had fewer, but larger meals. Blocking endogenous secretin with a neutralizing antibody attenuated brown fat thermogenesis during refeeding, and increased food intake of mice. Propranolol pretreatment efficiently attenuated the thermogenic action of the beta-3-adrenergic agonist, CL316,243, but did not inhibit the effects of secretin on brown fat thermogenesis and food intake.

Our results reveal the gut hormone secretin as the endocrine mediator of meal-associated thermogenesis in brown fat. The meal-induced rise of secretin levels in the blood circulation causes direct activation of brown fat. We hypothesize that the resulting rise in brain temperature conveys information on feeding status to the brain and promotes satiation. Thus, secretin triggers a so far unknown endocrine communication axis from the gut via brown fat to the brain which functions to control food intake (3). Pertaining to the role of brown fat as a heater organ in mammalian energy balance, the physiological role of this endocrine axis in the control of energy intake deserves more attention.

Susulic, V. S., Frederick, R. C., Lawitts, J., Tozzo, E., Kahn, B. B., Harper, M. E., Himms-Hagen, J., Flier, J. S., and Lowell, B. B. (1995) Targeted disruption of the beta-3-adrenergic receptor gene. *J Biol Chem* 270, 29483-29492

U Din, M., Saari, T., Raiko, J., Kudomi, N., Maurer, S. F., Laheesmaa, M., Fromme, T., Amri, E. Z., Klingenspor, M., Solin, O., Nuutila, P., and Virtanen, K. A. (2018) Postprandial oxidative metabolism of human brown fat indicates thermogenesis. *Cell Metab* 28, 207-216 e203

Li, Y., Schnabl, K., Gabler, S. M., Willershauser, M., Reber, J., Karlas, A., Laurila, S., Laheesmaa, M., M, U. D., Bast-Habersbrunner, A., Virtanen, K. A., Fromme, T., Bolze, F., O'Farrell, L. S., Alsina-Fernandez, J., Coskun, T., Ntziachristos, V., Nuutila, P., and Klingenspor, M. (2018) Secretin-activated brown fat mediates prandial thermogenesis to induce satiation. *Cell* 175, 1561-1574 e1512

Himms-Hagen, J. (1995) Role of brown adipose tissue thermogenesis in control of thermoregulatory feeding in rats: a new hypothesis that links thermostatic and glucostatic hypotheses for control of food intake. *Proc Soc Exp Biol Med* 208, 159-169

Schnabl, K., Westermeier, J., Li, Y., and Klingenspor, M. (2018) Opposing actions of adrenocorticotrophic hormone and glucocorticoids on UCP1-mediated respiration in brown adipocytes. *Front Physiol* 9, 1931

This work was funded by DFG grants to MK (KL973/11&12 and RTG1482) and the Else Kröner-Fresenius-Stiftung.

Conflict of interest: TUM has filed a patent for these findings.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Circadian rhythm in heart rate – role of intrinsic versus extrinsic factors

M.R. Boyett

Division of Cardiovascular Sciences, University of Manchester, Manchester, UK

It is well known that, in the human and other mammals, the heart rate shows an endogenous circadian rhythm over 24 h and the heart rate is lower during the sleep period than during the awake period, regardless of whether the mammal is diurnal as in the case of the human or nocturnal, for example, in the case of the mouse. This is widely attributed to the autonomic nervous system: an increase in sympathetic tone to support physical activity during the awake period and an increase in vagal tone during the sleep period (Massin *et al.*, 2000). However, there are problems with this interpretation. For example, (i) an increase in heart rate variability at night is cited as evidence of high vagal tone (Massin *et al.*, 2000), but we have argued that heart rate variability cannot be used to measure autonomic tone (Monfredi *et al.*, 2014), (ii) knockout of cardiac autonomic receptors - β -receptors (1, 2 and 3) and the muscarinic M_2 receptor - in the mouse is reported to have no effect on the circadian rhythm in heart rate (Swoap *et al.*, 2008) and (iii) a circadian rhythm in heart rate is still seen in the denervated heart of heart transplant patients (Idema *et al.*, 1994). As an alternative, we have tested the possibility that there is a circadian rhythm in the intrinsic pacemaker activity of the cardiac pacemaker, the sinus node, driven by a local circadian clock in the sinus node (Wang *et al.*, 2016). In the mouse, there was a circadian rhythm in the heart rate *in vivo* in the conscious telemetrized animal, but there was also a circadian rhythm in heart rate of the isolated denervated Langendorff-perfused heart and beating rate of the isolated denervated sinus node. In the sinus node, experiments (qPCR and bioluminescence recordings in mice with a *Per1* luciferase reporter) revealed functioning canonical clock genes, e.g. *Bmal1* and *Per1*. There is, therefore, a functioning circadian clock in the sinus node. In the sinus node, we identified a circadian rhythm in the expression of some key ion channels, most notably the pacemaker channel, HCN4 (mRNA and protein measured by qPCR and western blot). HCN4 is the major isoform responsible for the funny current (I_f) and the density of I_f measured by whole cell patch clamp in isolated sinus node cells showed a prominent circadian rhythm. Block of I_f in the isolated sinus node by 2 mM Cs^+ abolished the circadian rhythm in beating rate of the isolated sinus node. Disruption of the local circadian clock in the sinus node (by cardiac-specific knockout of *Bmal1*) abolished the circadian rhythm of HCN4, I_f density and beating rate of the isolated sinus node. Chromatin immunoprecipitation demonstrated that HCN4 is a potential transcriptional target of *Bmal1* establishing a pathway by which the local clock could regulate pacemaking. In conclusion, there is a circadian rhythm in intrinsic cardiac pacemaking as a result of a local circadian clock in the sinus node that drives rhythmic expression of HCN4. The data reveal a novel regulator of cardiac pacemaking. However, the

role of these intrinsic factors versus extrinsic factors, such as body temperature and even autonomic tone, has still to be determined.

IDEMA, R.N., VAN DEN MEIRACKER, A.H., BALK, A.H., BOS, E., SCHALEKAMP, M.A. & MAN IN'T VELD, A.J. (1994). Decreased circadian blood pressure variation up to three years after heart transplantation. *American Journal of Cardiology* 73, 1006-1009.

MASSIN, M.M., MAEYNS, K., WITHOFS, N., RAVET, F. & GERARD, P. (2000). Circadian rhythm of heart rate and heart rate variability. *Archives of Disease in Childhood* 83, 179-182.

MONFREDI, O., LYASHKOV, A.E., JOHNSEN, A.B., INADA, S., SCHNEIDER, H., WANG, R., NIRMALAN, M., WISLOFF, U., MALTSEV, V.A., LAKATTA, E.G., ZHANG, H. & BOYETT, M.R. (2014). Biophysical characterisation of the under-appreciated and important relationship between heart rate variability and heart rate. *Hypertension* 64, 1334-1343.

SWOAP, S.J., LI, C., WESS, J., PARSONS, A.D., WILLIAMS, T.D. & OVERTON, J.M. (2008). Vagal tone dominates autonomic control of mouse heart rate at thermoneutrality. *American Journal of Physiology-Heart and Circulatory Physiology* 294, H1581-1588.

WANG, Y., D'SOUZA, A., JOHNSEN, A., OLIESLAGERS, S., COX, C., BUCCHI, A., WEGNER, S., GILL, E., CARTWRIGHT, E., WISLOFF, U., MARTINS, P.D.C., DIFRANCESCO, D., DOBRZYNSKI, H., PIGGINS, H. & BOYETT, M. (2016). Circadian rhythm in heart rate is due to an intrinsic circadian clock in the sinus node. *European Heart Journal* 37, 618.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA034

Direct recordings of cardiac sympathetic and vagal parasympathetic nerve activity to clarify the underlying mechanisms of circadian heart rhythm.

C.T. Bussey

Department of Physiology, University of Otago, Dunedin, New Zealand

Like many biological processes, heart rate exhibits a significant cyclical variation over a 24 h period (circadian rhythm). The mechanism of this tight regulation is debated, with the prevailing opinion suggesting that it is controlled by autonomic neural signalling from the brain. However, an alternate mechanism of altered ion channel signalling within the heart's own pacemaker cells has been proposed. Currently, no data exists to directly address whether a circadian rhythm exists in autonomic nervous signalling, preventing any conclusive determination of the mechanism behind heart rate variations. However, we have recently established protocols to undertake specific, direct and concurrent recordings of cardiac sympathetic and vagal parasympathetic nerve activity.

The right cardiac sympathetic nerve can be isolated via a thoracotomy, and the right vagal nerve similarly exposed via a neck incision, in order to place platinum bipolar recording electrodes to directly record autonomic nerve activity. Using male Sprague-Dawley rats (350-450g) nerve activity was assessed at Zeitgeber time (ZT) 3 and ZT15, when previous experiments have shown heart rate differences peak. ZT0 indicates lights-on and ZT12 indicates lights off under a 12 h:12

h light:dark lighting regime. Measures were undertaken under sodium pentobarbital anaesthesia (80 mg/kg) delivered intraperitoneally, maintained with a variable pentobarbital infusion at ~ 0.6 mg/kg/min via the jugular vein, and rats were temperature controlled and mechanically ventilated.

Emerging data suggests that while a subtle shift in the balance between sympathetic and parasympathetic inputs to the heart may contribute to circadian heart rate regulation, autonomic changes are unlikely to account for the significant heart rate cycle. Neither cardiac sympathetic (ZT3: 35.3 ± 10.1 vs. ZT15: 52.3 ± 14.0 Hz, mean $\pm$ SEM, $n=6$, unpaired t-test NS) nor vagal parasympathetic (ZT3: 30.7 ± 10.9 vs ZT15: 26.0 ± 10.1 Hz, mean $\pm$ SEM, $n=8$, unpaired t-test NS) nerve firing rate suggest a primary role for autonomic modulation in circadian heart rate regulation. Currently, the underlying mechanisms of the circadian rhythm in heart rate are debated due a lack of data to conclusively delineate the importance of neural and pacemaker processes. Direct autonomic nerve recordings provide crucial insight into the fundamental mechanisms of heart rate regulation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA035

Effect of disruption of the normal circadian rhythm on the heart

Y. Wang

Medical & Human Sciences, University of Manchester, Manchester, UK

Disruption of the normal circadian rhythm is a problem in modern life. Shift workers, frequent time-zone travellers and patients with sleep disorders are all affected by different degrees of circadian disruption. Many people are also affected by social jet lag (misalignment between biological and social time). Circadian disruption can cause cardiac rhythm disturbances, including sinus node bradyarrhythmia and tachyarrhythmia, impaired heart rate variability, ventricular dysfunction with frequent ectopic beats and even sudden cardiac death. Day time sleep is the common way for such people to restore their sleep, but day time sleep cannot provide the same cardiovascular restorative effect as night time sleep. Excessive daytime sleepiness after night shift is a prodromal symptom of myocardial infarction. Currently, there is little understanding of how circadian disruption affects the heart rhythm and poor guidance for the affected population on how to lower any risk. In this study, the effect of circadian disruption on the pacemaker of the heart, the sinus node, was investigated. In the mouse, circadian disruption was caused using a chronic “jet-lag” protocol. Mice were grouped-housed under a 0700 lights on (zeitgeber time, ZT, 0), 1900 lights off (ZT 12) cycle (Greenwich mean time, GMT, +0 h) for three days, moved to a 2300 lights on, 1100 lights off cycle (GMT+8 h) for another three days, and then moved back to the GMT+0 h time zone for another three days. These cycles were repeated for 30 days.

The ECG was recorded in the anaesthetised mouse under 1.5% isoflurane at 1 L/min flow rate with respiratory rate 80-100 breath/min. Circadian disruption caused a significant prolongation of the RR interval (corresponding to a decrease in the heart rate) and impairment of heart rate variability. The ECG from the anaesthetised mouse showed the occurrence of atrial bigeminy (a normal sinus beat followed by a premature atrial ectopic beat and then pause). The incidence and duration of atrial bigeminy progressively increased during the 30-day circadian disruption protocol: atrial bigeminy was observed in 1 out of 20 mice at Day 0 and 7 out of 20 mice at Day 30, and the duration of runs of atrial bigeminy increased from 2.7 s at Day 0 to 11.2 s at Day 30. Poincaré plots confirmed an increased occurrence of atrial ectopic beats at Day 30. At Day 30, the sinus node was dissected at one of four time points over 24 h and the beating rate was measured. Frequent bigeminy in sinus node beating rate and a dampened circadian rhythm in the beating rate over 24 h. This revealed a circadian rhythm in the intrinsic beating rate of the sinus node. The circadian rhythm in the beating rate was dampened and shifted in the circadian disrupted mice as compared to a control group. In the isolated denervated sinus node, atrial bigeminy was observed in the circadian disrupted group, but not in the control group.

To understand the mechanisms underlying the disturbances in heart rhythm, at Day 30 the pacemaker current, I_f (funny current), was measured in isolated sinus node myocytes at 6 time points over 24 h. There was a circadian rhythm in I_f density, but the circadian rhythm in circadian disrupted mice was dampened and shifted as compared to the control group. The effect of I_f on pacemaking can be estimated by measuring the decrease in beating rate of the isolated sinus node on blocking I_f by 2 mM Cs^+ . The decrease in beating rate on blocking I_f shows a circadian rhythm consistent with the circadian rhythm in I_f . This circadian rhythm was also dampened and shifted in the circadian disrupted mice. In the circadian disrupted mice, the change in beating rate on blocking I_f showed a circadian rhythm with a peak at ZT 6 and a trough at ZT 18 compared to ZT 12 and ZT 0 in the control group. The ion channels, HCN1 and HCN4, are responsible for I_f . At Day 30, HCN1 and HCN4 mRNA were measured at 6 time points over 24 h; both showed a circadian rhythm and, once again, the circadian rhythm was dampened and shifted in the circadian disrupted group. We have previously shown that the circadian rhythm in HCN4 at least is driven by a local circadian clock in the sinus node (Wang *et al.*, 2016). At Day 30, mRNA for two circadian clock genes, *Bmal1* and *Clock*, were measured at the same time points and, as expected, both showed a circadian rhythm and, once again, the rhythms were drastically altered in the circadian disrupted mice. In summary, circadian disruption causes a disruption of the intrinsic circadian clock in the sinus node and we suggest that this is responsible for a disruption in the circadian rhythm in pacemaking ion channels. In turn, this may be responsible for an alteration in the circadian rhythm in heart rate and the occurrence of atrial bigeminy. Although not yet measured, it is possible that analogous changes are occurring throughout the heart and this may explain the occurrence of other cardiac arrhythmias with circadian disruption.

WANG, Y., D'SOUZA, A., JOHNSEN, A., OLIESLAGERS, S., COX, C., BUCCHI, A., WEGNER, S., GILL, E., CARTWRIGHT, E., WISLOFF, U., MARTINS, P.D.C., DIFRANCESCO, D., DOBRZYNSKI, H., PIGGINS, H. & BOYETT, M. (2016). Circadian rhythm in heart rate is due to an intrinsic circadian clock in the sinus node. *European Heart Journal* 37, 618.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA036

Circadian Rhythms in the Heart

M. Young

Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Cardiovascular function exhibits dramatic fluctuations over the course of a normal day. Upon awaking, parameters such as heart rate and blood pressure rise steeply, and remain elevated throughout the day. Moreover, profound time-of-day-dependent rhythms are observed in cardiovascular-relevant organs (such as the heart) at molecular, biochemical, and histological levels. Classically, time-of-day-dependent fluctuations in cardiovascular parameters have been attributed to behavior-induced perturbations in neurohumoral factors, such as sympathetic tone. However, recent human- and animal-based studies suggest that intrinsic timekeeping mechanisms, known as circadian clocks, contribute towards these daily rhythms. Circadian clocks are cell autonomous molecular mechanisms, consisting of a series of positive and negative feedback loops, with a periodicity of approximately 24-hrs. These clocks confer the selective advantage of anticipation, allowing a cell/organ to prepare for a stimulus/stress prior to its onset. Circadian clocks have been identified in essentially all mammalian cells, including cardiomyocytes. Studies utilizing murine models of cardiomyocyte circadian clock genetic disruption suggest that this mechanism temporally regulates numerous biological processes known to be critical for cardiac function. These include transcription, protein turnover (synthesis and degradation), cellular signaling, electrophysiology, and metabolism. Moreover, the cardiomyocyte circadian clock appears to influence the sensitivity of the heart to both physiologic stimuli (e.g., insulin, fatty acids) and pathologic stresses (e.g., ischemia/reperfusion, pro-hypertrophic stimuli). Chronic disruption of this molecular timekeeper precipitates cardiac dysfunction, including increased cardiomyocyte size (hypertrophy), interstitial fibrosis, and contractile impairments. Interestingly, pharmacological strategies designed to target the circadian clock (e.g., small molecule agonists targeting the clock component REV-ERBa/b) have yielded promising beneficial effects in pre-clinical animal models of cardiac disease (e.g., heart failure). Collectively, these studies highlight the importance of the cardiomyocyte circadian clock in maintenance of normal cardiac physiology, and the potential of targeting this molecular mechanism for the future prevention/treatment of heart diseases.

Martino TA & Young ME (2015) *J Biol Rhythms* 30, 183-205

Van Laake LW et al (2018) *Eur Heart J* 39, 2326-2329

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA037

Cell-cell interactions in normal human lung development

E. Rawlins

Gurdon Institute, University of Cambridge, Cambridge, UK

Extensive work on mouse lung morphogenesis has shown that the branching tips of the developing epithelium comprise a multipotent progenitor population. These distal tip progenitors are maintained throughout embryonic development and initially generate bronchiolar-fated and subsequently alveolar-fated progeny. Many of the developmental cues which regulate lung branching, epithelial and mesenchymal differentiation and subsequent maturation have been investigated in the mouse lung. How many of the morphogenetic events and signals are conserved in human lung embryonic development? Can we develop improved models of *in vitro* human lung development that will facilitate drug screening and disease modelling? And gain insights into lung regeneration? To address these questions, we have been studying human embryonic lung development with a focus on the multipotent distal epithelial progenitor cells. Transcriptional analysis revealed broad similarity between the mouse and human tip epithelial populations, but with some surprising differences. To be able to perform functional experiments, we have developed an organoid-based culture system in which we can grow human embryonic distal tip cells isolated from 6-8 week gestation human lungs. We can self-renew these epithelial tips as karyotypically stable, genetically manipulable, organoids for at least 12 months allowing us to expand the scarce starting material. Moreover, we have developed methods for *in vitro* and *in vivo* differentiation of the cells, providing new platforms specifically for the study of human lung development.

Nikolic, M. Z. *et al.* Human embryonic lung epithelial tips are multipotent progenitors that can be expanded *in vitro* as long-term self-renewing organoids. *Elife* 6, doi:10.7554/eLife.26575 (2017).

Medical Research Council, Wellcome Trust

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA038

IPF-relevant cytokine cocktail induces a shift from alveolar to airway-like epithelial cells in an iPSC-derived model of alveolar epithelial progenitor differentiation

E. Schruf¹, V. Schroeder¹, H.Q. Le¹, E.L. Stewart¹, D. Raedel¹, R. Heilker¹, M. Dass¹, M.J. Webster¹, B. Stierstorfer¹, K. Quast¹, M. Frick² and J.P. Garnett¹

¹Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany and

²Institute of General Physiology, University of Ulm, Ulm, Germany

Idiopathic pulmonary fibrosis (IPF) is a fatal disease with no known cure that is characterized by progressive fibrotic remodeling of the lung. While the pathophysiology remains unclear, growing evidence suggests that a pro-fibrotic milieu and dysfunctional epithelial repair likely play key roles in IPF. An emergence of airway epithelial like cells of unknown origin is frequently observed in distorted alveolar regions in IPF, which is often referred to as bronchiolisation. We aimed to investigate if a pro-fibrotic environment could drive aberrant trans-differentiation of alveolar epithelial progenitors towards airway epithelial like cells.

We used an iPSC-derived air-liquid interface model of directed ATII cell differentiation and treated the cells with an IPF-relevant cocktail (IPF-RC), composed of cytokines previously described to be upregulated in IPF patient lungs. Values are means $\pm$ S.E.M., compared by two-tailed unpaired Student's t-test or Mann-Whitney U-test. Characterization by qRT-PCR, RNAseq, IF and TEM confirmed the ATII-like phenotype of the cultures. IPF-RC stimulation during differentiation resulted in significantly reduced expression of SFTPC (mRNA decreased by $79.1 \pm 3.5\%$, $p < 0.0001$, $N=9$) and increased airway related transcript expression, including KRT5, SCGB1A1, MUC5B and BPIFB1. In addition, treatment with IPF-RC resulted in elevated MMP-7 (increased to $151.2 \pm 21.6\%$ of control, $p < 0.05$, $N=3$) and MMP-10 (increased to $245.5 \pm 42.8\%$ of control, $p < 0.01$, $N=3$) concentrations in the culture medium. RNAseq revealed a significant overlap of deregulated transcripts between IPF-RC treated cultures and publicly available IPF patient datasets.

Thus, we have demonstrated using an iPSC-derived model of alveolar epithelial progenitor cell differentiation that an IPF-related mix of cytokines has the potential to impair ATII differentiation by driving a shift towards an airway epithelial like expression signature, as well as induce IPF biomarker secretion, resembling that observed in IPF lungs. Overall these findings indicate that aberrant alveolar epithelial stem cell differentiation could play a role in IPF-associated bronchiolisation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA039

Unlimited expansion of putative distal lung bud tip progenitor cells from human pluripotent stem cells and their ex vivo and in vivo engraftment

Y. Chen^{1,2,3}, H. Liu^{4,5}, N. Dorrello^{6,7}, K. Cunningham⁷, G. Vunjak-Novakovic^{7,4} and H. Snoeck^{4,5,8,9}

¹Medicine, University of Southern California, Los Angeles, CA, USA, ²Hastings Center for Pulmonary Research, University of Southern California, Los Angeles, CA, USA, ³Pulmonary, Critical Care and Sleep Medicine, University of Southern California, Los Angeles, CA, USA, ⁴Medicine, Columbia University Irving Medical Center, New York, NY, USA, ⁵Columbia Center for Human Development, Columbia University Irving Medical Center, New York, NY, USA, ⁶Pediatrics, Columbia University Irving Medical Center, New York, NY, USA, ⁷Biomedical Engineering, Columbia University Irving Medical Center, New York, NY, USA, ⁸Columbia Center for Translational Immunology, Columbia University Irving Medical Center, New York, NY, USA and ⁹Microbiology and Immunology, Columbia University Irving Medical Center, New York, NY, USA

End-stage lung disease is the third leading cause of death in the United States. Lung transplantation is the only definitive treatment to replace lungs in end-stage patients. However, complications are frequent, and there is a severe shortage of organs. Stem cell-based therapy supplying exogenous stem/progenitor cells to help lung repair or ultimately replacing diseased lung tissue have become prime translational goals. One of the major challenges before stem cell-based therapies can be realized is generating sufficient quantities of the appropriate progenitor and mature cells. We have previously shown that three-dimensional branching lung organoids can be generated from human pluripotent stem cells (hPSCs, including embryonic stem cells and induced pluripotent stem cells). Here we show that we can isolate and grow cells from these organoids with a phenotype and expression pattern compatible with human distal lung bud tip progenitor cells (SOX2+SOX9+). These cells, which we call putative distal tip progenitors (pDTPs) could be maintained as a homogenous population, passaged for > 12 months, frozen and thawed. The cells maintained active proliferation and expanded > 90 fold in 2 weeks even at passage 38. *In vitro* expanded pDTPs attach to, flatten and survive for at least 7 days in de-epithelialized rat lungs *ex vivo* and for at least 2 weeks in injured immunodeficient mouse lung *in vivo* after delivery in the airway. Furthermore, they can be differentiated *in vitro* into mature basal cells (NGFR+P63+CD104+CD49f+), the stem cells of airway, and further differentiated into ciliated, goblet, and club cells. The *in vitro* expanded basal cells can engraft and repopulate de-epithelialized mouse tracheas *ex vivo*. The capacity to generate an unlimited number of distal lung bud tip progenitor cells and basal cells from hPSCs provides a potential source of cells for stem cell therapies and regenerative medicine in the lung.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA040

Macrophages and fibroblasts as niches in the adult lung

J. Rock¹ and A. Lechner²

¹Center for Regenerative Medicine, Boston University, Boston, MA, USA and ²University of California, San Francisco, San Francisco, CA, USA

Our underlying hypothesis is that many pathological features of lung disease occur when endogenous capacity of the lung to repair itself following mild to moderate injury is impaired. We use in vivo animal models and in vitro assays to identify putative stem/progenitor cell populations in adult lungs and the micro-environmental cues that modulate their behaviors. We have shown that type 2 alveolar epithelial cells (AEC2) are a major source of new alveolar epithelium in a mouse model of lung growth following pneumonectomy (PNX). To characterize the regenerative niche, we performed single cell RNA sequencing on several cell types following PNX, including CD115+ myeloid cells and Pdgfra+ fibroblasts. We have used in vivo gain and loss of function experiments and in vitro assays to demonstrate that recruited monocytes and alternatively activated macrophages promote the regenerative response post-PNX. We hope that further characterization of the molecular regulation of lung regeneration will identify new cell and molecular therapies to prevent or reverse debilitating pathology in patients with lung disease.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA041

Regulation of the hepatic feeding response by combined action of insulin and glucocorticoid receptor signaling.

L. Grøntved, S. Præstholt, M. Siersbæk, A. Kalvisa and C. Correia

Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark

The liver is an essential organ regulating metabolic homeostasis in response to fluctuations of metabolites induced by daily rhythms of food intake. Homeostasis is maintained by precise dynamic regulation of signaling pathways controlling a wealth of enzymatic reactions regulating lipid, bile acid, xenobiotic, amino acid and glucose metabolism in hepatocytes. Here, precise temporal expression of hepatic enzymes is crucial, where a major part of circadian hepatic protein expression is regulated by precisely timed gene transcription (1). This is controlled by complex interactions between rhythmic endocrine signaling, oscillating metabolites and

intrinsic circadian networks (2). Genetic disruption of the intrinsic circadian clock has profound impact on circadian gene transcription in liver, yet rhythmic gene transcription can be restored by controlled feeding regimens (3–5), likely regulated by putative food-entrainable oscillators (6).

We aim to understand mechanisms controlling circadian hepatic gene expression with a specific focus on molecular cues coming from daily feeding cycles. The experimental approaches are centered on functional genomics technologies seeking to characterize differential transcriptomic and cistromic regulation in response to food intake. We use a mouse model, where C57BL/6 mice are trained to night restricted feeding in a 12-hour day/night cycle (food available from ZT12 to ZT24). At the day of the experiment we collect livers from various pre- and post-prandial timepoints. To measure the effect of feeding we keep a cohort of mice unfed from ZT12-ZT24. Using integrated RNA-seq, DNase-seq and H3K27Ac ChIP-seq analysis we can identify hundreds of circadian regulated genes and enhancers controlled by feeding. Bioinformatic analysis of the putative feeding regulated enhancers suggests a functional role of transcription factors such as GR, FOXO1, CREB and HNF4. Focusing on feeding repressed gene expression shows that post-prandial suppression of enhancer activity is associated with reduced genome-wide GR and FOXO1 occupancy of chromatin correlating with reduced serum corticosterone levels and increased serum insulin levels. Despite substantial pre-prandial co-occupancy of feeding regulated enhancers by GR and FOXO1, selective genetic or pharmacological disruption of post-prandial corticosteroid or insulin signaling demonstrates that these signaling pathways selectively operate specific feeding regulated gene programs. Importantly, combined regulation of these signaling pathways showed to be a major determinant of repressed gene expression in response to feeding (7). Interestingly, genetic disruption of the glucocorticoid receptor in liver also perturbed a subset of genes induced by feeding, including *Gck* important for glucose uptake by the liver. Using primary hepatocytes, we find that glucocorticoid pre-treatment is necessary for subsequent insulin induced *Gck* expression and ChIP shows GR occupancy of two enhancers near the *Gck* gene in liver and primary hepatocytes. This suggest that *Gck* is directly regulated by GR and indicate that glucocorticoids and insulin cooperate to repress and induce post-prandial gene expression in the liver.

Atger F, Gobet C, Marquis J, Martin E, Wang J, Weger B, Lefebvre G, Descombes P, Naef F, Gachon F. Circadian and feeding rhythms differentially affect rhythmic mRNA transcription and translation in mouse liver. *Proc Natl Acad Sci U S A* 2015;112(47):E6579-88.

Panda S. Circadian physiology of metabolism. *Science* 2016;354(6315):1008–1015.

Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc Natl Acad Sci U S A* 2009;106(50):21453–21458.

Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* 2000;14(23):2950–2961.

Chaix A, Lin T, Le HD, Chang MW, Panda S. Time-Restricted Feeding Prevents Obesity and Metabolic Syndrome in Mice Lacking a Circadian Clock. *Cell Metab* 2019;29(2):303–319.e4.

Challet E. Keeping circadian time with hormones. *Diabetes Obes Metab* 2015;17 Suppl 1:76–83.
Kalvisa A, Siersbæk MS, Præstholm SM, Christensen LJL, Nielsen R, Stohr O, Vettorazzi S, Tuckermann J, White M, Mandrup S, Grøntved L. Insulin signaling and reduced glucocorticoid receptor activity attenuate postprandial gene expression in liver. *PLoS Biol* 2018;16(12):e2006249.

Independent Research Fund Denmark and the Novo Nordisk Foundation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA042

Regulation of lipid metabolism by the nuclear receptor PPAR α

S. Kersten

Human Nutrition and Health, Wageningen University, Wageningen, Netherlands

In recent years, insights into the mechanisms underlying the biological effects of fatty acids have improved considerably and have provided the foundation for the emerging concept of fatty acid sensing. Fatty acid sensing can be interpreted as the property of fatty acids to influence biological processes by serving as signalling molecules. An important mechanism of fatty acid sensing is via stimulation of DNA transcription via activation of peroxisome proliferators-activated receptors (PPARs) α , β/δ and γ . Apart from fatty acids and various other lipid species, the PPAR α subtype is activated by a class of chemicals referred to as peroxisome proliferators. One of the tissues that expresses high levels of PPAR α is the liver. Studies in mice have shown that PPAR α serves as the master regulator of hepatic lipid metabolism during fasting. In addition, PPAR α suppresses inflammation and the acute phase response. Comparatively little is known about PPAR α in human liver. Here I will cover the role and regulation of PPAR α in human liver. The main outcomes are: 1) the level of PPARA mRNA expression in human and mouse liver is similar. 2) Expression of PPARA in human liver is reduced in patients with non-alcoholic steatohepatitis or infected with the hepatitis C virus. 3) PPAR α in human liver is able to effectively induce the expression of numerous genes involved in numerous lipid metabolic pathways, including microsomal, peroxisomal and mitochondrial fatty acid oxidation, fatty acid binding and activation, fatty acid elongation and desaturation, synthesis and breakdown of triglycerides and lipid droplets, lipoprotein metabolism, gluconeogenesis, bile acid metabolism, and various other metabolic pathways and genes. 4) PPAR α activation in human liver causes the down-regulation of a large number of genes involved in various immunity-related pathways. 5) Peroxisome proliferators do not promote tumour formation in human liver as opposed to mouse liver because of structural and functional differences between human and mouse PPAR α . 6) In addition to helping to correct dyslipidemia, PPAR α agonists may hold promise as a therapy for patients with cholestatic liver diseases, non-alcoholic fatty liver disease, and/or type 2 diabetes.

In addition, the functional implications of PPAR α activation of specific target genes will be discussed, focusing on the role of PPAR α in the regulation of lipid droplets. Lipid droplets can be found in nearly all cell types and may expand during certain (patho)physiological conditions. The synthesis and breakdown of triglycerides and their deposition in lipid droplets is governed by a diverse set of enzymes and lipid droplet-associated proteins. These proteins serve structural roles in and around lipid droplets and regulate the activity of key lipogenic and lipolytic enzymes. Many lipid droplet-associated proteins, including members of the PLIN family, CIDEA, HILPDA, FITM1, FITM2, and G0S2 are under direct transcriptional control of PPARs. Upregulation of lipid droplet-associated proteins by PPARs provides a mechanism to link uptake of lipids to regulation of lipid storage capacity.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA043

Blood flow distribution between respiratory vs. locomotor muscles during exercise

W. Sheel

University of British Columbia, Vancouver, BC, Canada

Skeletal muscle blood flow and metabolism are tightly coupled during exercise. Under conditions of strenuous whole-body exercise, there are alterations in the sympathetic and parasympathetic branches of the autonomic nervous system which serve to ensure appropriate perfusion of a large and contracting muscle mass and to also maintain arterial blood pressure. Exercise-induced sympathoexcitation can also result in some vasoconstriction in the contracting musculature. Appropriate blood flow and oxygen delivery are required to sustain muscular contraction and locomotion. It is important to recognize that during exercise, the metabolic demands of both locomotor and respiratory muscles need to be considered where the respiratory musculature is required generate sustained and high levels of force to ensure appropriate levels of ventilation to ensure blood gas homeostasis. As such, the muscles of breathing must receive their 'fair share' of cardiac output. It is now well established that performing sustained heavy exercise can result in fatigue in both the limb locomotor and respiratory muscles. In this presentation a summary of the inter-relationships between limb and respiratory muscles during exercise will be made. The case will be made that the evidence to date in humans supports the hypothesis that the high level of sympathetic vasoconstrictor activity emanating from both limb and respiratory muscle metaboreceptors in heavy intensity exercise constrains blood flow and O₂ transport, thereby promoting both diaphragm and locomotor muscle fatigue during whole body exercise at high intensity. Emphasis will be given to newer studies that experimentally manipulate respiratory muscle work to evaluate muscular fatigue and blood flow distribution (1).

Sheel AW, Boushel R and Dempsey JA. Competition for blood flow distribution between respiratory and locomotor muscles: implications for muscle fatigue. *Journal of Applied Physiology* 125(3):820-831, 2018.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA044

Pulmonary vascular dysfunction and exercise intolerance in heart failure

B.J. Taylor¹, B.A. Borlaug², T.P. Olson² and B. Johnson²

¹*School of Biomedical Sciences, University of Leeds, Leeds, UK* and ²*Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN, USA*

Background. Pulmonary hypertension (PH) is a hallmark of heart failure (HF), developing in ~60% of patients with left ventricular systolic dysfunction and ~70% of patients with isolated left ventricular diastolic dysfunction. The pathogenesis of PH in HF is complex, with two distinct subsets of PH due to HF identified: 1) isolated post-capillary PH (IpcPH) and 2) combined pre- and postcapillary PH (CpcPH) (Vachieri *et al.*, 2019). Patients with HF universally complain of exertional intolerance, but the causes may differ between patients with different phenotypes. Exercise introduces a substantial stress to the lungs, where elevations in venous return and cardiac output (Q) increase pulmonary blood volume and blood flow by 50% and 300%, respectively (Flamm *et al.*, 1990). The healthy, highly compliant and low resistance pulmonary vasculature can readily accommodate these marked increases in blood volume and flow with inevitable but relatively small increases in pulmonary vascular pressures. However, this reserve to accommodate increased pulmonary blood flow is likely compromised in HF patients, particularly those with PH, with a consequent impairment in right ventricular reserve and aerobic capacity (Gorter *et al.*, 2018).

Aim. To determine: 1) the impact of pulmonary hypertension on the pulmonary haemodynamic response to exercise in patients with HF; 2) whether the pulmonary haemodynamic response to exercise is different in patients with IpcPH vs. CpcPH; and 3) the relationship between the pulmonary haemodynamic response to exercise and exercise capacity in HF patients.

Methods. Thirty-nine stable HF patients undergoing right-heart catheterisation performed exhaustive incremental exercise (10 W every 3 min starting from 10 W) on a recumbent cycle ergometer. Systolic, diastolic and mean pulmonary arterial pressure (sPAP, dPAP and mPAP), and pulmonary capillary wedge pressure (PCWP) were measured beat-by-beat at end expiration and averaged over 5-10 cardiac cycles. Q was determined via direct Fick. Transpulmonary pressure gradient (TPG; mPAP – PCWP), pulmonary vascular resistance (PVR; TPG/Q), and pulmonary arterial compliance [PAC; stroke volume/(sPAP – dPAP)] were calculated.

Results. *The effect of PH on the haemodynamic response to exercise in HF:* At peak exercise, the ratio mPAP to Q (i.e. unit increase in pressure for a unit increase in blood flow) was greater in HF patients with PH vs. HF patients without

PH (9.7 ± 3.4 vs. 5.2 ± 1.5 mmHg/L/min⁻¹, $P < 0.001$). By contrast, PAC at peak exercise was lower in HF patients with PH compared to HF patients without PH (1.2 ± 0.3 vs. 1.9 ± 0.2 mL/mmHg, $P = 0.004$). Importantly, both a greater mPAP-Q ratio ($r^2 = -0.549$, $P < 0.001$) and a lower absolute PAC ($r^2 = 0.632$, $P < 0.001$) at peak exercise were significantly related to impaired exercise capacity (i.e. lower peak oxygen consumption, $VO_{2\text{peak}}$) across all HF patients. *Effect of PH subtype on the haemodynamic response to exercise in HF:* Post catheterization, patients were classified as having no PH ($n = 11$), lpcPH ($n = 11$), or CpcPH ($n = 17$). At peak exercise, mPAP was greater in CpcPH vs. lpcPH and no PH (57 ± 8 vs. 46 ± 10 and 34 ± 11 mmHg, $P \leq 0.029$). PCWP at peak exercise was greater in CpcPH compared to no PH (30 ± 7 vs. 19 ± 8 mmHg, $P < 0.001$), but similar between CpcPH and lpcPH (30 ± 7 vs. 28 ± 10 mmHg, $P = 0.215$). The absolute increase in mPAP, PCWP and TPG from rest to peak exercise was not different between no PH, lpcPH and CpcPH. By contrast, the slope of the mPAP-Q, PCWP-Q and TPG-Q relationship during exercise was greater in CpcPH vs. lpcPH vs. no PH (mPAP-Q: 7.2 ± 3.2 vs. 4.1 ± 3.9 vs. 2.6 ± 1.7 mmHg/L/min⁻¹; PCWP-Q: 4.2 ± 1.4 vs. 3.3 ± 1.2 vs. 2.9 ± 1.5 mmHg/L/min⁻¹; TPG-Q: 4.3 ± 2.1 vs. 3.2 ± 1.8 vs. 2.6 ± 1.4 mmHg/L/min⁻¹); however, only the differences between no PH and CpcPH were statistically significant. The slope of the mPAP-Q and the TPG-Q relationship in response to exercise was inversely related to $VO_{2\text{peak}}$ across all HF patients (mPAP-Q: $r = -0.603$, $P = 0.02$; TPG-Q: $r = -0.592$, $P < 0.01$).

Conclusion. The pulmonary haemodynamic response to exercise in HF with PH is characterized by an exaggerated rise in pulmonary vascular pressures and limited or no pulmonary vascular reserve, both of which appear to be key determinants of exercise limitation in these patients. This pulmonary vascular limitation to exercise is further worsened by the development of combined pre- and postcapillary PH (CpcPH). Mechanistically, PH likely contributes to exercise intolerance in HF through a substantial increase in right ventricular afterload that restricts right ventricular contractile reserve and forward-flow of blood from the right ventricle to the pulmonary vasculature and systemic circulation.

Flamm SD, Taki J, Moore R, Lewis SF, Keech F, Maltais F, Ahmad M, Callahan R, Dragotakes S, Alpert N & et al. (1990). Redistribution of regional and organ blood volume and effect on cardiac function in relation to upright exercise intensity in healthy human subjects. *Circulation* 81, 1550-1559.

Gorter TM, Obokata M, Reddy YNV, Melenovsky V & Borlaug BA. (2018). Exercise unmasks distinct pathophysiologic features in heart failure with preserved ejection fraction and pulmonary vascular disease. *Eur Heart J* 39, 2825-2835.

Vachieri JL, Tedford RJ, Rosenkranz S, Palazzini M, Lang I, Guazzi M, Coghlan G, Chazova I & De Marco T. (2019). Pulmonary hypertension due to left heart disease. *Eur Respir J* 53.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA045

Respiratory influences on oxygen transport and exercise performance – a cystic fibrosis perspective

Z.L. Saynor

Department of Sport and Exercise Science, University of Portsmouth, Portsmouth, UK

The lungs, airways and respiratory muscles of healthy humans are typically considered 'overbuilt' for the ventilatory and gas-exchange demands imposed by exercise. However, respiratory disease can compromise oxygen (O_2) delivery to the locomotor muscles and cause premature peripheral muscle fatigue during exercise. Cystic fibrosis (CF) is an autosomal recessive condition caused by mutations to the CF transmembrane conductance regulator (CFTR) gene. CF is traditionally associated with chronic respiratory infections and the development of bronchiectasis, resulting in respiratory failure, hypoxaemia and hypoventilation. However, CFTR protein is expressed in multiple organs and, thus, this complex condition can pose significant alterations to central and localised O_2 delivery, as well as the ability to effectively extract and utilise O_2 at the myocyte level, even when pulmonary function is preserved. Understanding the mechanisms that can limit the ability to exercise in people with CF is important, as aerobic exercise function is linked to quality of life, risk of being hospitalised with pulmonary exacerbation and, ultimately, prognosis. Since intense exercise poses considerable challenge to arterial O_2 content and/or blood flow and its distribution to the working skeletal muscle, evaluating the exercise physiology of people with CF has helped us elucidate potential underlying mechanisms by which the disease can limit exercise performance. Experimental manipulations that would enhance O_2 delivery to the locomotor muscle in healthy individuals have also offered insight into the putative mediators of aerobic exercise (dys)function in this population. In healthy individuals, skeletal muscle blood flow and metabolism are tightly coupled. However, aerobic exercise function and, more specifically, skeletal muscle oxidative metabolism can be limited by central and peripheral factors in chronic disease states. This talk will provide a contemporary overview of the central and peripheral contributors of CF pathophysiology that modulate the dynamic balance between O_2 delivery-to-utilisation during exercise. The case will be made that at the mild end of the CF disease spectrum, the human body has the ability to physiologically compensate for some degree of dysfunction, in an effort to maintain the metabolic demands of lower intensity exercise. However, with more severe dysfunction, a pharmacological 'helping hand' may be needed to improve exercise capacity and, thus, quality of life.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA046

The effect of manipulating respiratory muscle loading on respiratory and locomotor muscle blood flow regulation during exercise in health and chronic disease

I. Vogiatzis

Sport, Exercise and Rehabilitation, Northumbria University Newcastle, Newcastle upon Tyne, UK

In healthy individuals exercising heavily the 'dogma' has implicated a significant role of fatiguing respiratory muscle work in the sympathetically mediated vasoconstriction of exercising limb muscle vasculature (1). In the absence of actual respiratory muscle blood flow measurements, it was suggested that the reduction in limb blood flow with fatiguing respiratory muscle work was directed towards the respiratory muscles (2). Use of near-infrared spectroscopy over the left 7th intercostal space and the light-absorbing tracer indocyanine green dye injected intravenously, made it possible for the first time to quantify respiratory muscle blood flow at different levels of isocapnic hyperpnoea in humans (3). Subsequent studies were designed to test the 'dogma' of blood flow redistribution between the respiratory and locomotor muscles during exercise in healthy trained individuals and those with lung disease.

In healthy trained individuals this was achieved by exacerbating exercise-induced diaphragmatic muscle fatigue via reductions in systemic oxygen delivery (hypoxia), whilst simultaneously assessing intercostal and quadriceps muscle blood flow during heavy, near-maximal exercise (4). Despite the greater degree of diaphragmatic fatigue in hypoxia compared to normoxia, blood flow to the intercostal and quadriceps muscles was not significantly different between the two conditions. Nonetheless, diaphragmatic muscle blood flow was not possible to be assessed in these experiments to verify potential blood flow redistribution between the diaphragm and the limb muscles.

In patients with chronic obstructive pulmonary disease (COPD) studies were designed to reduce the work of breathing during exercise via supplementation of oxygen (causing an increase in systemic oxygen delivery through an increase in arterial oxygen content), or heliox (also increasing systemic oxygen delivery via an increase in cardiac output). In these experiments both respiratory and locomotor muscle blood flow and oxygen delivery were simultaneously assessed during exercise (5). None of the respiratory muscle unloading strategies (oxygen or heliox administration) was associated with a decrease in respiratory (intercostal and abdominal) muscle blood flow compared with exercise in room air, whilst both ergogenic strategies significantly increased quadriceps, and respiratory (both intercostal and abdominal) muscle oxygen delivery (5). Similarly to the experiments in trained athletes, diaphragmatic muscle blood flow was not assessed.

Different approaches to unload the respiratory muscles via proportional assist ventilation strategies have been associated with improved leg muscle oxygenation in COPD (6) and chronic heart failure (CHF) patients (7). However, actual respiratory and/or leg muscle blood flow measurements were not available in these studies. In this presentation the case will be made that the available evidence is still inconclusive to support the hypothesis of blood flow redistribution between the respiratory and locomotor muscles as the basis for improved exercise capacity under conditions of respiratory muscle unloading in patients with COPD or CHF. Emphasis will be given to studies that reduce the work of breathing via pharmacological and non-pharmacological interventions in patients with COPD and CHF and the effect of these interventions on patients exercise tolerance and peripheral muscle oxygen availability.

Sheel AW, Derchak PA, Morgan BJ, Pegelow DF, Jacques AJ, Dempsey JA. Fatiguing inspiratory muscle work causes reflex reduction in resting leg blood flow in humans. *J Physiol*. 2001 Nov 15;537(Pt 1):277-89.

Dempsey JA, Romer L, Rodman J, Miller J, Smith C. Consequences of exercise-induced respiratory muscle work. *Respir Physiol Neurobiol*. 2006 Apr 28;151(2-3):242-50. Review.

Guenette JA, Vogiatzis I, Zakynthinos S, Athanasopoulos D, Koskolou M, Golemati S, Vasilopoulou M, Wagner HE, Roussos C, Wagner PD, Boushel R. Human respiratory muscle blood flow measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol*. 2008 Apr;104(4):1202-10.

Vogiatzis I, Athanasopoulos D, Boushel R, Guenette JA, Koskolou M, Vasilopoulou M, Wagner H, Roussos C, Wagner PD, Zakynthinos S. Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists. *J Physiol*. 2008 Nov 15;586(22):5575-87.

Louvaris Z, Vogiatzis I, Aliverti A, Habazettl H, Wagner H, Wagner P, Zakynthinos S. Blood flow does not redistribute from respiratory to leg muscles during exercise breathing heliox or oxygen in COPD. *J Appl Physiol*. 2014 Aug 1;117(3):267-76.

Borghi-Silva A, Oliveira CC, Carrascosa C, Maia J, Berton DC, Queiroga F Jr, Ferreira EM, Almeida DR, Nery LE, Neder JA. Respiratory muscle unloading improves leg muscle oxygenation during exercise in patients with COPD. *Thorax*. 2008 Oct;63(10):910-5.

Borghi-Silva A, Carrascosa C, Oliveira CC, Barroco AC, Berton DC, Vilaça D, Lira-Filho EB, Ribeiro D, Nery LE, Neder JA. Effects of respiratory muscle unloading on leg muscle oxygenation and blood volume during high-intensity exercise in chronic heart failure. *Am J Physiol Heart Circ Physiol*. 2008 Jun;294(6):H2465-72.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA047

cBIN1: from T-tubule Folds to Dyad Organization, Microparticles and Clinical Utility

T. Hong

Cedars-Sinai Medical Center, Los Angeles, CA, USA

Bridging integrator 1 (BIN1) is a membrane curvature protein which is richly spliced. We have identified a cardiac muscle splice variant (BIN1+13+17) now named cBIN1¹. In the past decade, it has been found that cBIN1 is responsible for calcium channel trafficking to cardiac transverse-tubules (T-tubules)² as well as organizing microfolds of cardiac T-tubule membrane¹. The folds affect the local flow of ions¹ and help arrange cardiac dyads between L-type calcium channels and ryanodine receptors³. In heart failure, cBIN1 is transcriptionally decreased^{4,5}, affecting T-tubule architecture, and negatively impacting calcium transients as well as cardiac function. Most recently, we found that normalization of cBIN1 in failing hearts can maintain microdomain structure, thereby preserving or even rescuing cardiac function. On the other hand, cBIN1-microfolds are externally released from normal cardiomyocytes⁶. Using electron microscopy imaging with immunogold labeling, we found that cardiac origin cBIN1 exists in extracellular membrane vesicles known as microparticles. In human plasma, cBIN1 detection by an enzyme-linked immunosorbent assay (ELISA), is decreased in humans with heart failure, a condition with reduced cardiac muscle cBIN1. The clinical utility is significant. A cBIN1 Score (CS) based on plasma cBIN1 levels was studied in patients with heart failure. In our first cohort of heart failure with preserved ejection fraction, CS outperforms current biomarkers for the diagnosis of heart failure, and also can accurately predict future hospitalization in heart failure patients⁷. The data indicate that myocardial cBIN1 not only can serve as a target for new heart failure therapy development, but also is continuously released into blood. The blood-based CS can therefore serve as a clinical biomarker of cardiomyocyte remodeling, aiding in the management of heart failure patients.

1. Hong T *et al.* (2014). *Nat Med* 20, 624-632.
2. Hong T *et al.* (2010). *PLoS Biol* 8, e1000312.
3. Fu Y *et al.* (2016). *Circulation*. 133, 388-397.
4. Hong T *et al.* (2012). *Heart Rhythm* 9, 812-820.
5. Caldwell JL *et al.* (2014). *Circ Res* 115, 986-996.
6. Xu B *et al.* (2017). *PLoS Biol* 15, e2002354.
7. Nikolova AP *et al.* (2018). *JAMA Cardiol* 3, 1206-1210.

The continuous success of this work is due to the contribution from all the coauthors and collaborators, as well as the funding support from agencies including National Institute of Health, American Heart Association, and Department of Defense.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA048

Disordered yet functional atrial t-tubules following recovery from heart failure.

J. Caldwell, J. Clarke, C. Pinali, D. Eisner, A. Trafford and K. Dibb

University of Manchester, Manchester, UK

Transverse (T)-tubules are vital for the synchronous rise of systolic calcium. Heart failure (HF) results in atrial t-tubule loss and dyssynchronous calcium release. In the ventricle, recovery from HF is associated with t-tubule restoration and normalisation of systolic calcium. The mechanisms that control atrial t-tubules however, remain largely unknown, thus we aim to determine; i) if atrial t-tubule loss in HF can be recovered, ii) the effect of atrial t-tubule restoration on systolic calcium, iii) proteins involved in t-tubule recovery.

Isoflurane anaesthetized (2–4% v/v in oxygen) sheep were instrumented with a pacemaker and pacing lead. Post-operative analgesia (meloxicam 0.5 mg/kg) and antibiotics (enrofloxacin 2.5 mg/kg) were provided for 48 hours. HF was induced by ventricular pacing at 210 bpm and, in a subset of animals pacing was terminated and the sheep allowed to recover for 5 weeks. Atrial myocytes were isolated and tissue samples taken from control, HF and recovered sheep following euthanasia with pentobarbitone (200 mg/kg iv; n=9 per group). T-tubule density was assessed using di-4-ANEPPS and intracellular calcium measured by confocal microscopy of Fluo-8AM loaded cells. To determine the involvement of specific proteins in t-tubule recovery, vectors encoding t-tubule proteins were transiently expressed in neonatal rat ventricular myocytes (NRVM). Data presented as mean $\pm$ S.E.M and statistical significance taken as $p < 0.05$, determined using mixed model analysis.

Atrial t-tubules were lost in HF (reduced by $-81 \pm 5.6\%$, $p < 0.001$) and the initial rise of systolic calcium was restricted to the surface sarcolemma. Cessation of tachypacing resulted in recovery of cardiac function and recovery of atrial tubule density to control values. The organization of recovered tubules was altered however, with recovered tubules being predominately longitudinally orientated, longer and more branched. Despite structural changes, the recovered tubules were functional; calcium was firstly released along the tubules, followed by propagation to the rest of the cell. Expression of t-tubule associated proteins BIN1, Tcap and MTM1 was decreased in heart failure ($-24.8 \pm 8.6\%$, $-49.4 \pm 9.6\%$; $-24.5 \pm 7.9\%$ respectively; $p < 0.05$), with both Tcap and MTM1 fully restored to control levels following recovery from HF ($p < 0.01$). BIN1 trended towards recovery with expression being no different to control. 48hrs transfection with BIN1 in NRVM led to the development of tubules, the structure of which was altered by coexpression with MTM1 and Tcap (n=7 litters).

Atrial t-tubules are lost in a sheep model of HF; this is associated with dyssynchronous rise of systolic calcium. T-tubule proteins BIN1, MTM1 and Tcap also decrease

during HF. Recovery of HF leads to upregulation of these proteins and restoration of atrial tubules, which are once again the main site for the rise of systolic calcium. Furthermore our data suggest Tcap and MTM1 play a role in shaping BIN1 induced tubule structures in NRVMs.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA049

Microdomain signaling around ryanodine receptors in cardiac disease

E. Dries

Cardiovascular Sciences, KU Leuven, Leuven, Belgium

Abnormal cellular Ca^{2+} handling and excitation-contraction coupling are associated with reduced contractile function and life-threatening arrhythmias in heart failure (HF). One of the mechanisms contributing to this process is aberrant Ca^{2+} release through ryanodine receptors (RyRs).

In HF and myocardial infarction (MI), the loss of sarcoplasmic reticulum Ca^{2+} through 'leaky' RyRs is due to post-translational modifications of these receptors, which lead to alterations in RyR Ca^{2+} sensitivity and open probability. Cellular remodeling and alterations in microdomain signaling can further augment spontaneous Ca^{2+} release through 'leaky' RyRs, resulting in contractile dysfunction and arrhythmia.

We further investigated how local signaling pathways involving reactive oxygen species (ROS), Ca^{2+} /calmodulin-dependent kinase II (CaMKII) and nitric oxide (NO) modulate different subsets of RyRs (i.e. coupled and non-coupled RyRs) in healthy and diseased hearts. We studied RyR organization and function in healthy, myocardial infarction (MI) and human HF cardiomyocytes. Our data show a specific microdomain-dependent modulation of dyadic RyRs in the healthy heart, which is subject to remodeling with disease. In MI and HF, altered microdomain modulation of RyRs enhances spontaneous Ca^{2+} release from non-dyadic sites, dependent on CaMKII activation and mitochondrial ROS production. This specific modulation may generate a substrate for arrhythmia that appears to be responsive to selective pharmacological inhibition.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA050

Understanding Dyadic Plasticity in Cardiomyocytes

W.E. Louch

Institute for Experimental Medical Research, University of Oslo, Oslo, Norway

Contraction of the heart is reliant on the shortening of individual cardiomyocytes, which is triggered by Ca^{2+} release from the sarcoplasmic reticulum (SR). This Ca^{2+} release process is initiated at sub-cellular structures called dyads, where L-type Ca^{2+} channels within t-tubules face Ryanodine Receptors (RyRs) in the SR. Despite the fundamental role of dyads in triggering the heartbeat, their precise functional arrangement remains unclear. In the present work, emerging techniques for 3D super-resolution imaging¹ and electron microscopy have been employed to reveal the nanoscale arrangement of key dyadic proteins and membranes. This work has shown that dyadic structure and function are highly malleable. Indeed, during development, dyads are formed gradually, with progressive assembly of both t-tubules and SR and precise trafficking of L-type Ca^{2+} channels and RyRs.² During diseases such as heart failure, dyads are broken down with a reversion to an immature phenotype, including both disorganization of t-tubules and dispersion of RyRs which reduce the efficiency of Ca^{2+} -induced Ca^{2+} release.^{2,3} What signals control this dyadic plasticity? We have observed that the physical stress placed on the myocardial wall is a key regulator of t-tubule structure. Importantly, the relationship between t-tubule density and wall stress is bell-shaped, with the healthy adult heart positioned on the rising phase of this curve. Thus, modest increases in workload result in compensatory remodeling of cardiomyocytes, as cells grow new t-tubules to augment Ca^{2+} cycling and contractility. However, markedly elevated wall stress, as occurs in the dilated failing heart, triggers decompensatory remodeling associated with reduced expression of the dyadic anchor junctophilin-2 and t-tubule loss.⁴ On the adjacent dyadic membrane, new data indicate that plasticity of RyR localization and function are regulated by phosphorylation of the channel. We have specifically observed that prolonged activation of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) results in dispersion of RyR clusters, resulting in distributed channel arrangements and inefficient triggering of Ca^{2+} release reminiscent of heart failure. Indeed, CaMKII inhibition was observed to rescue RyR organization and function in failing cells, suggesting that hyperactivity of this kinase has complex pathological actions in this disease. Taken together, our data demonstrate that plasticity of dyadic structure/function is afforded by malleability of t-tubule and SR organization and the Ca^{2+} -handling proteins within these membranes. Understanding the signals that regulate this plasticity presents an important therapeutic opportunity to strengthen the heartbeat in cardiac patients.

Shen X, van den Brink J, Hou F, Colli D, Le C, Kolstad TR, MacQuaide N, Carlson CR, Kekenus-Huskey PM, Edwards AG, Soeller C, and Louch WE. 3D dSTORM Imaging Reveals Novel Detail of Ryanodine Receptor Localization in Rat Cardiac Myocytes (2019) *J Physiol*, 597:399-418.

Lipsett DB, Frisk M, Aronsen JM, Nordén ES, Buonarati OR, Cataliotti A, Hell JW, Sjaastad I, Christensen G, and Louch WE. Cardiomyocyte substructure reverts to an immature phenotype during heart failure (2019) *J Physiol*, 597:1833-1853.

Kolstad TR, van den Brink J, MacQuaide N, Lunde PK, Frisk M, Aronsen JM, Norden ES, Cataliotti S, Sjaastad I, Sejersted OM, Edwards AG, Lines GT, and Louch WE. Ryanodine receptor dispersion disrupts Ca^{2+} release in failing cardiac myocytes (2018) *eLife*, 7:e39427.

Frisk M, Ruud, M, Espe EKS, Aronsen JM, Røe ÅT, Zhang, L, Norseng PA, Sejersted OM, Christensen G, Sjaastad I, and Louch WE. Elevated ventricular wall stress disrupts cardiomyocyte t-tubule structure and calcium homeostasis (2016) *Cardiovasc Res*, 112:443-451.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA051

Molecular basis for dynamic change in the bicarbonate permeability of anion channels

M. LEE

Department of Pharmacology and Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul, Korea (the Republic of)

Anion channels are an essential component of the cells for keeping them alive and mediating their diverse functions. Although many anions can permeate anion channels, chloride and bicarbonate are the two most abundant anions that can be the charge carrier of anion channels in animal cells. Increasing evidence indicates that bicarbonate permeation through anion channel is involved in many basic biologic processes ranging from epithelial fluid secretion to neuronal excitation. Interestingly, ion selectivity of anion channels is not fixed and can be dynamically altered under specific cellular conditions. For example, the N-terminal region of WNK1 increases the bicarbonate permeability ($P_{\text{HCO}_3}/P_{\text{Cl}}$) of CFTR anion channel via a $[\text{Cl}^-]_i$ -dependent physical association and that defects in this process predispose to CFTR mutation-related disorders. By employing an integrated study of combined molecular, physiological, structural, and mathematical approaches, we provide evidence that electric permittivity and channel pore diameter are cardinal features, which determine the ion selectivity of anion channels. Pore size change affects the bicarbonate permeability of anion channels by altering energy barriers of size-exclusion and ion dehydration of bicarbonate permeation. These findings provide key insights into the mechanism of how the ion permeation and selectivity of anion channels are determined.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Anion channels of the Anoctamin/TMEM16 family as modulators of Ca^{2+} signalling and mucin secretion

R. Benedetto, I. Cabrita, J. Ousingsawat, R. Schreiber and K. Kunzelmann

Department of Physiology, University of Regensburg, Regensburg, Germany

Anoctamins, or TMEM16 proteins (TMEM16A to K), are a family of Ca^{2+} -activated chloride channels (CaCC) and phospholipid scramblases. TMEM16A was identified in 2008 as the *bona fide* CaCC in airways and a number of epithelial tissues. It has been shown to tether the cortical endoplasmic reticulum (ER) to the plasma membrane.

The presence of TMEM16A and TMEM16F augments Ca^{2+} store release upon stimulation of G-protein coupled receptors (GPCRs) detected by PI-G-CaMP2, whereas others TMEM16 proteins (D, H, I) largely reduced Ca^{2+} signals. We found a pronounced inhibition of ATP-induced Ca^{2+} signals after siRNA knockdown of endogenous TMEM16F and TMEM16K and, inversely, a strong augmentation of Ca^{2+} signals in TMEM16F and TMEM16K overexpressing cells.

The role of TMEM16A in intestinal epithelial cells has been previously shown (Schreiber, R. *et al*, 2014). In addition we observed that lack of expression of TMEM16K in TMEM16K^{-/-} mice, inhibits Ca^{2+} release (n=6, males and females).

Recent results suggested that TMEM16A and TMEM16F control membrane exocytosis by setting Ca^{2+} levels near the plasma membrane, and/or by controlling the intracellular Cl^- concentration. Generation of TMEM16A tissue specific knock out mice in both airways and intestine allowed us to study the role of TMEM16A in exocytosis *in vivo*. Airway-ciliated and intestinal epithelial-specific knockout of TMEM16A leads to accumulation of mucus in airway secretory cells and intestinal goblet cells, respectively. Acute ATP-induced mucus secretion in airway club cells is inhibited when TMEM16A is knocked out in ciliated cells, possibly as a result of compromised release of prosecretory cytokines (n=16, males and females). In contrast, cholinergic stimulated mucus secretion was independent of TMEM16A (n=23, males and females). The data demonstrate a previously unrecognized role of TMEM16A for membrane exocytosis and describe a novel, ATP-driven pathway for intestinal mucus secretion (Benedetto *et al*, 2019).

Benedetto, R., Cabrita, I., Schreiber, R., & Kunzelmann, K. (2018). *TMEM16A is indispensable for basal mucus secretion in airways and intestine*. *The FASEB Journal*, fj.201801333RRR. doi:10.1096/fj.201801333rrr

Cabrita, I., Benedetto, R., Fonseca, A., Wanitchakool, P., Sirianant, L., Skryabin, B. V., ... Kunzelmann, K. (2017). *Differential effects of anoctamins on intracellular calcium signals*. *The FASEB Journal*, 31(5), 2123–2134. doi:10.1096/fj.201600797rr

This work was supported by Cystic Fibrosis Trust (SRC 013), Personalized Therapies for All (DFG KU756/14-1), and Gilead Stiftung.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA053

The Volume Regulated Anion Channel: from molecular identification to physiological roles and 3D structure.

S. Bertelli^{1,2}, R. Barbieri², P. Gavazzo² and M. Pusch²

¹SISSA, Trieste, Italy and ²Istituto di Biofisica, Genova, Italy

The Volume regulated anion channel (VRAC) is a key player in the cell-intrinsic regulatory processes which tend to restore the original cell volume upon osmotic challenges. This is achieved mediating the swelling-induced release of halide ions and organic osmolytes that subsequently drive water efflux through the membrane. VRAC is ubiquitously found in all vertebrate cells. In addition to its role in volume regulation, a large amount of literature suggests its importance in various other physiological and pathophysiological processes including cell proliferation, migration and apoptosis¹.

The molecular entity of VRAC was unknown until 2014, when two groups, using similar genome-wide siRNA screens, independently identified the leucine-rich repeat-containing protein 8A (LRRC8A) as an essential VRAC subunit^{2,3}. Voss et al.² further found that VRAC is a heteromer composed of closely related members of the LRRC8 family (LRRC8A-E), containing the obligatory LRRC8A subunit and at least one among the LRRC8B-E subunits.

Recently, 4 Cryo-EM structures revealed the general architecture of VRAC by determining the structure of the homomeric LRRC8A complexes⁴⁻⁷. While homomeric LRRC8A channels are not physiologically relevant, the general architecture is likely conserved for heteromeric channels.

The proteins are hexamers, in which four transmembrane domains of each subunit contribute to the formation of a relatively loosely packed, membrane imbedded, ion pore, followed by a cytoplasmic leucine rich repeat domain, that, as predicted from its sequence, consists of 16 structural repeats, each containing a β -strand followed by an α -helix. In three of the four published structures, the LRR domains assembled as trimers of dimer, while the fourth structure exhibited hexameric symmetry.

One of the leading hypotheses to explain how the channel responds to a hypotonic stimulus is the sensing of a low intracellular ionic strength¹. However, the mechanism of activation is not fully resolved and little is known about the actual, physiologically relevant stoichiometry.

Indeed, it is known that the subunit composition of VRACs influences the biophysical properties of their anion transport such as single-channel conductance, outward rectification, and depolarization-dependent inactivation kinetics¹. Also ROS sensitivity is strictly subunit specific; using fluorescently tagged LRRC8 proteins in oocytes it was found that LRRC8A-LRRC8E heteromeric channels are dramatically

activated by oxidation of intracellular cysteines, whereas LRRC8A-LRRC8C and LRRC8A-LRRC8D heteromers are inhibited¹.

The aim of this project is to provide deeper insight into the mechanism of activation of VRAC with particular emphasis on the role of the intracellular LRR domains. To address this major point, first we investigated the role of oxidation, transfecting different subunits in a cell line in which all 5 LRRC8 genes had been knocked out. Interestingly we observed that oxidation through the application of Chloramine T was sufficient to activate LRRC8A-LRRC8E heteromeric channels even in the absence of a pre-volume stimulation.

To elucidate if oxidation acts directly on the LRRC8 channel-forming proteins or on regulatory factors we exploited two positively charged molecules known to react with cysteine residues and blocks their further oxidation. The inclusion of MTSET(+) or MTSES(-) in the pipette solution led to a dramatic increase of LRRC8A/LRRC8E mediated currents, followed by a complete inactivation of the channel. The subsequent application of hypotonic solution and Chloramine-T failed to reactivate the channels, suggesting that activation of LRRC8A/LRRC8E by chloramine-T is due to the oxidation of intracellular cysteines.

Our results show that LRRC8 channels are directly modulated by oxidation in a subunit-dependent manner, confirming what was previously observed in oocytes. We are currently systematically mutating all intracellular cysteines of LRRC8E hoping to obtain insight on the specific residues responsible of the different effects mediated by ROS respectively on LRRC8C and LRRC8E.

Okada Y. *et al.* (2019). Cell Volume-Activated and Volume-Correlated Anion Channels in Mammalian Cells: Their Biophysical, Molecular, and Pharmacological Properties. *Pharmacol Rev*; 71:49–88.

Voss FK *et al.* (2014). Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. *Science*; 344: 634-8.

Qiu Z *et al.* (2014). SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel. *Cell*; 157: 447-58.

Deneka D *et al.* (2018). Structure of a volume-regulated anion channel of the LRRC8 family. *Nature*; 558: 254-9.

Kasuya G *et al.* (2018). Cryo-EM structures of the human volume-regulated anion channel LRRC8. *Nature structural & molecular biology*; 25: 797-804.

Kern DM *et al.* (2019). Cryo-EM structures of the DCPIB-inhibited volume-regulated anion channel LRRC8A in lipid nanodiscs. *eLife*; 8.

Kefauver JM *et al.* (2018). Structure of the human volume regulated anion channel. *eLife*; 7.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA054

The Consequences of Altered Wnt Signaling in the Cystic Fibrosis IntestineR.A. Woode², A. Strubberg², N. Walker¹, J. Liu¹ and L.L. Clarke^{2,1}

¹Dalton Cardiovascular Research Center; Department of Biomedical Sciences, University of Missouri, Columbia, MO, USA and ²Biomedical Sciences, University of Missouri, Columbia, MO, USA

Introduction: Cystic fibrosis (CF) patients and CF mouse models have an increased risk for gastrointestinal tumors^{1,2}. CF mice show augmented intestinal proliferation of unknown etiology, which together with an altered intestinal environment may predispose to neoplastic development. Here, we examined the involvement of canonical Wnt/ β -catenin signaling in proliferation of the intestinal stem cell (ISC) population of the CF mouse intestine. Directed cell migration, cell polarity and tight junction (TJ) remodeling must keep pace with increased epithelial proliferation. CF patients and CF mouse models also exhibit increased intestinal barrier permeability which likely contributes to other manifestations of CF disease^{3,4}. Therefore, we also examined the involvement of non-canonical Wnt signaling, in particular components of the planar cell polarity (PCP) pathway, in TJ remodeling in the CF mouse intestine. A unifying hypothesis to altered Wnt signaling in the CF crypt epithelium is based on our previous finding that CF crypts have sustained increases in intracellular pH (pH_i) due to a coincident intracellular retention of Cl^- and HCO_3^- resulting from loss of the CF transmembrane conductance regulator CFTR anion conductance. An alkaline pH_i increases the plasma membrane association of disheveled (Dvl), a mutual transducer of both canonical and non-canonical Wnt signaling, thereby facilitating initial Wnt signaling cascades.

Methods: In these studies we employed transgenic mouse models crossed to wild-type (WT) and Cfr knockout (CF) sex-matched littermate mice to estimate membrane association of Dvl2-dGFP in cultured organoids by confocal fluorescence microscopy. All animal experiments were performed on an approved IACUC protocol. Tight junction permeability was assessed by 3K fluorescent dextran in spherical intestinal enteroids (2-3 days post-passage). Immunofluorescence (IF) was used to investigate localization of the Rho GTPase Cdc42 in WT and CF enteroids. Matched Caco2 cells with WT CFTR and CFTR knocked out (KO) by gene-editing, which show a hyperproliferative phenotype, were investigated for changes in GTP-bound (active) form of Cdc42.

Results: Increased intestinal proliferation in CF mice in vivo was recapitulated in CF enteroids relative to WT. Studies further showed increased proliferation of Lgr5+ ISCs in CF mice crossed to Lgr5-eGFP reporter mice as compared to WT. CF enteroids expressing Dvl2-eGFP in a Dvl2 KO background and membrane dTomato showed increased membrane association of Dvl2 beginning in crypt-base columnar cells (CBC) and extending as well as amplifying in the apical region of crypt cells of the transit-amplifying zone (TAZ) as compared to WT. The increased membrane association of Dvl2-eGFP in CF enteroid crypts was reversed by treatment with

sphingosine, a cation membrane lipid, and by two-day exposure to pH 6.4 medium, which also decreased active b-catenin, relative to untreated CF enteroids. IF showed expression of the PCP Wnt receptor Frizzled 6 and increased TJ localization of the PCP pathway effector Cdc42. Western blots indicated reduced expression of the TJ scaffold protein ZO-1 and unchanged to slightly increased protein expression of Cdc42. Tight junction transcriptome measured by RT-qPCR Taqman array plates showed decreases in ZO-1, partitioning defective polarity proteins Par 3 and Par6b and, paradoxically, decreased Cdc42 mRNA relative to WT. Spherical CF enteroids showed increased “leak” permeability to 3K-dextran, as compared to WT. ELISA based studies of CF Caco2 cells indicating increased GTP-bound Cdc42 and a proliferative phenotype by exhibiting multiple filopodia, a hallmark of Cdc42 activity.

Conclusions: The above findings support the hypothesis that loss of CFTR in the proliferative compartment of intestinal crypts results in an alkaline pH_i and increased membrane association of the Wnt transducer Dvl. The consequences may include a) facilitation of Wnt/ β -catenin signaling and a contribution to ISC hyperproliferation in the CF intestine, thereby increasing the potential for DNA replication errors; and b) facilitation of non-canonical Wnt PCP signaling involving increased activation of the Rho GTPase Cdc42 and increased “leak” permeability of the CF intestine, thereby providing a propensity for barrier dysfunction in the CF intestine. Together, altered Wnt signaling resulting from dysregulated pH_i in the proliferative crypt compartment may contribute to the increased risk of intestinal cancer and impaired intestinal barrier permeability in CF patients.

Neglia JP, Fitzsimmons SC, Maisonneuve P, et al. The risk of cancer among patients with cystic fibrosis. *N.Engl.J.Med.* 1995;332:494-499.

Than BLN, Linnekamp JF, Starr TK, et al. CFTR is a tumor suppressor gene in murine and human intestinal cancer. *Oncogene* 2016.

Hallberg K, Grzegorzczak A, Larson G, et al. Intestinal permeability in cystic fibrosis in relation to genotype. *Journal of Pediatric Gastroenterology & Nutrition* 1997;25:290-5.

De Lisle RC. Disrupted tight junctions in the small intestine of cystic fibrosis mice. *Cell Tissue Res* 2014;355:131-42.

Supported by NIDDK and CF Foundation. Gene-edited Caco2 cells were a kind gift from Dr. Mitchell Drumm, Case Western Reserve University.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA055

Chemotherapy-Induced Pathophysiology of Muscle Spindles in RatsT.C. Cope^{1,2}, S.N. Housley¹, P. Nardelli¹, D.I. Carrasco¹ and R.K. Powers³

¹*School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA,*
²*Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA and* ³*Department of Physiology and Biophysics, University of Washington, Seattle, WA, USA*

Descriptions of proprioceptive-like disorders that persist years after chemotherapy in cancer survivors led us to preclinical investigation of muscle spindles. We find a distinctive defect in mechanosensory encoding by muscle spindles in rats five weeks following treatment with a human-scaled regime of oxaliplatin (OX), a standard treatment in human cancer patients. Specifically, group Ia muscle spindle afferents, though responsive to dynamic muscle stretch, lose the capacity to sustain firing during static stretch ^[1,2]. Our evidence suggests that defective firing originates in the spindle primary ending, but not as a result of structural degeneration typically held responsible for chronic sensory disorders induced by chemotherapy. An alternate possibility of functional impairment in the generation of receptor and/or action potentials gains support in finding that acute administration of drugs (riluzole and phenytoin) that block slowly inactivating inward Na currents replicate OX-induced firing defects. From these observations, we hypothesize that chronic sensory encoding defects caused by chemotherapy result from channelopathy in the primary sensory endings of muscle spindles.

We address our hypothesis using the OX-treatment protocol described above. In attempt to advance translational relevance, we incorporate a rat model of colon cancer that best reproduces features of human colon cancer ^[3]. Results from treated rats receiving OX treatment were compared with control rats of the same species that were cancer free, untreated, and aged matched. Three kinds of studies were performed on rats in each group. Electrophysiological data were obtained in terminal experiments performed *in vivo* on isoflurane-anesthetized rats ^[2]. Action potentials evoked by repeated trials of ramp-hold-release stretch of triceps surae muscles were sampled from single group Ia axons in dorsal roots. The two remaining experiment types were performed on tissues harvested from rats. First, broad transcriptional profiling of fresh dorsal root ganglia was performed using whole genome microarrays (log₂ expression). Second, sections of fixed muscles were exposed to monoclonal antibodies and imaged by fluorescent confocal microscopy to reveal relative expression levels (mean fluorescent intensity) and distribution of relevant channels ^[4].

Spindle afferents sampled from treated rats exhibited the defective firing response profile described above. Means and standard deviations of firing during the hold phase of stretch in OX-treated (n=10 spindles) vs. control (n=11) groups,

respectively, were 69 ± 16 pps vs 93 ± 22 pps ($p < 0.001$, ANOVA) for average firing rate and 723 ± 365 ms vs 991 ± 20 ms ($p < 0.02$, ANOVA) for firing duration. Similarity with our earlier findings demonstrates generalizability to both sexes, two rat species, and the presence of cancer.

Immunohistochemical examination of muscle spindles began with NaV1.6. Loss of this ion channel and the persistent inward current it generates [5] might have been responsible for the defect in sustained firing as in other neuron types [6]. This explanation was excluded, however, in finding robust labeling for NaV1.6 in the primary sensory terminals and heminodes of muscle spindles comparable to controls. All attempts at replication were successful (26 OX-treated spindles and 32 control spindles, 5 rats each).

Consideration of other possible explanations opened us to the complexity of enumerable biophysical mechanisms in muscle spindles that might impair firing. To confront this problem, we turned to transcription profiling, which enabled comprehensive examination of gene expression for all ion channels. Data confirmed equivocal NaV1.6 expression levels between control (6.83 ± 0.32) and treated groups (6.20 ± 0.31 $p > 0.05$, linear fixed effects model (LM)). Among the results, expression of the KCNC3 exhibited distinct downregulation in OX treated relative to control rats, respectively, 6.25 ± 0.14 vs 7.07 ± 0.06 ($p < 0.001$, LM). Downstream expression of KCNC3's protein product, Kv3.3, revealed for the first time in control muscle spindles (88.4 ± 53 , $n = 12$ spindles) was also decreased ($p < 0.0001$, ANOVA) after OX treatment (41.3 ± 30 , $n = 9$ spindles). Decreased Kv3.3 expression is noteworthy for its capacity to impair high frequency repetitive firing [7]. Moreover, this finding supports our hypothesis and provides the first demonstration of channelopathy in muscle spindle after OX treatment.

Results of these ongoing studies suggest new ideas relevant to treating sensory disorders with chemotherapy. Nonetheless, we are far from identifying the molecular mechanism(s) of defective firing. The complexity the muscle spindle's molecular composition draws attention to the need for comprehensive biophysical models to determine how these ion channels determine transduction and encoding by the primary sensory terminal.

Bullinger KL, Nardelli P, Wang Q, Rich MM, Cope TC. Oxaliplatin neurotoxicity of sensory transduction in rat proprioceptors. 2011 Aug; 106(2):704-9

Vincent JA, Wiczerzak KB, Gabriel HM, Nardelli P, Rich MM, Cope TC. A novel Path to Chronic Proprioceptive Disability with Oxaliplatin: Distortion of Sensory Encoding. *Neurobio Dis.* 2016 Nov;95:54-65

Irving AA, Yoshimi K, Hart ML, Parker T, Clipson L, Ford MR, Kuramotor T, Dove WF, Amos-Landgraf JM. The utility of Apc-mutant rats in modeling human colon cancer. *Dis. Model Mech.* 2014 Nov;7(11):1215-25

Carrasco DI, Vincent JA, Cope TC. Distribution of TTX-sensitive voltage-gated sodium channels in primary sensory endings of mammalian muscle spindles. *J. Neurophysiol.* 2017 Apr;117(4):1690-1701

Chen Y, Yu FH, Sharp EM, Beacham D, Scheuer, and Catterall WA. Functional properties and differential neuromodulation of Na(v)1.6 channels. *Mol Cell Neurosci* 2008 Aug;38(4): 607-15.

Raman IM, Springer LK, Meisler MH, Bean BP. Altered subthreshold sodium currents and disrupted firing patterns in Purkinje neurons of Scn8a mutant mice. 1997 Oct;19(4):881-91
Zhang Y and Kaczmarek LK. Kv3.3. potassium channels and spinocerebellar ataxia. *J. Physiol.* 2016 Aug;594(16):4677-84.

NIH, NCI R01 CA221363-01; NIH, NICHD R01 HD090642

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA056

Altered muscle spindle function in mouse models of muscular dystrophy

B. Watkins¹, L. Gerwin¹, S. Rossmann¹, C. Haupt¹, H. Brinkmeier², R. Bittner³ and S. Kröger¹

¹LMU Munich, Munich, Germany, ²University Medicine Greifswald, Karlsburg, Germany and ³Medical University of Vienna, Vienna, Austria

Muscular dystrophies comprise a heterogeneous group of neuromuscular diseases characterized by progressive degeneration and weakness of skeletal muscles. We investigated the hypothesis that an altered proprioceptive feedback contributes to the instable gait and frequent falls in patients with muscular dystrophy. Proprioceptive information is generated by muscle spindles, complex sensory organs found in almost every muscle that detect changes in muscle length and the speed of muscle stretching. We analyzed muscle spindle function and morphology in wildtype-, as well as in dystrophin-, utrophin- and dysferlin-deficient mice. To obtain muscle tissue for immunohistochemistry, mice were deeply anaesthetized using ketamine and xylazine. After transcardial perfusion with 4% paraformaldehyde, the muscle was dissected, sectioned in a cryostat, stained with the appropriate antibodies and analyzed using confocal microscopy. Immunofluorescence staining demonstrated the presence of all three proteins in the central region of muscle spindles from wildtype- and their absence in spindles from the respective mutant mice. Dystrophin was concentrated in the sarcolemma of intrafusal fibers but excluded from the contact region with the sensory neuron. In dystrophin-deficient mice, dystrophin was undetectable but utrophin expression was severely upregulated, potentially compensating the absence of dystrophin. The total number and the overall morphology of muscle spindles in soleus muscles of dystrophin-deficient, Dysf^{-/-} and utrophin-deficient mice appeared unchanged, demonstrating that intrafusal muscle fibers are less sensitive to degeneration compared to extrafusal muscle fibers. Proprioceptive sensory neuron responses to stretch were assayed using an isolated muscle-nerve preparation from the EDL muscle. To this end, mice were sacrificed by cervical dislocation and the EDL muscle together with the deep peroneal branch of the sciatic nerve were dissected and placed in a tissue bath containing oxygenated

artificial cerebrospinal fluid. Extracellular recordings during ramp-and-hold stretches as well as during sinusoidal vibrations revealed that dystrophin-deficient and Dysf^{-/-} mice have an increased resting discharge and a lower action potential firing rate during sinusoidal vibrations particularly at low frequencies. Utrophin-deficient mice reacted to ramp-and-hold stretches and sinusoidal vibrations similar to wildtype mice.

These results demonstrate that dystrophin and dysferlin are required for normal muscle spindle function and suggest that an impaired proprioceptive feedback might contribute to the instable gait and the frequent falls in patients with muscular dystrophy. The increase in the resting discharge results in an elevated general muscle tone, which might add to the degeneration of extrafusal fibers in muscular dystrophy patients.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA057

Diet induced obesity alters muscle spindle afferent mechanosensation and Hoffmann's Reflex excitability in adult mice of both sexes

K.A. Wilkinson

San Jose State University, San Jose, CA, USA

Obesity is associated with many co-morbidities, including impaired balance and an increased risk of falling. While the increase in weight and change in center of mass contribute to balance instability, there is growing evidence that changes in sensory function also occur during obesity. Due to the importance of muscle spindle afferent sensory information to proprioception and motor control, we tested the hypothesis that diet induced obesity could alter both muscle spindle afferent mechanosensation and motor neuron excitability to Group Ia muscle spindle afferent sensory input. For all experiments C57BL/6 mice of both sexes were fed either a control (CON; 10% kcal fat, Diet D12450J, Research Diets) or a high fat diet (HFD; 60% kcal fat, Diet D12492) for 5-15 weeks starting 4 weeks after birth. To determine whether muscle spindle afferent passive mechanosensation was affected by diet induced obesity, we used an *in vitro* extensor digitorum longus muscle-nerve preparation. We tested the response of individual muscle spindle afferents to ramp and hold stretch and sinusoidal vibration (CON n=10M & 11F; HFD n=12M & 11F). High fat feeding for 10 weeks led to decreased firing rates during all points of stretch measured compared to control in mice of both sexes (highest firing rate during ramp, 0.5 s and 3.5 s into hold phase of stretch; ANOVA, $p < 0.001$ for all 3 measures). Muscle spindle afferent dynamic mechanosensation was moderately more affected in male mice, where muscle spindle afferents in high fat fed animals were less likely to entrain to vibration than controls (logistic regression, $p = 0.01$; Elahi et al., 2018). The changes in muscle

spindle afferent mechanosensation that we observed could potentially contribute to balance instability during obesity if not compensated with increased gamma motor neuron tone and/or reflex excitability. To determine whether processing of muscle spindle afferent sensory information was also altered by diet induced obesity, we measured the anesthetized Hoffmann's or H-reflex (125 mg/kg ketamine, 12.5 mg/kg xylazine), the electrical analog of the muscle stretch reflex. Mice of both sexes were tested after 5, 10, or 15 weeks on either a CON or HFD (n=9-15/group). We used three quantitative measures of H-reflex excitability: (1) H-reflex latency; (2) the percentage of motor neurons recruited from electrical stimulation of Group Ia muscle spindle afferents (Hmax/Mmax); and (3) rate-dependent depression (RDD), the decrease in H-reflex amplitude to high frequency stimulation (20 stimuli at 5 Hz). A HFD did not significantly alter H latency (GLS, $p=0.16$) or Hmax/Mmax ratios (GLS, $p=0.05$), but RDD was significantly lower in HFD compared to CON groups (GLS, $p<0.001$). Interestingly, HFD males exhibited lower RDD than controls only after 5 and 10 weeks of feeding, but females showed progressive decreases in RDD that were only significant at 10 and 15 weeks on the HFD. These results suggest that high-fat feeding increases H-reflex excitability, but that there is a sex difference in the progression of these changes. Ongoing work is focused on understanding the underlying mechanism(s) of the changes we observed during high fat feeding and whether these changes alter muscle stretch reflex strength and/or balance. Future studies should investigate whether similar changes to the muscle proprioceptor system occur in humans and contribute to balance instability in individuals with obesity.

Elahi, LS, *et al.* (2018) *PloS one* 13.5: e0196832.

This work was supported by NIH 1SC3GM127195 and California State University Program in Education and Research in Biotechnology New Investigator and Research Development Grants.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA058

Shifted vascular optimization: The emergence of a new arteriolar behaviour with chronic metabolic disease.

J. Frisbee

Medical Biophysics, University of Western Ontario, London, ON, Canada

Preceding years of dedicated investigation on linkages between vascular (dys) function under conditions of elevated cardiovascular disease risk and tissue/organ performance have produced results and insights that frequently suffer from limited correlation and causation. Reaching out from this challenge, it was proposed that this may reflect a "level of resolution" argument and that altered

hemodynamic behavior in vascular networks could be a stronger predictor of functional outcomes than higher resolution measures. Using this approach, we have determined that an attractor that describes the spatial and temporal shift in perfusion distribution at successive arteriolar bifurcations within the skeletal muscle is a strong predictor of functional outcomes within animals and provides novel insight into fundamental mechanistic contributors to altered patterns of intra-muscular perfusion. This lecture will focus on the applicability and utility of the attractor in models of cardiovascular and metabolic disease risk of increasing severity. We will also discuss the utility of the attractor in terms of understanding the effectiveness of aggressive interventions for reversing established vasculopathy and perfusion impairments.

Supported by NIH, AHA, CIHR and NSERC.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA059

Perivascular adipose tissue – an immune cell metropolis

S. Withers^{1,2}, R. Forman², S. Saxton², W. Agace³, C. Lawrence², M. Svensson-Frej³, K. Else², S. Cruickshank² and A. Heagerty²

¹University of Salford, Greater Manchester, UK, ²University of Manchester, Manchester, UK and ³Lund University, Lund, Sweden

The perivascular adipose tissue environment plays a key role in regulating its function. How immune populations residing in adipose tissue communicate with other cells has significant implications in health and disease. We have shown that healthy perivascular adipose tissue has a relaxant effect which this is lost in obesity. In obesity, adipocytes become enlarged, hypoxic and inflamed. Immune cell profiles residing in perivascular adipose tissue become dysregulated; our data show decreased eosinophil numbers compared with control. Therefore, we further investigated the role of eosinophils in mediating normal perivascular adipose tissue function.

We used wire myography to assess vascular contractility of mesenteric blood vessels (~200 micrometer diameter). Animals deficient in eosinophils demonstrated a loss of the relaxant effect of perivascular adipose tissue and had increased mean arterial pressure and blood glucose levels, similar to obese mice. This effect was independent of other immune cells. Reconstitution of eosinophils restored perivascular adipose tissue function and physiological measures. Reconstituting eosinophils derived from knockout models demonstrated that the loss of PVAT function was due to reduced bioavailability of adiponectin and adipocyte-derived nitric oxide. This effect was mediated via β_3 adrenoceptors activation by catecholamines. We further identified eosinophils as a novel source of these mediators.

In summary, we demonstrate that eosinophils are important regulators of healthy perivascular adipose tissue function. Our findings identify a novel therapeutic avenue for the treatment of obesity and type 2 diabetes.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

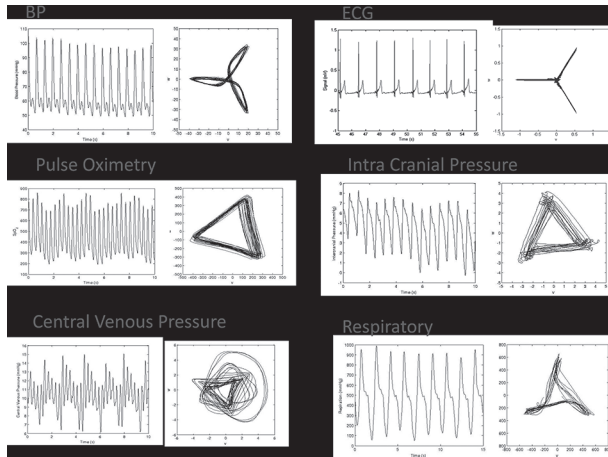
SA060

Extracting new information from old waveforms: Attractor reconstruction where maths meets medicine

M. Nandi

Pharmacology; Drug Discovery Group, King's College London, London, UK

Periodic physiological waveform data such as blood pressure and ECG are routinely sampled between 100-1000Hz in preclinical research and in the clinical setting. However, much of the data is disregarded, such that the detail contained within the shape of the waveform is usually ignored and focus tends to reside in only reporting minimum, maximum and rate values. We have developed a novel mathematical method to quantify previously undetectable changes in the cardiovascular system. Our method applies a non-biased approach, taking all of the raw waveform data and plotting it three dimensionally in phase space (using Takens' Embedding Theorem). This generates an 'attractor' which dynamically changes as it tracks through the raw time series data. The 3D attractor is then viewed down one line, reducing it to 2 dimensions. Density is then added to represent variability. Dynamic changes in the size, shape, density and other measures of the attractor, reflect subtle changes in the waveform shape and variability and hence physiological changes of the system. This method provides a unique approach for quantifying the dynamic changes of the entire waveform increasing the sensitivity of detecting perturbations within the physiological system, which are otherwise undetectable using conventional analysis. In particular, we are interested in ascertaining whether this approach can provide earlier evidence of ensuing clinical deterioration in a patient, facilitating more timely diagnosis and disease management. In the setting of critical care, this could be lifesaving.



Attractor reconstruction analysis of 6 periodic physiological waveforms from humans: ^aBlood pressure (BP); ^bElectrocardiogram (ECG); ^aPulse Oximetry; ^aIntra Cranial Pressure; ^aCentral Venous Pressure and ^aRespiratory pressure. As the shape of input signal/waveform differs, so each generated attractor is unique and distinct. The attractor tracks through the data over time. Changes in the shape and dimensions of the attractor reflect perturbations in the upstroke and down stroke of the input waveform. Waveform data obtained from: ^a <http://bsp.pdx.edu> and ^b Physionet.

Physiol Meas. 2018 Oct 30;39(10):104008. doi: 10.1088/1361-6579/aae46a.

A novel method to quantify arterial pulse waveform morphology: attractor reconstruction for physiologists and clinicians.

Nandi M¹, Venton J, Aston PJ.

Physiol Meas. 2018 Mar 1;39(2):024001. doi: 10.1088/1361-6579/aaa93d.

Beyond HRV: attractor reconstruction using the entire cardiovascular waveform data for novel feature extraction.

Aston PJ¹, Christie MI, Huang YH, Nandi M.

Beyond HRV: Analysis of ECG Signals Using Attractor Reconstruction

<http://www.cinc.org/archives/2017/pdf/091-096.pdf>

Prof Philip Aston

Dr Jenny Venton

Ms Jane Lyle

Dr Esther Bonet Luz

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

SA061

Multi-scale, multi-domain analysis of microvascular flow dynamicsA.J. Chipperfield¹, G.F. Clough² and M. Thanaj¹¹Bioengineering Science, University of Southampton, Southampton, UK and ²Faculty of Medicine, University of Southampton, Southampton, UK

An adequate delivery of blood flow through a vascular network, commensurate with the metabolic demands of the tissue, is dependent on neural, humoral and local vaso-mechanisms that determine vascular tone and thus temporal and spatial flow patterns within the microvascular network. As alteration or attenuation of these flow patterns may be a contributor to disease risk, quantitative *in vivo* information on the temporal behaviour and spatial distribution is necessary to the understanding of network functionality and adaptivity with stressors or disease. Time and frequency domain analysis of Laser Doppler (LD) flowmetry signals has been extensively used to describe and characterise microvascular flows. Spectral analysis of the frequency components of LD signals has been shown to reflect the influence of the spontaneous, rhythmic oscillations of both local (endothelial, neurogenic and myogenic) and systemic origin. Variation in amplitude and spectral content have been associated with microvascular function decline in metabolic and CVD¹. However, time and frequency domain analysis alone have proved insufficient for the consistent interpretation of microvascular function.

Recently, methods to assess regularity and randomness, have been applied to LD signals. Chaotic network attractor analysis has shown a decline in adaptability of network flow in rodent models of metabolic and CVD² and Lempel-Ziv (LZ) microvascular perfusion complexity reduced in a primate model of diabetes³. In previous work, we have shown that an altered spatial heterogeneity and temporal stability of network perfusion is associated with a decrease in both LZ complexity and sample entropy under differing haemodynamic states in healthy humans. Complexity and sample entropy were dependent on the time-scale of the perfusion measurement⁴.

Time, frequency and complexity domain analysis of microvascular blood flow can provide robust parameters that provide a better understanding of the relationship between microvascular perfusion and CVD risk and account, to some degree, for the temporal scale of their origin in terms of the local and systemic physiological activity. The synchronicity of rhythms in the modulators of skin blood flux contributes to the complexity of microvascular blood flow and this reduces with CVD risk as flow becomes more homogenous and predictable. These multiple domain analyses and findings provide a platform from which to investigate microvascular impairment in the skin.

Clough GF, Kuliga KZ and Chipperfield AJ. (2016) Flow motion dynamics of microvascular blood flow and oxygenation—Evidence of adaptive changes in obesity and type 2 diabetes mellitus/insulin resistance. *Microcirculation*, 24 doi: 10.1111/micc.12331.

Frisbee JC, Goodwill AG, et al. (2016) Microvascular perfusion heterogeneity contributes to peripheral vascular disease in metabolic syndrome. *J Physiol*, 594: 2233-43.

Tigno XT, Hansen BC, et al. (2011) Vasomotion becomes less random as diabetes progresses in monkeys. *Microcirculation*, 18: 429-439.

Thanaj M, Chipperfield AJ, Clough GF (2018). Analysis of microvascular blood flow and oxygenation: Discrimination between two haemodynamic steady states using nonlinear measures and multiscale analysis. *Computers in Biology and Medicine*, 102, 157-167.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C001

Effect of a Cardiotoxic Pollutant-Phenanthrene on the Cardiac Function of Brown Trout (*Salmo trutta*)

M.O. Ainerua^{1,2}, J. Tinwell¹, S.N. Kompella¹, E. Sørhus³, K. white⁴, B. vanDongen⁴ and H. Shiels¹

¹Cardiovascular Division, University of manchester, Manchester, UK, ²Animal and Environmental Biology, University of Benin, Benin City, Nigeria, ³Institute of marine Research, Bergen, Norway and ⁴School of Earth Atmospheric and Environmental Sciences, University of Manchester, Manchester, UK

Phenanthrene (Phe) is a three-ringed polyaromatic hydrocarbon which is formed from incomplete combustion of hydrocarbons and is also a component of crude oil. Previous studies have shown Phe to be cardiotoxic to marine fishes. This study investigated and show significant cardiotoxic effects of Phe across multiple levels of cardiac organization in the brown trout, a sentinel freshwater species. Exposure of whole heart (fish mass 332.4±29.6g; n=10; mean heart mass 0.61±0.05; n=10) with 15 µM Phe showed significant prolongation of ~8.6 % and ~13.2 % for the QT interval (Control - 349±10; Phe - 379±9 ms) and the monophasic action potential (MAP) duration (Control - 386±15; Phe - 437±24 ms) respectively ($p < 0.05$; 2-way ANOVA) at 0.5 Hz, recorded using Langendorff heart perfusion and contact electrodes. Phe (15 µM) also significantly decreased the force of contraction by approximately 21 % in isolated ventricular muscle strip preparations (Control - 109±8; Phe - 80±9; $p < 0.05$; 2-way ANOVA) in a force-frequency trial. This suggests that Phe is able to reduce ventricular contracting force by altering cellular calcium cycling. This finding was supported by a significant reduction of ~39 % in the cellular calcium transient following same Phe concentration (15 µM) exposure in isolated ventricular myocytes assessed as mean change in peak fluorescence divided by baseline fluorescence ($\Delta F/F_0$) (Control - 0.18 ± 0.02; Phe - 0.11 ± 0.01 $p < 0.05$; one-way ANOVA). Single cell voltage clamp revealed a Phe-dependent reduction of ~38 % in the L-type Ca^{2+} current (I_{CaL}) (Control - 6.8 ± 0.4 pA/pF; Phe - 4.2 ± 0.3 pA/pF; $p < 0.01$; unpaired t-test) which accounts, at least in part, for the reduced Ca^{2+} transient and force. The prolongation of MAPD and QT interval suggest that Phe could also play a role in the inhibition of repolarizing K^+ currents which was confirmed by a significant 70 % reduction (Control - 3.3 ± 0.4 pA/pF; Phe - 1.0 ± 0.3 pA/pF; $p < 0.01$; unpaired t-test) in the delayed rectifier K^+ current (I_{Kr}) following 10 µM Phe exposure. Using a dose-response curve the IC_{50} for Phe on I_{Kr} was 7.2 ± 0.7 µM. Together our data indicate that Phe is cardiotoxic to freshwater salmonids and operates via similar mechanisms to those identified for marine teleosts.

We appreciate the Nigerian petroleum technology development Funds (PTDF) for providing support as part of a PhD studentship

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C002

TREK1 regulates K⁺ efflux during LPS-induced inflammasome activation

C. Waters¹, C. Immanuel^{2,1}, B. Teng³, B. Dong¹, E. Gordon¹, K. Parthasarathi³, S. Cormier⁵, E. Fitzpatrick⁶ and A. Schwingshackl⁴

¹Physiology, University Of Kentucky, Lexington, KY, USA, ²Pediatrics, University of Tennessee Health Science Center, Memphis, TN, USA, ³Physiology, University of Tennessee Health Science Center, Memphis, TN, USA, ⁴Pediatrics, UCLA, Los Angeles, CA, USA, ⁵Biological Sciences, Louisiana State University, Baton Rouge, LA, USA and ⁶Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, TN, USA

Bacterial and viral infections in the lungs promote an adaptive immunity response involving inflammasome formation and activation in macrophages. The most severe infections can lead to acute respiratory distress syndrome (ARDS), a devastating illness with high mortality and few therapeutic options. Inflammasome signaling involves both a priming stimulus to assemble the components and a second activation signal that causes cleavage and release of pro-inflammatory mediators such as IL-1 β . The activation step is dependent upon a decrease in intracellular K⁺, but the identity of the efflux channel is unclear. In this study we investigated the potential role of a two-pore potassium channel, TREK1, in inflammasome signaling. Mice that were deficient in TREK1 expression (TREK1^{-/-}) exhibited decreased lung injury compared with wild type (wt) mice when exposed to aerosolized LPS, a potent inflammasome stimulus. To evaluate inflammasome signaling, we used bone marrow-derived macrophages (BMDMs) and alveolar macrophages (AMs) exposed to LPS for priming followed by ATP for activation. Expression of NLRP3 increased in BMDMs from wt mice following LPS priming, and this was diminished in TREK1^{-/-} BMDMs and in wt BMDMs treated with a specific TREK1 inhibitor (Spadin). AMs from TREK1^{-/-} secreted significantly less IL-1 β compared with wt AMs following LPS+ATP. Intracellular K⁺ (measured using Asante green as an indicator) was increased in TREK1^{-/-} compared with wt BMDMs following LPS treatment. Wt BMDMs treated with Spadin exhibited intracellular K⁺ similar to untreated wt, while the TREK1 activator BL1249 promoted decreased K⁺. When cells were subsequently treated with ATP, there was a rapid decrease in intracellular K⁺ in wt BMDMs that was diminished by TREK1 inhibition. There was a much larger decline in intracellular K⁺ in TREK1^{-/-} BMDMs following ATP, but the concentration remained higher than that in wt BMDMs. These results suggest that TREK1 regulates NLRP3 inflammasome priming and activation through mechanisms involving K⁺ efflux.

We gratefully acknowledge the technical support of Charlean Luellen, James Tatum, and Joseph Kennedy. This work was supported by NIH grants HL123540 and HL131526.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C003

Anti-hypertensive properties and mechanisms of action of the extract and fractions from *Persea americana* Mill. leaf in rats

J.A. Badejo

Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria

Hypertension is a chronic cardiovascular disease characterised by persistent elevated arterial blood pressure. Current therapies are expensive and linked with many adverse effects, hence the need for alternative remedies. *Persea americana* leaf is used in ethnomedicine to treat hypertension, with limited scientific justification. This study evaluated the antihypertensive properties and mechanisms of action of fractions from the leaf of *P. americana* in rats.

Persea americana methanol leaf extract (PAM) was partitioned with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. Thirty noradrenaline-induced hypertensive Wistar rats were treated i.v as follows (n=5): control (normal saline; 0.6mL/kg), PAM (100mg/kg), PAM fractions (100mg/kg); *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The PAM ethyl acetate fraction (PEtOAc) was fractionated on silica gel using column chromatography. Four pooled Column Fractions of Ethyl Acetate: (CFEt1, CFEt2, CFEt3 and CFEt4) were evaluated intravenously at 25mg/kg for antihypertensive activity in noradrenaline- or *N*^G-nitro-L-arginine methyl-ester (L-NAME)-induced hypertension in rats. Mean Arterial Pressure (MAP) was used as index for antihypertensive activity. The effect of PAM (100 mg/mL) and CFEt3 (25 mg/mL) were also tested on noradrenaline-induced contraction of thoracic aortic rings (n=6). The Angiotensin Converting Enzyme (ACE) inhibition by PAM, CFEt3 and captopril (100µg/mL) was also investigated. Values are means ± S.E.M., compared by One-Way Analysis of Variance at $p < 0.05$. The constituents of CFEt3 were identified by GC-MS.

Results showed PEtOAc as the most active column fraction of PAM. Percentage reductions in noradrenaline-induced raised MAP were 31.6%, 32.0% and 32.3% for PAM (100mg/kg), PEtOAc (100mg/kg), and CFEt3 (25 mg/kg) respectively. Only PAM and CFEt3 caused 21.2% and 23.3% reductions respectively in L-NAME-induced raised MAP. Additionally, PAM (100 mg/kg) and CFEt3 compared to control significantly ($p < 0.05$) reduced noradrenaline contraction of endothelium-intact (74.4% and 65.0%) and endothelium-denuded (36.6% and 27.8%) aortic rings respectively. The percentage *in vitro* inhibition of ACE by PAM, CFEt3 and Captopril were 68.1 ± 1.9 , 78.7 ± 2.9 and 59.6 ± 5.0 respectively. GC-MS analysis revealed 11-Tetradecyn-1-ol acetate (16.6%) and 14-methyl-(Z)-8-Hexadecenal (16.5%) as major components of CFEt3.

The results suggested that *Persea americana* leaf methanol extract and its ethyl acetate column fraction 3 possess *in vitro* relaxant and *in vivo* antihypertensive

activities in rats probably mediated through inhibition of vascular alpha adrenoceptors and angiotensin converting enzyme.

Keywords: *Persea americana*, Angiotensin Converting Enzyme, Mean Arterial Pressure

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C004

Inhibition of voltage-gated Na⁺ currents by eleclazine in adult rat atrial and ventricular myocytes

R.E. Caves^{1,4}, H. Cheng^{1,3}, S.C. Choisy¹, B. Clennell¹, C. McNiff¹, B. Mann¹, J.T. Milnes², J.C. Hancox¹ and A.F. James¹

¹School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, UK, ²Xention Ltd, Cambridge, UK, ³School of Medical Sciences, University of Manchester, Manchester, UK and ⁴Biosciences, Aston University, Birmingham, UK

Atrial fibrillation (AF), the most prevalent clinical arrhythmia, is associated with increased mortality and morbidity. Voltage-gated Na⁺ channel blockers are highly effective in cardioversion and maintenance of sinus rhythm in patients with early onset AF but carry a significant risk of lethal ventricular tachyarrhythmias (Camm, 2012; Lafuente-Lafuente *et al.*, 2015). It has been suggested that atrial-ventricular differences in voltage-gated Na⁺ currents may be exploited for atrial-selective anti-arrhythmic drug action for the suppression of AF without risk of ventricular side-effects (Hancox *et al.*, 2016). Eleclazine (GS-6615) is a putative anti-arrhythmic drug, selective for the late component of the Na⁺ current, that has been shown to be safe and well-tolerated in patients and has properties similar to the prototypical atrial-selective Na⁺ channel blocker, ranolazine (Caves *et al.*, 2017; El-Bizri *et al.*, 2018). The present study investigated atrial-ventricular differences in voltage-gated Na⁺ currents and their inhibition by eleclazine. Procedures were approved by local ethics committee and performed in accordance with UK legislation. Atrial and ventricular myocytes were isolated from adult male Wistar rat hearts. The fast and late components of voltage-gated Na⁺ currents (respectively, I_{Na} & I_{NaL}) were recorded at room temperature (~22 °C) using whole-cell patch clamp recording techniques. I_{Na} was recorded using symmetrical external and internal [Na⁺] (5 mM). I_{NaL} was recorded in separate experiments using an external [Na⁺] of 70 mM (internal [Na⁺] = 5 mM). I_{NaL} was activated by superfusion with the sea anemone toxin, ATX-II (3 nM). The half-maximal voltages of activation (-47.0±1.4 mV, $n=10$) and inactivation (-94.3±0.5 mV, $n=11$) of atrial I_{Na} were more negative than the corresponding values for ventricular I_{Na} (respectively, -41.5±1.3 mV, $n=10$, $P<0.05$ and -87.2±0.4 mV, $n=11$, $P<0.0001$; Student's *t*-test). There was no difference between cell types in the maximally activated Na⁺ conductance density. There was no difference between atrial and ventricular myocytes in the eleclazine inhibition of I_{NaL} (IC_{50} s ~200 nM for both) in the presence of ATX-II. Eleclazine

(10 μM) inhibited I_{Na} in atrial and ventricular myocytes in a use-dependent manner consistent with preferential activated state block. There was no difference between atrial and ventricular myocytes in the use-dependent inhibition of I_{Na} . However, eleclazine produced an instantaneous inhibition of I_{Na} in atrial but not in ventricular myocytes. In summary, differences exist between rat atrial and ventricular myocytes in the biophysical properties of I_{Na} . The more negative voltage-dependence of I_{Na} activation and inactivation underlies the instantaneous inhibition by eleclazine in atrial myocytes. Eleclazine warrants further investigation as an atrial-selective anti-arrhythmic drug.

Camm J. (2012). Antiarrhythmic drugs for the maintenance of sinus rhythm: Risks and benefits. *Int J Cardiol* 155, 362-371. doi: 10.1016/j.ijcard.2011.06.012

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

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

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

Lafuente-Lafuente C, Valembois L, Bergmann J-F & Belmin J. (2015). Antiarrhythmics for maintaining sinus rhythm after cardioversion of atrial fibrillation. *Cochrane Database Sys Rev* 3, CD005049. doi: 10.1002/14651858.CD005049

This work was supported by the British Heart Foundation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C005

The Ultrastructure of the Atrioventricular Node

T. sritharan¹, M.R. Boyett² and A. Kitmitto²

¹Medical School, University of Manchester, Manchester, UK and ²The Institute of Cardiovascular Science, The University of Manchester, Manchester, UK

Introduction: The atrioventricular node (AV node) is responsible for the conduction of action potentials from the atria to the ventricles and ensuring a delay between atrial and ventricular contraction to allow complete diastolic ventricular filling. Despite a century worth of research, the AV node ultrastructure is unknown and hence the understanding of its electrophysiological properties is not well understood. Therefore, we applied 3-D imaging techniques to provide greater insight into the nano-architecture of AV nodal cells and the mode of communication between adjacent nodal cells

Methods: Serial block-face scanning electron microscopy (SBF-SEM) (1), a high-resolution imaging technique was performed on rabbit AV nodal tissue. SBF-SEM

produced 1200 electron micrograph images on the rabbit AV node and they were analysed using image analysis software (2) to generate a novel three-dimensional reconstruction of the cells and their intracellular organelles.

Results: The AV node contains a complex arrangement of heterogeneously-shaped cells arranged in variable orientation and were surrounded by longer, parallel-running rod-shaped cells. The rod-shaped cells could represent the transitional cells surrounding the heterogeneously-shaped cells of the node responsible for the delay in action potential transmission through the node. Both cell populations were either mono- or bi-nucleated and contained clustered mitochondria and small vesicles. The cells mainly contact adjacent cells via intercalated discs and interdigitating prominences. The AV nodal cells were morphologically different in terms of size and shape and contained larger areas of intercalated discs compared to sinoatrial nodal cells and Purkinje cells.

Conclusion: We have generated the first computer 3-D model of AV nodal cells to show how the ultrastructure of the AV node influences slow propagation of action potential through the node. The significance of nodal cell geometry and arrangement and intercalated disc distribution are discussed. The data have provided the groundwork for electrophysiological experiments to investigate and further understand the functional characteristics of the cells described in this study.

Denk W & Horstmann H. (2004). *PLoS Biology* 2, 1900-9.

Kremer J *et al.* (1996). *Journal of Structural Biology* 116, 71-6.

Gregory Funge. Luke Stuart. Sunil Logantha. Tobias Starborg. Shu Nakao. Il-Young Oh. Mark Boyett. Ashraf Kitmitto.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C006

Prediction of paroxysmal atrial fibrillation in the equine athlete using heart rate adjusted complexity analysis of normal sinus rhythm ECGs.

V. Alexeenko^{1,2}, J.A. Fraser², M. Bowen³, C.L. Huang^{4,5}, C. Marr⁶ and K. Jeevaratnam^{1,2}

¹Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK, ²Department of PDN, University of Cambridge, Cambridge, UK, ³Faculty of Medicine & Health Sciences, University of Nottingham, Nottingham, UK, ⁴Physiological Laboratory, University of Cambridge, Cambridge, UK, ⁵Department of Biochemistry, University of Cambridge, Cambridge, UK and ⁶Rosdals Equine Hospital and Diagnostic Centre, Exning, UK

Introduction and aim

Both human and equine athletes have a propensity for atrial fibrillation (AF) even in the absence of gross structural abnormalities of the heart. Risk of AF in both species is increased by extreme training. This condition often manifests as paroxysmal AF (PAF) in which ECG detection of the infrequent self-terminating

fibrillation episodes is hampered by long periods of normal sinus rhythm between them. We hypothesised that the proarrhythmic background present in paroxysmal AF might be detectable by complexity analysis of short segments of normal sinus rhythm ECG.

Methods

Non-invasive ECG recordings were collected during routine clinical work while horses were exercised as part of their established performance assessment programmes. All subjects were Thoroughbred horses of racing age and undergoing actual race training (control group: 83 healthy horses; PAF group: 10 horses diagnosed with PAF from previous ECGs). Horses were atraumatically fitted with telemetric ECG recorders (Televet 100).

Artefact-free 60-sec strips of lead II ECG were excised from telemetric ECGs and converted to binary strings using threshold crossing (TC), beat detection (BD) and a novel feature detection (FD) coarse-graining algorithms. To limit the errors caused by complexity variability at higher heart rates, strips were analysed in a 25-60 bpm range. Since a number of subjects did not provide any 60-second artefact-free normal sinus rhythm ECGs in this range, some control subjects were excluded giving a final data set of 52 subjects in the control group and 10 in the PAF group. Lempel-Ziv '76 (LZ76) and Lempel-Ziv '78 (LZ78) complexity of the resulting binary strings was calculated. An unpaired two-sided t-test and ANOVA test with Tukey correction were used for group comparisons. Receiver operating curve analysis was used to assess the performance of diagnostic tests, with area under curve (AUC) as the metric.

Results

LZ76 (but not LZ78) complexity in combination with the FD coarse-graining algorithm is sensitive to the presence of PAF, although it did not attain a diagnostic level of accuracy (AUC = 0.79). Therefore, we introduced a heart-rate-corrected complexity metric D_{LZ76} which greatly improved sensitivity and specificity (fig. 1a), with AUC of 0.956 (Fig. 1b). The average D_{LZ76} for the controls group was 0.0118 ± 0.0095 bit/sample and for the PAF group it was -0.00954 ± 0.0103 ($p < 0.001$).

Conclusions

Our results suggest that pro-arrhythmic background present between AF episodes in PAF might be detected using the appropriately chosen ECG complexity evaluation methods. This might be developed into a fully automated method to detect PAF risk in horses by analysis of normal sinus rhythm ECG.

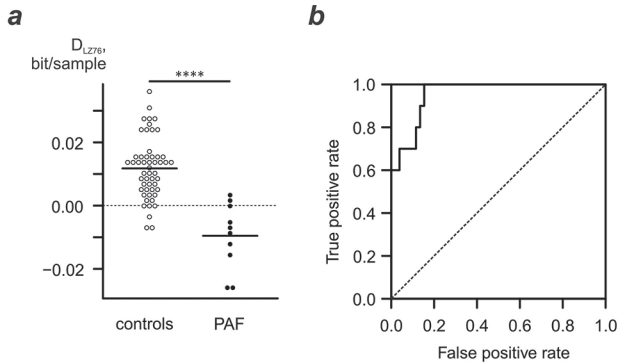


Fig.1. Heart rate adjusted complexity is indicative of PAF. *a.* combined complexity/heart rate metric values. *b.* Receiver operating curve for D_{L276} metric.

This study was funded by PetPlan Charitable Trust, grant S17-447-485 and the EPSRC Impact Acceleration Award to the University of Surrey

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C007

Increased cytoplasmic Ca^{2+} sensitivity of cardiac ryanodine receptors from the arrhythmic RyR2-P2328S mouse is independent of adrenergic challenge and FKBP12/12.6 regulation.

S.C. Salvage^{1,3}, E.M. Gallant², N.A. Beard⁴, S. Ahmad³, H. Valli³, J.A. Fraser³, C.L. Huang^{3,1} and A. Dulhunty²

¹Department of Biochemistry, University of Cambridge, Cambridge, UK, ²Eccles Institute of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia, ³Physiology Department, University of Cambridge, Cambridge, UK and ⁴Centre for Research in Therapeutic Solutions, University of Canberra, Canberra, ACT, Australia

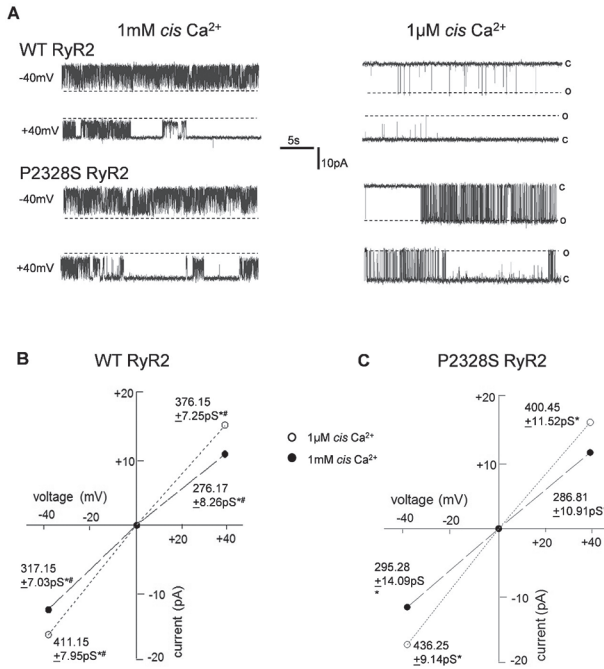
The cardiac ryanodine receptor (RyR2) P2328S mutation is a cause of both catecholaminergic polymorphic ventricular tachycardia (CPVT) [1], leading to fatal ventricular arrhythmias, and atrial arrhythmias including atrial fibrillation. *In vivo*, RyR2 can exist in various phosphorylation states and in association with regulatory proteins such as FKBP12/12.6 thought to affect channel stability [2,3].

Wild-type (WT) and homozygous RyR2-P2328S inbred 129/Sv mice, age matched across 3–7 months were killed by cervical dislocation in accordance with the UK Animals (Scientific Procedures) Act 1986; hearts were rapidly excised and snap frozen in liquid N_2 . Homotetrameric RyR2-containing sarcoplasmic reticular

(SR) vesicle preparations (each 5–7 hearts) were incorporated into lipid bilayers for single channel recordings and used for co-immunoprecipitation (Co-IP) and western blot (WB). Single channel recordings assessed open probability (P_o), mean open time, mean closed time and event frequency at a range of cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_{cyto}$); 0.1 μM – 1 mM. Recordings with multiple channels were only used for I²F (mean current normalised to maximum current - P_o equivalent) determination. Anti-RyR2 Co-IPs were performed and samples run on SDS-PAGE, transferred to PVDF and probed with antibodies for RyR2, FKBP12/12.6 as well as the S2808 and S2814 phosphorylated forms of RyR2. Data are means $\pm$ SEM compared by student's t-test (channel data) or ANOVA with Tukey post hoc (Western blots). Concentration dependences of P_o were fit to a Hill equation.

Activity in WT and P2328S RyR2 channels (Fig 1) was similar at 1 mM $[Ca^{2+}]_{cyto}$ (P_o at -40 mV; 0.55 ± 0.08 vs 0.56 ± 0.07 , respectively), but P2328S was significantly more active than WT at 1 μM $[Ca^{2+}]_{cyto}$ (0.24 ± 0.08 vs 0.028 ± 0.07 , respectively). This was associated with a >10-fold shift in the AC_{50} for Ca^{2+} -activation from $\sim 3.5 \mu M$ Ca^{2+} in WT RyR2 to ~ 320 nM in P2328S channels and an unexpected >1000-fold shift in IC_{50} for inactivation from ~ 50 mM in WT channels to $\leq 7 \mu M$ in P2328S channels, within systolic $[Ca^{2+}]$ levels. Unexpectedly, the shift in Ca^{2+} -activation was not associated with changes in S2808 or S2814 phosphorylation, or FKBP12/12.6 bound to the channels.

These data demonstrate that *in vivo* derived P2328S-RyR2 channels are more sensitive to cytoplasmic Ca^{2+} concentrations within the lower diastolic range (0.1–1 μM). This was in the absence of adrenergic challenge and without altered FKBP12 binding or RyR2 S2808/S2814 hyperphosphorylation. This suggests an increased predisposition to CPVT and an alternative mechanism for enhanced channel activity not dependent on hyperphosphorylation or loss of FKBP12/12.6-mediated stabilisation of RyR2 channels.



Activity of WT and P2328S RyR2 at 1 mM and 1 μM cytoplasmic (*cis*) Ca²⁺

Laitinen, P. J., Brown, K. M., Piippo, K., Swan, H., Devaney, J. M., Brahmbhatt, B., Donarum, E. A., Marino, M., Tiso, N., Viitasalo, M., Toivonen, L., Stephan, D. A., and Kontula, K. (2001) Mutations of the Cardiac Ryanodine Receptor (RyR2) Gene in Familial Polymorphic Ventricular Tachycardia. *Circulation* 103, 485–490

Brillantes, A. M. B., Ondrias, K., Scott, A., Kobrinsky, E., Ondriašová, E., Moschella, M. C., Jayaraman, T., Landers, M., Ehrlich, B. E., and Marks, A. R. (1994) Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 77, 513–523

Heijman, J., Wehrens, X. H. T., and Dobrev, D. (2013) Atrial arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia—is there a mechanistic link between sarcoplasmic reticulum Ca(2+) leak and re-entry? *Acta Physiol. (Oxf)*. 207, 208–211

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Cigarette Smoke Activates Calcium Influx in Human Airway Smooth Muscle Cells

J. Lin¹, M. Taggart², R. Tarran³ and M. Gray¹

¹*Institute of Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, UK,*

²*Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK and*

³*Marsico Lung Institute, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

Introduction

Tobacco smoking is the largest risk factor for developing chronic obstructive pulmonary disease (COPD), and is associated with airway hyperresponsiveness, which in turn is linked to abnormal Ca^{2+} homeostasis in airway smooth muscle (ASM). However, whether cigarette smoke (CS) directly alters or regulates Ca^{2+} signaling in human ASM cells (hASM) is not fully known. In airway epithelial cells, acute CS exposure increases $[\text{Ca}^{2+}]_i$ (Rasmussen et al, 2014). We speculate that CS disrupts Ca^{2+} homeostasis in hASM, leading to exacerbated ASM contraction especially under inflamed conditions in COPD.

Methods

Human lung tissue was provided from the Royal Victoria Infirmary, Newcastle (ethical approval ref 11109/2016). Primary hASM were isolated and maintained in culture up to 8 passages, and subjected to Ca^{2+} imaging studies using fura-2-AM. ASM were exposed to either whole CS (1 cigarette) or CS extract (CSE; 1 cigarette bubbled in 25ml buffer solution) from 3R4F reference cigarettes (University of Kentucky) with the particulate phase filtered out. All data are presented as mean $\pm$ SEM, and n numbers are independent experiments on cells from the same donor. Ca^{2+} response amplitudes are expressed as change in the fura-2 fluorescence ratio ($F_{340/380}$), and rates expressed as change in $F_{340/380}$ per minute (fitted using linear regression).

Results

We found that both CS and 10% CSE elevated $[\text{Ca}^{2+}]_i$ in hASM, which was abolished by removal of extracellular Ca^{2+} , suggesting that both CS and CSE activate Ca^{2+} influx pathways in cultured hASM (Fig. 1). Using a calcium-addback approach, we found that CSE-induced Ca^{2+} influx was poorly reversible after washing out CSE for 20 minutes (influx amplitude and rate following each addback before vs. after washout (n=7 for both sets) 0.19 ± 0.04 vs. 0.16 ± 0.04 , $p=0.16$ and 0.20 ± 0.04 vs. 0.14 ± 0.04 , $p<0.05$; Wilcoxon matched-pairs signed rank test). In addition, pre-treatment with cyclopiazonic acid to activate store-operated Ca^{2+} entry (SOCE) did not abolish CS- and CSE-induced Ca^{2+} influx. Furthermore, CSE-induced Ca^{2+} influx was not reduced by flunaril, an inhibitor of voltage-gated Ca^{2+} channels (VOCC; influx amplitude and rate DMSO (n=13) vs. flunaril-treated (n=16) 0.13 ± 0.03 vs. 0.12 ± 0.03 , $p=0.97$ and 0.08 ± 0.02 vs. 0.10 ± 0.03 , $p=0.47$; Mann-Whitney test), but was significantly attenuated by the lanthanide ion Gadolinium, Gd^{3+} (influx amplitude and rate untreated (n=11) vs. Gd^{3+} -treated (n=6) 0.43 ± 0.05 vs. 0.12 ± 0.03 , $p<0.0005$ and 0.43 ± 0.07 vs. 0.10 ± 0.03 , $p<0.0005$; Mann-Whitney test).

Conclusion

Whole CS and CSE activate Ca^{2+} influx pathways in hASMC that are independent from SOCE and VOCC. The poorly reversible activation of Ca^{2+} influx may lead to long-term elevation of hASMC $[\text{Ca}^{2+}]_i$ in chronic smokers, contributing to airway hyperresponsiveness. Further investigations into the identity of Ca^{2+} influx channels activated, and the direct effects of CS/CSE on ASM contractile mechanisms are on-going.

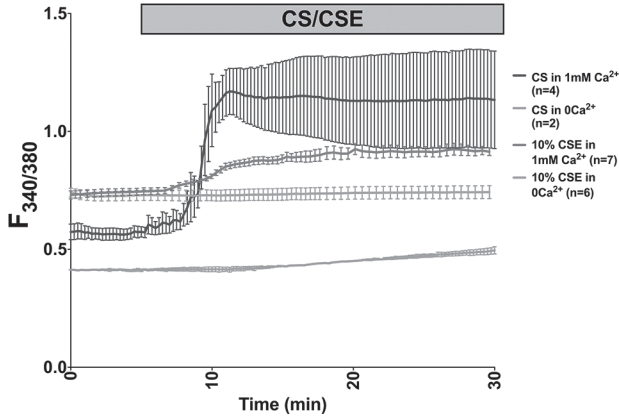


Figure 1: Whole CS and 10% CSE elevate $[\text{Ca}^{2+}]_i$ in hASMC, and requires extracellular Ca^{2+} . Data plotted as mean $\pm$ SEM.

Rasmussen, J.E., Sheridan, J.T., Polk, W., Davies, C.M. and Tarran, R., 2014. Cigarette smoke-induced Ca^{2+} release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. *Journal of Biological Chemistry*, 289(11), pp.7671-7681.

This PhD project is funded by the JJ Bequest Studentship from Newcastle University.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C009

Cystic fibrosis-transmembrane conductance regulator limits F-actin formation and promotes morphological alignment with flow in human lung microvascular endothelial cells.

A.J. Causer¹, M. Khalaf², E. Rote², K. Brand², S. Bailey³, M. Cummings⁴, A. Shepherd¹, Z.L. Saynor¹ and J. Shute²

¹Department of Sport & Exercise Science, University of Portsmouth, Portsmouth, UK,

²School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, UK, ³School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, UK and ⁴Department of Diabetes and Endocrinology, Queen Alexandra Hospital, Portsmouth, UK

Problem statement: Flow mediated dilation is compromised in cystic fibrosis (CF), demonstrating endothelial dysfunction in response to shear stress. Inhibition of endothelial CF-transmembrane conductance regulator (CFTR) activity causes a pro-inflammatory and pro-oxidant state. Inflammation and oxidative stress cause endothelial barrier dysfunction by re-organising the endothelial actin cytoskeleton. It is currently unknown, however, whether CFTR inhibition causes F-actin polymerisation. The present study, therefore, aimed to investigate the implications of CFTR inhibition on F-actin polymerisation and morphological alignment following exposure to shear stress in human lung microvascular endothelial cells (HLMVEC) *in vitro*.

Methods: HLMVEC were cultured in the absence and presence of the pharmacological CFTR inhibitor GlyH-101 (20 μ M), during 16 h, 24 h and 48 h of exposure to shear stress (11.1 dynes/cm²) or static conditions. Nitrite and endothelin (ET)-1 were analysed in cell media supernatants. Morphological changes in response to shear stress were detected using Crystal Violet staining. Cortical rim, perinuclear and stress fibre F-actin reformation were detected using Rhodamine Phalloidin (red) and Hoechst 33342 (blue) staining. Values are means $\pm$ SEM.

Results: Exposure to 48 h of shear stress increased [nitrite] (+146.2 $\pm$ 2.0% vs. static conditions; $p < 0.05$), whereas 48 h (-57.9 $\pm$ 3.6% vs. static conditions; $p < 0.05$) and 24 h (-63.5 $\pm$ 5.6% vs. static conditions; $p < 0.05$) of shear stress lowered [ET-1] in HLMVEC supernatant. Notably, 16 h or 24 h of shear stress had no significant effect on [nitrite], nor did 16 h of shear stress significantly affect [ET-1] ($p > 0.05$). The addition of GlyH-101 did not alter [nitrite] or [ET-1] (all $p > 0.05$). Shear stress over 48 h induced cellular alignment with the direction of flow; however, the addition of GlyH-101 prevented such alignment (Figure 1). GlyH-101 increased cytosolic F-actin polymerisation under static conditions ($p < 0.05$). GlyH-101 also caused a re-distribution of F-actin from the cortical rim to cytosolic stress fibres under both static and shear stress conditions over 24 h (Figure 2).

Conclusions: Functional CFTR appears to limit cytosolic F-actin polymerisation, while maintaining a cortical rim distribution that is important for cellular integrity and CFTR function. Inhibition of CFTR, with increased F-actin stress fibre formation, prevented

HLMVEC alignment with flow independent of [nitrite] and [ET-1]. CFTR, therefore, plays an important role in the regulation of actin dynamics in HLMVEC and may contribute to the endothelial perturbations that are evident even in relatively mild CF.

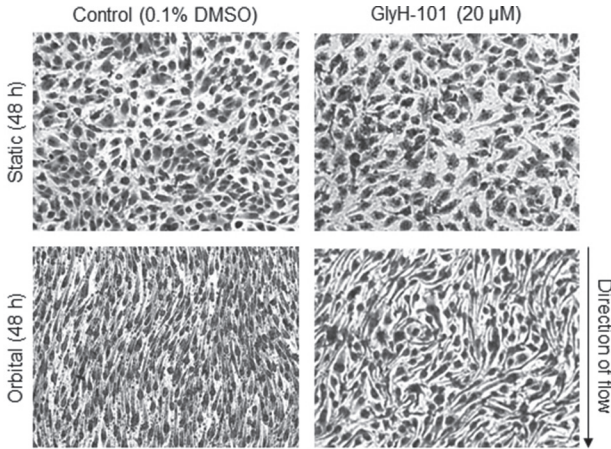


Figure 1. Crystal violet staining of human lung microvascular endothelial cells cultured in the absence (Column 1) or presence (Column 2) of GlyH-101, following 48 h of static incubation (Row 1) or 11.1 dynes/cm² of shear stress (Row 2).

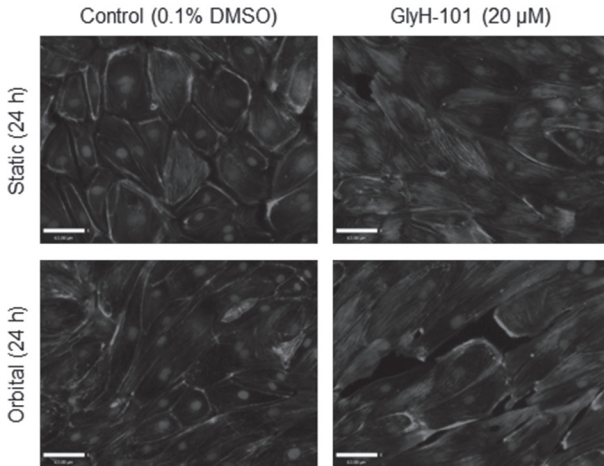


Figure 2. F-actin staining of human lung microvascular endothelial cells cultured in the absence (Column 1) or presence (Column 2) of GlyH-101, following 24 h of static incubation (Row 1) or 11.1 dynes/cm² of shear stress (Row 2).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Carotid body chemosensitivity is enhanced during moderate exercise in human hypertension

K.A. Hope³, L. Ratcliffe⁴, A. Burchell¹, T. Hinton^{1,3}, B. Chant³, A. Nightingale^{1,3}, J.F. Paton² and E.C. Hart³

¹Bristol Heart Institute, University Hospitals Bristol NHS Foundation Trust, Bristol, UK,

²Department of Physiology, Faculty of Medical & Health Sciences, University of Auckland,

Auckland, New Zealand, ³School of Physiology, Pharmacology & Neuroscience,

University of Bristol, Bristol, UK and ⁴Department of Nephrology, Lister Hospital, East & North Herfordshire NHS trust, Stevenage, UK

Hypertension is the main risk factor contributing to burden of disease globally (1). At rest, hypertensive humans (HTN) demonstrate increased carotid body (CB) chemosensitivity compared to normotensive (NTN) controls, revealed by a raised Hypoxic Ventilatory Response (HVR (2). HTN also show an exaggerated Blood Pressure (BP) response during exercise (exercise pressor response; EPR; (3)). The CB's play a role in the EPR in hypertensive rats (4) but their role in the EPR of humans is unknown. This study aimed to examine HVR during exercise in HTN compared to NTN & whether CB blockade with dopamine can blunt EPR in HTN.

8 NTN (2 women) & 12 HTN (5 women) participants completed a 25W.min⁻¹, ramped upright cycle ergometer exercise test to obtain peak oxygen consumption (VO₂peak). HVR was assessed at rest & during moderate exercise (≈45% VO₂peak) using a transient, poikilocapnic technique with titration of nitrogen to inspired air to achieve oxygen saturations (SpO₂) of 87% for 2-3min. HVR was calculated by linear regression of the change in minute ventilation (V_E) divided by the change in SpO₂ during hypoxia (5). CB Blockade was assessed during exercise by comparison of BP during intravenous infusion of 2mcg.kg⁻¹.min⁻¹ of dopamine with an infusion of 0.9% saline as a vehicle control. Unpaired T tests compared demographic data while HVR & CB blockade data were compared by 2-way ANOVAS with repeated measures. Alpha was set at P<0.05. Data are mean±SD.

There was no difference between groups in age (NTN: 61±8 vs HTN: 65.3±7.6yrs; P=0.2429), Body Mass Index (BMI) (NTN: 25.6±3.7 vs HTN: 26±4kg.m⁻²; P=0.7923), VO₂peak (NTN: 24.6±12 vs HTN: 23.1±9mL.min⁻¹.kg⁻¹; P=0.7722) or level of exercise used to assess HVR & CB blockade (NTN: 43.8±14.5 vs HTN: 47.3±17.9%VO₂peak; P=0.6709). At rest, HVR did not differ between groups (NTN: -0.4±0.3 vs HTN: -0.6±0.6L.min⁻¹.%SpO₂⁻¹; P=0.8716). During moderate exercise, HVR remained unchanged from rest in NTN (-0.5±0.3 L.min⁻¹.%SpO₂⁻¹; P=0.5725) but increased by 58.8% in HTN (-1.5±1L.min⁻¹.%SpO₂⁻¹; P=0.0007). HVR was also significantly higher in HTN than NTN during exercise (P=0.0086, Figure 1). CB blockade (dopamine vs saline) during exercise did not alter systolic BP (SBP), mean arterial pressure (MAP) or diastolic BP (DBP) in NTN (SBP: 127.2±11.2 vs 130.8±15.4mmHg; P=0.7942; MAP: 91.7±9.3 vs 91.2±9.9mmHg; P=0.9882; DBP: 73.6±11.4 vs 71.4±8.1mmHg; P=0.7733) but significantly reduced MAP & DBP in HTN (SBP:

143.2 vs 131.5mmHg; $P=0.0637$; MAP: 101.1 ± 17.3 vs 95.3 ± 13.6 mmHg; $P=0.0076$; DBP: 86.0 ± 7.5 vs 77.0 ± 16.4 mmHg; $P=0.0124$).

These data indicate that HVR is elevated in middle-aged hypertensives during moderate exercise and that the CB's could play a role in the exaggerated pressor response to exercise in people with hypertension, which has been shown to be associated with worse outcomes but is not normalised by current antihypertensive drugs.

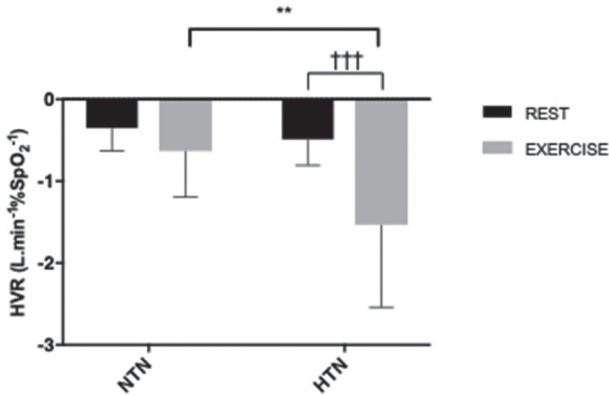


Figure 1. Carotid Body (CB) chemosensitivity as measured by Hypoxic Ventilatory Response (HVR) in Normotensive (NTN) and Hypertensive (HTN) humans at rest and during exercise. Values are mean $\pm$ SD. ** $P<0.01$ NTN vs HTN during exercise, † $P<0.001$ HTN rest vs exercise.

Bromfield S & Muntner P. (2013). High blood pressure: the leading global burden of disease risk factor and the need for worldwide prevention programs. *Curr Hypertens Rep* 15, 134-136.

Trzebski A, Tafil M, Zoltowski M & Przybylski J. (1982). Increased sensitivity of the arterial chemoreceptor drive in young men with mild hypertension. *Cardiovasc Res* 16, 163-172.

Chant B, Bakali M, Hinton T, Burchell AE, Nightingale AK, Paton JFR & Hart EC. (2018). Anti-hypertensive Treatment Fails to Control Blood Pressure During Exercise. *Hypertension* 72, 102-109.

Pijacka W, Katayama PL, Salgado HC, Lincevicius GS, Campos RR, McBryde FD & Paton JFR. (2018). Variable role of carotid bodies in cardiovascular responses to exercise, hypoxia and hypercapnia in spontaneously hypertensive rats. *J Physiol* 596, 3201-3216.

Goldberg S, Ollila HM, Lin L, Sharifi H, Rico T, Andlauer O, Aran A, Bloomrosen E, Faraco J, Fang H & Mignot E. (2017). Analysis of Hypoxic and Hypercapnic Ventilatory Response in Healthy Volunteers. *PLoS ONE* 12, e0168930-0168916.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Orai3 expression increases during chemotherapy in lung adenocarcinoma and is involved in resistance to chemotherapy

H. AbouDaya, F. Hague and H. OUADID-AHIDOUCH

LPCM-EA46-67, Université Picardie Jules Vernes, Amiens, France

Lung cancer is the first cause of cancer death in men and the second in woman. The first therapeutic line of action against lung adenocarcinoma is surgery. However only 15% of cases are operable, thus chemotherapy is considered. Cisplatin-based combination chemotherapy regimens are currently used as a front-line therapy in the treatment of non-small cell lung cancer. Unfortunately, such treatment often leads to chemoresistance and hence therapy failure. Thus, it is urgent to investigate the molecular mechanisms involved in this resistance. Cancer cells are known to exhibit a high rate of proliferation, migration ability and resistance to apoptosis. These processes are proved to be controlled by calcium and calcium-permeable ion channels. Store Operated Calcium Channels (SOCs) represent a major calcium entry pathway in non-excitabile cells and are shown to be implicated in drug resistance in different types of cancer. Recently, we showed that Orai3 is overexpressed in lung adenocarcinoma and is correlated to high tumor grade and controls cell proliferation via the Akt pathway (Ay et al., 2013). We also showed that Orai3 constitutes a predictive marker of metastasis and survival in resectable lung adenocarcinoma (Benzerdjeb et al., 2016). Furthermore, Orai3 overexpression confers resistance to chemotherapeutic drugs via the pro-survival PI3K/Sgk-1/Sek-1 pathway in breast cancer cells (Hasna et al., 2018). Hence, we aimed to investigate the Orai3 involvement in resistance to chemotherapy in lung adenocarcinoma cell lines.

Experiments were conducted on two lung adenocarcinoma cell lines, H23 and A549. The expression of the SOC actors was studied using RT-qPCR and confirmed by Western-Blot. The inhibition of the expression of the SOC actors was done via transfection using (siOrai1, siOrai3 and siStim1) vs. siControl (electroporation, AMAXA). Cellular viability and mortality were assessed (48 hours or 4 days after treatment with Cisplatin) using MTT and Trypan blue methods. Calcium imaging experiments were also conducted to measure the functional effect of the treatment obtained at the level of the channels (quantification of the SOC entry). We found that Cisplatin treatment increased Orai3 expression, SOC entry, and favours cell survival in both cell lines. However, in contrast to H23 cells where Orai3 constitutes a native SOC channel, in A549 cell line, Orai3 doesn't contribute to SOC entry but upon the treatment with Cisplatin, it becomes a SOC channel. Moreover, we found that the long term treatment with Cisplatin leads to morphological change of A549 cells along with increased stem cell marker, expression (Nanog) suggesting a stem cell enrichment process. In conclusion, we demonstrated that Orai3 channel becomes overexpressed during drug treatment in lung adenocarcinoma cell lines and contributes to Cisplatin resistance and the ability of conferring resistance is related to the SOC activity of the channel.

Ay AS et al (2013). *J PloSONE* 8, e7288.

Benzerdjeb N et al (2016). *J Oncotarget* 7, 81588–81597.

Hasna J et al (2018). *J Cell Death Differ* 24, 691-705.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C012

Adjustments in purinergic metabolism maintain the contribution of adenosine and ATP to carotid body chemosensory activity in ageing

S. Conde, M.J. Ribeiro, J. Prieto-Lloret, B.F. Melo, E.C. Monteiro and J.F. Sacramento
CEDOC, NOVA Medical School, Lisboa, Portugal

Adenosine and ATP are key neurotransmitters involved in carotid body (CB) response to hypoxia (1). CB exhibits a decline in its functionality in ageing (2), however, several diseases associated with ageing present increased CB activity (3,5). Here the effect of ageing was investigated on characteristics involved in CB function and on the contribution of adenosine and ATP to CB chemosensory activity. Experiments were performed in 3 and 24 months male Wistar rats anesthetized with pentobarbitone (60 mg/kg, ip). Ageing was investigated on: the number of type 1 and type 2 CB cells; the expression of SNAP25, tyrosine hydroxylase (TH), CD73 and ENT1 transporter in the CB and of A_{2A} adenosine and P2X₂ ATP receptors in the petrosal ganglion; CB basal and hypoxic release of adenosine and ATP; the contribution of adenosine and ATP to carotid sinus nerve (CSN) electrophysiological responses to hypoxia and hypercapnia and on spontaneous ventilation and in response to ischemic hypoxia by occlusion of the carotid common artery (OCC). Student t-test and one- and two-way Anova tests were used.

Ageing decreased by: 49.0% TH immune-positive cells ($p < 0.01$), but not the number of type 2 cells; 52.5% and 42.2% the expression of P2X₂ and A_{2A} receptors in petrosal ganglion; 46.7% ($p < 0.05$), 56.2% ($p < 0.01$) and 68.1% ($p < 0.001$), the basal and 10 and 2%O₂-hypoxic release of ATP (3M 20%O₂ = 5.1 ± 0.8 pmol/CB; 10%O₂ = 9.6 ± 1.5 pmol/CB; 2%O₂ = 18.0 ± 2.0 pmol/CB); 97.5% ($p < 0.001$), 98.2% ($p < 0.001$), and 98.1% ($p < 0.001$) the basal and 10 and 2%O₂-hypoxic release of adenosine (3M 20%O₂ = 25.8 ± 3.9 pmol/CB; 10%O₂ = 62.8 ± 6.2 pmol/CB; 2%O₂ = 43.8 ± 4.9 pmol/CB) from CB. Ageing increased by 87.1% CD73 immune-positive cells ($p < 0.01$) and by 83.7% and 400.5% the expression of SNAP25 ($p < 0.01$) and ENT1 ($p < 0.01$) in the CB. Ageing did not modify basal CSN activity (basal activity: 3M = 2.2 ± 0.4 spikes/s; 24M = 1.9 ± 0.2 spikes/s) or the activity in response to hypercapnia, but decreased by 77.8% ($p < 0.01$), 67.8% ($p < 0.001$) and 51.9% ($p < 0.001$) the CSN-electrophysiological activity in response to 7%O₂, 5%O₂ and 0%O₂. Bilateral OCCs during 5, 10 and 15s induced an increase in ventilation proportional to the duration of ischemia (OCC 5s = $59.8 \pm 4.2\%$; OCC 10s = $139.8 \pm 10.6\%$; OCC 15s = $23.9 \pm 15.2\%$), an effect decreased by ageing. ATP contributes with $\approx 50\%$ to the ventilatory responses to all ischemic

intensities tested in young and in aged rats; the contribution of adenosine was dependent on the intensity of ischemia, being maximal in ischemias of 5s (50%) and much smaller in 15s ischemias.

Our results demonstrate that although ageing decrease the number of type 1 cells in the CB, this seems to impact only in the responses to hypoxia, and not on CB basal and hypercapnic activity. Additionally, both ATP and adenosine, contribute to CB chemosensory activity in ageing. Though CB function in response to hypoxia decreases with age the relative contribution of both ATP and adenosine for CB activity is maintained.

Conde SV, Monteiro EC, Rigual R, Obeso A, Gonzalez C (2012) Hypoxic intensity: a determinant for the contribution of ATP and adenosine to the genesis of carotid body chemosensory activity. *J Appl Physiol* (1985) 112, 2002-10.

Conde SV, Obeso A, Rigual R, Monteiro EC, Gonzalez C (2006) Function of the rat carotid body chemoreceptors in ageing. *J Neurochem* 99:711-23.

Ribeiro MJ, Sacramento JF, Gonzalez C, Guarino MP, Monteiro EC, Conde SV (2013) Carotid body denervation prevents the development of insulin resistance and hypertension induced by hypercaloric diets. *Diabetes* 62, 2905-16.

Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, Sobotka PA, Gourine AV, Paton JF (2012) Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. *J Physiol.* 590, 4269-77.

Del Rio R, Marcus NJ, Schultz HD. (2013) Carotid chemoreceptor ablation improves survival in heart failure: rescuing autonomic control of cardiorespiratory function. *J Am Coll Cardiol.* 62, 2422-2430.

This study was supported by the Portuguese Foundation for Science and Technology grant PTDC/SAU-ORG/111417/2009 to Silvia Conde.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C013

Ca²⁺/Calmodulin-Dependent Protein Kinase II Activation Promotes Ryanodine Receptor Dispersion and Dysfunction During Heart Failure

T.R. Kolstad^{1,2}, X. Shen^{1,2}, J. van den Brink³, E. Nordèn^{1,2}, I. Sjaastad^{1,2}, A. Cataliotti^{1,2}, C. Soeller⁴ and W.E. Louch^{1,2}

¹Institute for Experimental Medical Reserach, University of Oslo, Oslo, Norway, ²K.G. Jebsen Center for Cardiac Research, Oslo, Norway, ³Simula Research Laboratory, Oslo, Norway and ⁴Living Systems Institute, University of Exeter, Exeter, UK

In cardiomyocytes, efficient Ca²⁺ release from ryanodine receptors (RyRs) is essential for the triggering of rapid and forceful contraction. The elemental units of Ca²⁺ release, called Ca²⁺ sparks, result from the concerted opening of RyRs within functional groupings called Calcium Release Units (CRUs). We have recently shown that the organization of RyRs within the CRU is altered during heart failure (HF), as

RyR dispersion results in smaller and more fragmented CRUs (Kolstad *et al.*, 2018). This reorganization was linked to slowing of both spontaneous and triggered Ca^{2+} sparks, and increased diastolic Ca^{2+} leak. Since previous work has also implicated a role of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) in dysregulated RyR activity during HF, we presently examined whether CaMKII activation is linked to functional CRU reorganization in this disease. Ventricular myocytes were isolated from rats which had developed HF 6 weeks following myocardial infarction. Using a newly-developed method for quantitative 3D dSTORM microscopy (Shen *et al.*, 2019), we observed that internal RyR clusters in failing myocytes were smaller than those in cells from sham-operated animals (5.0 ± 0.3 vs 7.9 ± 0.5 RyRs/cluster, $P < 0.05$). CRUs, defined as RyR clusters with edge-to-edge distances < 100 nm, also displayed more distributed 3D conformations in HF, with a higher density of clusters but fewer RyRs overall. Western blotting indicated augmented RyR phosphorylation by CaMKII at serine-2814 in failing hearts. Treatment of isolated cells with the CaMKII inhibitor auto-inhibitory peptide (AIP, $10 \mu\text{M}$) significantly reversed the reduction in RyR cluster and CRU sizes in failing cells (RyRs/CRU = 20.8 ± 1.1 , 15.1 ± 1.3 , 19.3 ± 3.9 in sham, HF, and HF+AIP). Although AIP treatment did not alter RyR configuration in sham cells at baseline, RyR dispersion could be induced in non-failing cells by prolonged exposure to 100 nM isoproterenol. These effects which were mitigated in the presence of AIP, are consistent with a direct role of CaMKII in controlling RyR organization. Live-cell imaging of failing myocytes revealed Ca^{2+} sparks with slowed kinetics, as Ca^{2+} propagated through dispersed CRUs (time to peak = 7.3 ± 0.8 vs 10.5 ± 0.4 ms in Sham, HF), and increased diastolic Ca^{2+} leak. AIP treatment accelerated spark kinetics and reduced leak to sham values. Mathematical modelling of dSTORM-derived CRU geometries supported that combined CRU fragmentation and RyR sensitization following CaMKII phosphorylation reproduced the slowed spark kinetics and increased Ca^{2+} leak observed experimentally in HF. Taken together, our data indicate that CaMKII-dependent RyR phosphorylation during HF is linked to a physical reorganization of the channels, which critically impairs Ca^{2+} homeostasis in this condition.

Kolstad TR, van den Brink J, MacQuaide N, Lunde PK, Frisk M, Aronsen JM, Norden ES, Cataliotti S, Sjaastad I, Sejersted OM, Edwards AG, Lines GT, and Louch WE. Ryanodine receptor dispersion disrupts Ca^{2+} release in failing cardiac myocytes (2018) *eLife*, 7:e39427.

Shen X, van den Brink J, Hou F, Colli D, Le C, Kolstad TR, MacQuaide N, Carlson CR, Kekenus-Huskey PM, Edwards AG, Soeller C, and Louch WE. 3D dSTORM Imaging Reveals Novel Detail of Ryanodine Receptor Localization in Rat Cardiac Myocytes (2019) *J Physiol*, 597:399-418.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C014

Dual antiarrhythmic properties of phosphodiesterase-5 inhibitors suppress Ca^{2+} waves in systolic heart failure

D.C. Hutchings, G.W. Madders, C. Waddell, L. Woods, K. Dibb, D. Eisner and A. Trafford

Division of Cardiovascular Sciences, University of Manchester, Manchester, UK

Background:

Arrhythmias account for up to half of all deaths in heart failure (HF)(1). Despite therapeutic advances new treatments targeting contractile dysfunction and arrhythmias are urgently required. While there are several mechanisms of ventricular arrhythmia in HF, a crucial trigger at the cardiac myocyte level is increased occurrence of Ca release events from the sarcoplasmic reticulum (SR) causing Ca waves. Waves lead to activation of the Na^+ - Ca^{2+} exchanger (NCX) current causing after-depolarisations and eliciting triggered action potentials(2). Ca waves occur when SR content reaches a threshold level(3). Phosphodiesterase-5 (PDE5) inhibitors acutely suppress waves and triggered arrhythmias in control myocytes by reducing SR Ca(4). However whether their benefit extends to HF, where structural and electrophysiological remodelling are present, and following chronic treatment, is undetermined.

Aims:

Determine effect of both acute and chronic PDE5 inhibition on Ca handling and pro-arrhythmic Ca waves in a sheep model of rapid-pacing induced heart failure.

Methods:

Young (~18 months age) female welsh mountain sheep were used. HF was induced by rapid pacing via a pacemaker (210 beats/min) implanted 10-14 days prior under isoflurane general anaesthesia (2-3%). After 4 weeks of pacing animals were assigned to either HF, where animals underwent tachypacing to end-stage HF (typically 2-3 wks), or tadalafil-HF, where animals were treated with oral tadalafil 20mg for three weeks while pacing was continued. Left ventricular myocytes were isolated, voltage-clamped, and stimulated at 0.5Hz. Intracellular Ca was measured with Fura-2. Ca waves were induced by raising external Ca to 10mM. To determine threshold SR Ca content to induce waves, caffeine (10mM) was added immediately after a wave, and both wave and caffeine-induced NCX currents integrated.

Results:

HF increased propensity to Ca waves compared to control myocytes by decreasing threshold SR content (128 ± 16 ctrl vs 68 ± 7 $\mu\text{mol/L}$ HF, $p < 0.001$). In HF cells displaying waves, acute PDE5 inhibitor sildenafil ($1 \mu\text{M}$) reduced wave size in all cells (integrated INCX -36 %, $p < 0.05$) and abolished waves in 3/10 cells by reducing SR content below threshold. In chronic PDE5 inhibitor-treated HF, myocytes were resistant to Ca waves, with only 47% cells exhibiting waves compared with 80% of HF cells ($p < 0.05$, $n = 19-25$ cells). In contrast to acute PDE5 inhibition, this was

achieved by elevating threshold SR content to control levels, with waves occurring at $105 \pm 15 \mu\text{mol.l}^{-1}$ vs 70 ± 7 in HF ($p < 0.05$, $n = 8-18$ cells).

Conclusions:

PDE5 inhibition suppresses Ca waves in HF via two separate mechanisms; a reduction in SR Ca content after acute treatment, and via chronic remodelling effects after long-term treatment where threshold SR content is increased. Whether such chronic effects relate to modifications in RyR2 properties or structural changes affecting SR and t-tubule networks require further investigation.

1. Greene HL, Richardson DW, Barker AH, Roden DM, Capone RJ, Echt DS, et al. Classification of deaths after myocardial infarction as arrhythmic or nonarrhythmic (the Cardiac Arrhythmia Pilot Study). *Am J Cardiol.* 1989;63(1):1-6. Epub 1989/01/01.
2. Bers DM, Despa S, Bossuyt J. Regulation of Ca^{2+} and Na^{+} in normal and failing cardiac myocytes. *Annals of the New York Academy of Sciences.* 2006;1080:165-77. Epub 2006/11/30.
3. Diaz ME, Trafford AW, O'Neill SC, Eisner DA. Measurement of sarcoplasmic reticulum Ca^{2+} content and sarcolemmal Ca^{2+} fluxes in isolated rat ventricular myocytes during spontaneous Ca^{2+} release. *The Journal of physiology.* 1997;501 (Pt 1):3-16. Epub 1997/05/15.
4. Hutchings D P, Madders G, Woods L, Dibb K, Eisner D, Trafford A. Phosphodiesterase-5 inhibition with sildenafil suppresses triggered ventricular arrhythmias and calcium waves via reduced sarcoplasmic reticulum content in the large mammal. *Journal of molecular and cellular cardiology.* 2018;120(Supplement):1.

Funding: British Heart Foundation

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C015

Adult mitochondria show sexual dimorphism after prenatal hypoxia

K.T. Hellgren, A. Trafford and G.L. Galli

Cardiovascular, University of Manchester, Manchester, UK

While lifestyle and genetics are well-known risk factors for heart disease, the impact of the intrauterine environment on disease susceptibility is a relatively new concept. Insufficient oxygen supply to a foetus (prenatal hypoxia) has been linked with developmental programming and adverse effects in the adult heart of the offspring. Whole heart studies have suggested increased susceptibility to ischaemia reperfusion (I/R) injury but there are few studies that have investigated the underlying mechanisms. We set out to explore the effects of prenatal hypoxia on adult offspring cardiac mitochondria; we investigated mitochondrial oxygen consumption and reactive oxygen species (ROS) production.

Pregnant C57 mice were subjected to 14% oxygen (hypoxic group) for gestational days 6-18. A control group (normoxic) were kept in 21% oxygen. Both groups delivered in 21% oxygen (normoxia) and the offspring were kept in normoxia for 7 months before being killed by cervical dislocation. Mitochondrial respiration and ROS production were assessed in homogenized left ventricular tissue using an Oroboros

Oxygraph-O2k and O2k-Fluo LED2-Module. Data presented as mean $\pm$ SEM. Using a sequence of substrates and inhibitors, we found increased respiration and decreased ROS production in adult females previously subjected to prenatal hypoxia. The same protocol showed no significant differences in male respiration but increased ROS production in males from hypoxic pregnancies. The differences in female respiration were greatest during oxidative phosphorylation ($517 \pm 109 \text{ nmol O}_2^{-1}\text{sec}^{-1}\text{mg}$ in normoxic, $n=7$, compared to $957 \pm 112 \text{ nmol O}_2^{-1}\text{sec}^{-1}\text{mg}$ in hypoxic offspring, $n=8$). Female ROS production showed the greatest difference during oxidative phosphorylation without activation of complex II ($1.23 \pm 0.25 \text{ pmol H}_2\text{O}_2^{-1}\text{sec}^{-1}\text{mg}$ in normoxic, $n=7$ compared to $0.75 \pm 0.12 \text{ pmol H}_2\text{O}_2^{-1}\text{sec}^{-1}\text{mg}$ in hypoxic, $n=8$). Male ROS production also showed the greatest difference during oxidative phosphorylation without activation of complex II ($0.43 \text{ pmol H}_2\text{O}_2^{-1}\text{sec}^{-1}\text{mg}$ in normoxic, $n=5$, versus $0.93 \text{ pmol H}_2\text{O}_2^{-1}\text{sec}^{-1}\text{mg}$ in hypoxic, $n=8$). In addition, enzyme activity assays showed that citrate synthase remained unchanged between the treatments, whilst complex I and II were decreased in hypoxic females, 73% and 62% respectively, compared to normoxic females. Complex IV activity increased with 92% in hypoxic females compared to normoxic females. No differences in enzyme activity were found in males. Our findings show that females respond differently to prenatal hypoxia than males at the mitochondrial level. The increase in basal ROS production in the hypoxic males could help to explain the increased susceptibility to I/R injury in males. In contrast, the lower basal ROS production in females from hypoxic pregnancies may protect the heart from I/R injury. In addition, our study further proves the need to explore physiology and pathology in females as well as males.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C016

Substrate affects contractile behaviour of hiPSC derived cardiomyocytes

E. Huethorst^{1,2}, F.L. Burton¹, N. Gadegaard² and G.L. Smith¹

¹*Institute of Cardiovascular And Medical Sciences, University of Glasgow, Glasgow, UK* and ²*Division of Biomedical Engineering, University of Glasgow, Glasgow, UK*

Cell-cell and cell-substrate interactions can alter the contractile behaviour of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). HiPSC-CM are often cultured on rigid standard tissue culture plastic (TCP) in 2D sheets, in contrast to the more flexible 3D extracellular matrix of the myocardium. We investigated contractile behaviour of hiPSC-CM microtissues (MT) before (2D), during and after detachment (3D) from TCP. Spontaneously beating hiPSC-CM were seeded on thermosensitive Nunc UpCell plates (3 experiments, 3-7 MT per experiment). On day 0, after plates were cooled to 25°C, MT were lifted off the dish and transferred to a standard TCP dish. Video recordings of the MT were made before transfer at 37°C (D0₃₇) and 25°C (D0₂₅), directly after transfer (D0) and on days 1, 3, 5 and 7 after

transfer (D1-7). Video frames were subdivided into a 30x30 grid, and each grid square analysed using MUSCLEMOTION (Sala *et al.*, 2018). The percentage of grid squares with single and multiple transient peaks was calculated, and the ranges of start times (TStart) and contraction durations at 50% (CD50) were characterised by the 10-90th percentile difference (IP90). Values are reported as mean ranks $\pm$ SD, and time points were compared to D0₃₇, using Kruskal-Wallis and Dunn's multiple comparisons tests. After detachment, the MT-diameter decreased from ~3mm to 0.5-1mm as the structure became more 3D-like. Before detachment (D0₃₇), the percentage of transients with multiple peaks was 50 $\pm$ 13% (Fig 1). Multiple peaks became rarer during and after detachment (D0₂₅: 43 $\pm$ 13%; D0: 13 $\pm$ 17%), but this trend reversed during reattachment to TCP on D1-7 (D1: 27 $\pm$ 15%; D7: 54 $\pm$ 17%) (Fig 1). The range of CD50 values on D0₃₇ and D0 were similar (IP90=159 $\pm$ 27ms vs 128 $\pm$ 83ms) but were significantly decreased on D1 and D3 (IP90=D1: 95 $\pm$ 27ms; D3: 108 $\pm$ 31ms; p <0.01) and increased during reattachment (IP90=D5: 123 $\pm$ 32ms; D7: 130 $\pm$ 29ms) (Fig 2). Transients with multiple peaks also had higher CD50 values (1 peak: 307 $\pm$ 44ms; >1 peak: 348 $\pm$ 45ms; p <0.0001), but it is unclear whether this was due to intrinsic properties of cells or to mechanical interactions between neighbouring cells. The ranges of TStart values were significantly smaller on D3-7 (IP90=D3:21 $\pm$ 6ms, D7:20 $\pm$ 5ms; p <0.0001) compared to D0₃₇ and D0 (IP90=42 $\pm$ 9ms vs 40 $\pm$ 10ms), suggesting that the 3D structure increases synchronicity of the MT independently from TCP-adhesion. In this study, for both 2D and 3D culture, hiPSC-CM cultured on TCP exhibit complex contractile behaviour which is less complicated after detachment. This highlights the importance of cell-substrate interaction and the need to consider this factor in future studies.

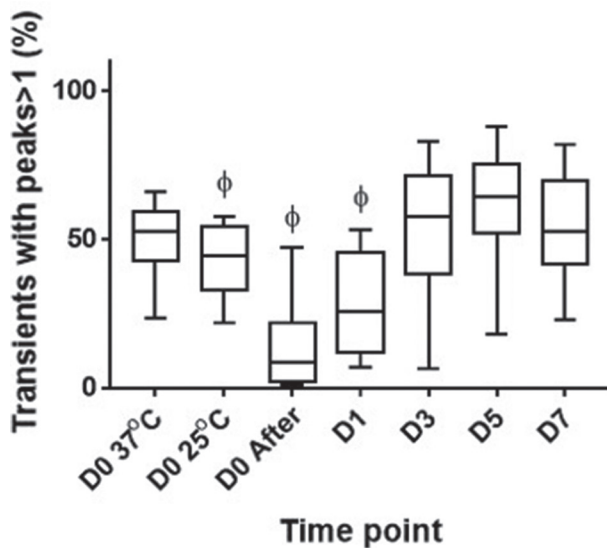


Figure 1. The percentage of contractility transients with multiple peaks on different time points.

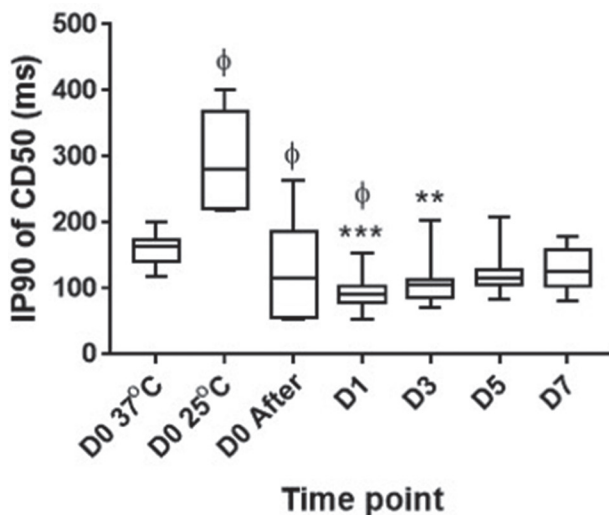


Figure 2. The IP90 range of CD50 values on different time points.

Sala, L., Van Meer, B. J., Tertoolen, L. G. J., Bakkers, J., Bellin, M., Davis, R. P., Denning, C., Dieben, M. A. E., Eschenhagen, T., Giacomelli, E., Grandela, C., Hansen, A., Holman, E. R., Jongbloed, M. R. M., Kamel, S. M., Koopman, C. D., Lachaud, Q., Mannhardt, I., Mol, M. P. H., Mosqueira, D., Orlova, V. V., Passier, R., Ribeiro, M. C., Saleem, U., Smith, G. L., Burton, F. L. & Mummery, C. L. 2018. MUSCLEMOTION: A Versatile Open Software Tool to Quantify Cardiomyocyte and Cardiac Muscle Contraction In Vitro and In Vivo. *Circ Res*, 122, e5-e16.

The British Heart Foundation

Dr. Xie He

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C017

Do repolarisation abnormalities disrupt calcium handling and contribute to impaired diastolic function in heart failure?

G.C. Anderson, J. Winter and M.J. Shattock

School of Cardiovascular Medicine and Sciences, King's College London, London, UK

Clinical studies have revealed an association between repolarisation abnormalities and diastolic dysfunction in heart failure with preserved ejection fraction (HFpEF)¹⁻³. It is unclear whether this delayed repolarisation may directly impair active myocardial relaxation by disrupting intracellular calcium (Ca) handling. Therefore, the present study aims to characterise the relationship between action

potential duration (APD/QT interval) and diastolic function. Dunkin Hartley guinea pigs (male, 500-750g) were anaesthetised (pentobarbitone, 160mg/kg, I.P.) and hearts excised and perfused with crystalloid buffer. Initial studies used optical mapping of Langendorff-perfused hearts (n=4) to demonstrate the relationship between APD and Ca transient duration. Hearts were paced at a range of cycle lengths, and incremental concentrations of E-4031 and HMR 1556 (max. 100nM E-4031 + 1000nM HMR 1556) were used to achieve pharmacological blockade of repolarising currents I_{Kr} and I_{Ks} . Voltage- and Ca-sensitive dyes (Rh237 and Rhod-2) were loaded to simultaneously record action potentials and Ca transients in the left ventricle (Fig. 1A&B). Separately, Langendorff-perfused hearts (n=6) were treated with E-4031 (100nM) and HMR 1556 (max. 300nM) or vehicle control during a paced protocol, and effects on electrophysiological and contractile activity monitored using an ECG and intraventricular balloon. Subsequently, isolated working hearts (n=6) were used to determine the effect of QT prolongation (100nM E-4031 + 30nM HMR 1556, or vehicle control) in the presence of sarco/endoplasmic reticulum Ca-ATPase (SERCA) inhibition (5 μ M cyclopiazonic acid). Function in the working heart preparation was monitored using a pressure-volume catheter positioned within the left ventricle. Results were analysed using two-way ANOVA with Sidak's *post hoc* tests, and effects with a p value of less than 0.05 relative to control were deemed statistically significant. A strong positive correlation was demonstrated between ventricular APD and Ca transient duration (Fig. 1C). In Langendorff-perfused hearts, QT prolongation did not impair systolic or diastolic function (Fig. 2A-C). In the working heart, QT prolongation did not lead to any additional impairment of function in the presence of SERCA inhibition (Fig. 3A-F). Our data show that delaying repolarisation (prolonging APD) leads to a substantial prolongation of the cytosolic Ca transient without affecting diastolic function. This also remains true in conditions of inhibited Ca reuptake, which mimics the impairment of cellular Ca handling commonly observed in the failing heart. Thus, while APD and QT interval changes in patients with HFpEF correlate with diastolic abnormalities, the present study suggests APD prolongation does not contribute to the functional impairment of ventricular relaxation.

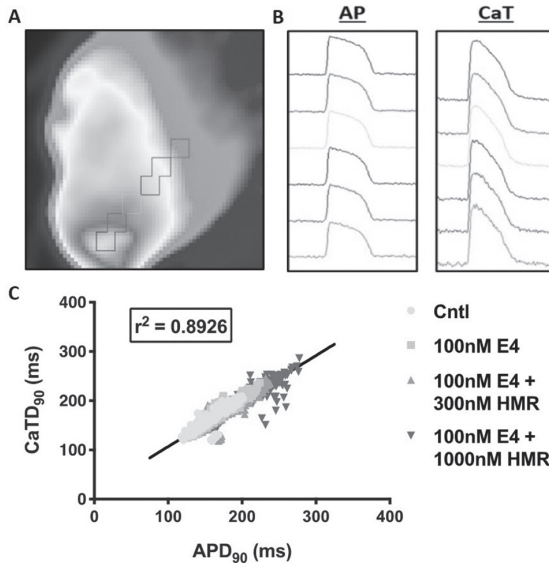


Figure 1. Relationship between action potential duration (APD) and calcium transient duration (CaTD) in the Langendorff-perfused guinea pig heart. (A) Representative image of the left ventricle obtained using optical mapping. (B) Representative averaged action potentials and calcium transients obtained from the regions shown in (A). (C) Effect of prolonging APD by altered pacing frequency or the use of E-4031 + HMR 1556 on CaTD. (n=4).

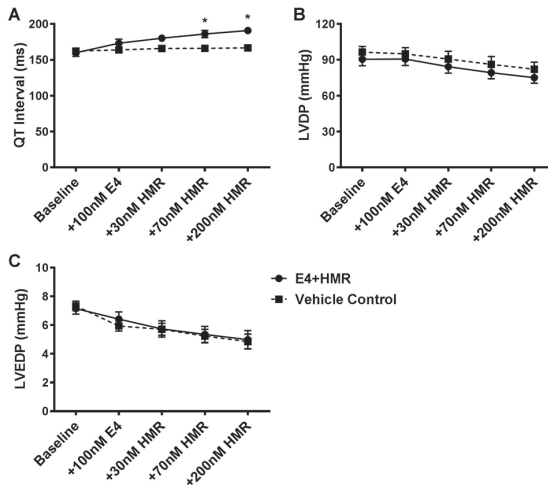


Figure 2. Impact of QT prolongation on function in the Langendorff-perfused guinea pig heart. Effect of cumulative doses of E-4031 and HMR 1556 on (A) QT interval, (B) left ventricular developed pressure (LVDP) and (C) left ventricular end diastolic pressure (LVEDP), relative to vehicle control. Two-way ANOVA with Sidak's *post hoc* test, *= $p < 0.05$. (n=6, values expressed as mean $\pm$ S.E.M.).

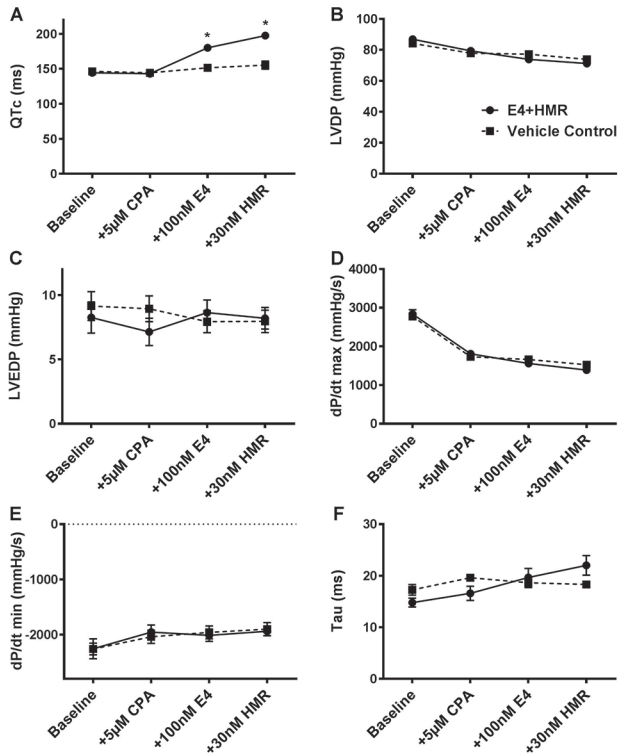


Figure 3. Impact of QT prolongation on function in the isolated working guinea pig heart in the presence of SERCA inhibition by cyclopiazonic acid (CPA). Effect of cumulative doses of E-4031 and HMR 1556 in the presence of 5μM CPA on (A) corrected QT interval, (B) left ventricular developed pressure (LVDP), (C) left ventricular end diastolic pressure (LVEDP), (D) dP/dt max, (E) dP/dt min and (F) time constant of relaxation (tau), relative to vehicle control in the presence of 5μM CPA. Two-way ANOVA with Sidak's post hoc test, *= $p < 0.05$. (n=6 values expressed as mean ± S.E.M).

Wilcox JE et al. (2011). *Am J Cardiol* 108, 1760-1766.

Sauer A et al. (2012). *Circ Arrhythm Electrophysiol* 5, 537-543.

Shah SJ et al. (2015). *Circulation* 131, 269-279.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C018

Antiarrhythmic effects of glucagon-like peptide-1

S. Mastitskaya¹, R. Ang¹, A. Tinker² and A.V. Gourine¹

¹Centre for Cardiovascular and Metabolic Neuroscience, University College London, London, UK and ²William Harvey Research Institute, London, UK

Incretin hormone glucagon-like peptide-1 (GLP-1) plays an important role in control of glucose metabolism and energy homeostasis. GLP-1R agonists also have significant beneficial effects on cardiovascular system (including anti-inflammatory, antiatherogenic, positive inotropic, glucose uptake stimulating, and vasodilatory effects). Despite significant research interest in cardiovascular biology of GLP-1, the presence of GLP-1 receptor in ventricular cardiomyocytes remains a controversial issue, and the effects of this peptide on the electrical properties of intact ventricular myocardium are unknown. We determined the effects of GLP-1R agonist exendin-4 (Ex4) on ventricular action potential duration (APD) and susceptibility to ventricular arrhythmia in the rat heart *in vivo* and *ex vivo*. Ventricular monophasic action potentials were recorded in anaesthetized (urethane) rats *in vivo* and isolated perfused rat hearts during sinus rhythm and ventricular pacing. *In vivo*, systemic administration of Ex4 (5 µg/kg intravenously) increased heart rate, and this effect was abolished by β-adrenoceptor blockade. Despite causing sympathetic activation, Ex4 increased APD during ventricular pacing by 7% ($P=0.044$; $n=6$) and reversed the effect of β-adrenoceptor agonist dobutamine on APD. In isolated perfused hearts, Ex4 (3 nM) increased APD by 14% ($P=0.015$; $n=6$) with no effect on heart rate. Ex4 also reduced ventricular arrhythmia inducibility in conditions of β-adrenoceptor stimulation with isoproterenol. Ex4 effects on APD and ventricular arrhythmia susceptibility were prevented in conditions of muscarinic receptor blockade or inhibition of nitric oxide synthase. These data demonstrate that GLP-1R activation effectively opposes the effect of β-adrenoreceptor stimulation on cardiac APD and reduces the ventricular arrhythmic potential in conditions of sympathetic activation. The effects of GLP-1R activation on ventricular electrical properties appear to be indirect, mediated by acetylcholine and NO, and, therefore, can be explained by recruitment of cardiac vagal neuron activity.

This work was supported by the British Heart Foundation (RG/14/4/30736 to Drs Gourine and Tinker; RG/15/15/31742 to Dr Tinker), European Union's Horizon 2020 research and innovation programme (Marie Skłodowska-Curie Grant No. 654691 to Dr Mastitskaya), Medical Research Council (MR/N02589X/1), and The National Institute for Health Research Barts Cardiovascular Biomedical Research Centre. Dr Gourine is a Wellcome Trust Senior Research Fellow (Ref: 200893).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C019

Comparison of the effectiveness of lectures based on problems and traditional lectures in physiology teaching in Sudan

N.A. Alaagib¹, O.A. Musa² and A.M. Saeed¹

¹*Department of Physiology, Faculty of Medicine University of Khartoum, Khartoum, Sudan* and ²*Department of Physiology, The National Rebat University, Khartoum, Sudan*

Lectures are one of the most common teaching methods in medical education. Didactic lectures were perceived by the students as the least effective method ⁽¹⁾. Teaching methods that encourage self-directed learning can be effective in delivering core knowledge leading to increased learning ⁽²⁾. Problem based learning (PBL) has been introduced as an active way of learning but it has some obstacles in developing countries where the intake is huge with minimum resources. This study introduces a new teaching approach: lectures based on problems (LBP) and evaluates its effectiveness compared to traditional lectures (TL) in physiology teaching. LBP and TL were applied in physiology teaching of medical students at University of Science and Technology during their study of introduction to physiology and respiratory physiology courses. Equal number of lectures was given as LBP and as TL in each course. In the steps of LBP a clinical problem is introduced to the whole class. Students in pairs analyze the problem for key words suggesting generally what the problem about and they share with the whole class what they have worked out. Students formulate learning objectives based on the scenario, and each pair of students should write down at least two learning objective. Then the instructor conducts a lecture and cover the intended objectives. The objectives of the problem can be achieved in more than one lecture with reference to the same problem. Later, in the small groups tutorial the problem is discussed again and students answer some questions based on the scenario. At the start of the next lecture, students are given quizzes on the previous one; answers are shared with the class.

Students were given quizzes at the end of each course which were used to compare the effectiveness of the two types of lectures. A questionnaire was used to assess students' satisfaction about LBP and the effect of the two methods on the students' attitude and practice towards learning physiology. Results showed that in LBP the students have better attention ($P= 0.002$) and more active role ($P= 0.003$) than in TL. Higher percentage of students think that LBP stimulated them to use references more ($P= 0.00006$) and to use the lecture time more effectively ($P= 0.0001$) compared to TL. However, there was no significant difference between LBP and TL in the awareness of the learning objectives. About 64% of students think that LBP is more enjoyable and it improved their understanding of physiology concepts. Comparison of the students' quiz marks showed that the means of the students' marks in the introduction to physiology and respiratory courses were higher in the quizzes of LBP than in TL with a significant difference between them ($P=.000$), ($P=.006$) respectively. These results suggest that LBP is an effective and active

learning method that achieved some of the objectives of PBL with the minimum resources and it can be used to improve the effectiveness of the lectures.

Table 1: Students' responses to certain items regarding the two type of the lectures

Item	In LBP	In Traditional Lectures	In both equally
More aware to the learning objectives	47.9%	29.5%	22.6%
More active role	53.4%	26.7%	19.9%
Better attention during the lecture	51.4%	24.0%	24.6%
Use the lecture time more effectively and efficiently	58.2%	24%	17.8%
Stimulated more to use references and study resources	58.2%	21.9%	19.9%
Think will score higher exam marks	35.6%	38.4%	26.0%

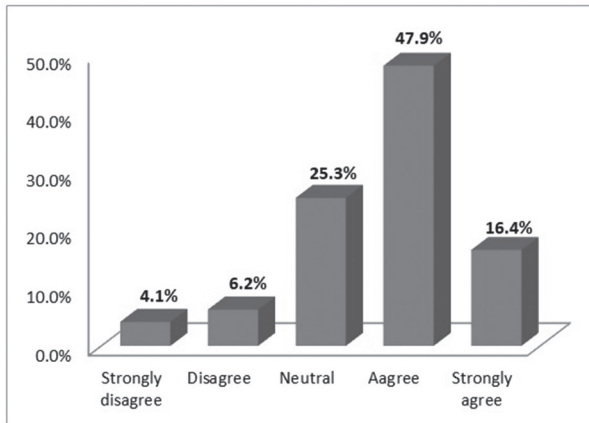


Figure 1: LBP improved understanding of physiological concepts

(1) Butler JA. Use of teaching methods within the lecture format. Medical teacher. 1992;14(1):11-25.

(2) Wolff M, Wagner MJ, Poznanski S, Schiller J, Santen S. Not another boring lecture: engaging learners with active learning techniques. Journal of Emergency Medicine. 2015;48(1):85-93.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Digital resources and student attainment, using learning analytics to support student success

L. Robson

Biomedical Science, University of Sheffield, Sheffield, UK

Students often don't instinctively know how to approach their studies, but the self-directed nature of higher education means it is not always easy to identify struggling students before they get to the module assessment. One of the areas that could help is learning analytics, data on what students do, when and how. However, while there is a wealth of information on these, what is not always clear is how these can be used to support students (Daniel, 2015). The aim of the current research was to examine whether student engagement with the virtual learning environment and lecture captures was correlated with student performance.

Students in the second year of their studies in Biomedical Science were monitored during the teaching period and just before the examinations in semester 1 of a whole academic year module. Data on their access to the virtual learning environment (VLE), formative feedback quizzes and lecture captures was recorded, and compared to student attainment in a multiple choice examination. Ethical approval was obtained from the University of Sheffield. Statistical analysis utilised either parametric or non-parametric tests, as appropriate for the data (normal distribution or not), and significance was assumed at the 5% level.

During teaching there was a significant positive correlation between the number of hours on the VLE versus grade in the MCQ examination ($r=0.38$, $n = 168$). Students achieving the highest grades spent 69.2 ± 4.20 hours on the VLE ($n=50$), while students achieving the lowest grades spent 36.1 ± 6.53 hours ($n=9$). Students who attempted the formative assessment quizzes during the teaching period were more likely to achieve a higher examination grade, 58.1 ± 1.49 ($n=85$) versus 66.9 ± 1.10 ($n=83$), for students who did not or did access the formative quizzes, respectively. The formative quiz grade appeared to be positively related to attainment. Both the number of lecture capture views and the mean percentage of videos watched were positively correlated with examination performance, $r = 0.29$ and 0.33 , respectively ($n=168$). Students achieving the highest grades had a mean number of views of 43.7 ± 6.40 ($n=50$) early in the semester, compared to 11.8 ± 3.92 ($n=9$) for those with the lowest examination grades. This difference was maintained by the start of the examinations, 157 ± 20.8 versus 37.2 ± 13.2 for the high and low achieving students, respectively.

These data suggest that student engagement with digital resources such as the VLE, formative quizzes and lecture captures is linked to academic attainment. Therefore, promotion of the use of these may have the potential to impact on the performance of previously lower achieving students. Moving forward, these data will be used to provide an evidenced based approach to help identify struggling students and shape how they engage with digital resources.

Daniel B (2015). *British Journal of Educational Technology* 46, 904-920

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C021

Contesting TESTA: Time on assessed tasks is important for student learning

N.S. Freestone

Chemical and Pharmaceutical Sciences, Kingston University, Didcot, UK

With the onset of student fees, universities are placing more emphasis on student progression and outcomes. This has led to the fear of “grade inflation” as universities try to gain a competitive advantage by emphasising positive student metrics. Consequently, the proportion of “good” degrees (Firsts and upper second class) awarded has greatly increased so that now 75% of all students graduate with a “good” degree. From 1994/95 to 2011/12 “good” degrees awarded increased by 113% (Bachan, 2017).

As a corollary to this increase a decline in academic standards and student effort has been reported. In terms of student effort the TESTA project (Transforming the Student Experience Through Assessment) has been influential in reducing summative coursework within many programmes. Much of the theoretical and intellectual justification for the TESTA project comes from Graham Gibbs who has demonstrated that students strategically game the system and adopt surface learning strategies to progress. Gibbs argues cogently that current assessment practices are neither effective for student learning nor efficient in terms of staff workloads. At the same time Gibbs has emphasised the concept of “time on task” (Chickering and Gamson, 1987). Here student effort is positively correlated to achievement. However, the move to reduce summative assessment might reduce student effort with negative impacts on student attainment. The use of institutional algorithms allows students to achieve higher degree classifications than they might have previously (Yorke et al, 2008). Thus, degree classifications currently do not provide a sound basis for indicating the quality of educational outcomes at UK institutions (House of Commons, 2009). Rather they are a way of enhancing an institution’s overall ‘status’.

In the current environment then can “real” student attainment changes be monitored?

Here we use the pedagogical gold standard of a standardised national test to evaluate the impact of a perceived decline in student effort on attainment. Pharmacy students in the UK undertake a year in practice preparing for their pre-registration exam. How effectively individual Schools of Pharmacy prepare their students for this exam can therefore be indirectly measured. Our hypothesis is that Schools of Pharmacy that teach their programmes via more, but smaller modules (in terms

of academic credits) will result in students doing more work, resulting in better outcomes in the national exam.

Indeed, those courses that taught using more modules per year achieved higher average student marks for their students than those teaching via fewer modules (89.3 ± 1.8 versus $83.6 \pm 1.6\%$ respectively; $p < 0.05$).

Whilst the move to improve student learning through appropriate assessment should be a primary concern of all involved in UK HE teaching these results indicate that thought must be used to ensure that such changes are not detrimental to students.

Bachan, R. (2017) Grade Inflation in UK Higher Education. *Studies in Higher Education* 42:1580-1600

Chickering, AW., Gamson, ZF. (1987) Seven Principles for Good Practice in Undergraduate Education. *American Association of Higher Education Bulletin* 39:3-7

Yorke, M., Woolf, H., Stowell, M., Allen, R., Haines, C., Redding, M., Scurry, D., Taylor-Russell, G., Turnbull, W., Walker, L. (2008) Enigmatic Variations: Honours Degree Assessment Regulations in the UK. *Higher Education Quarterly* 62:157-180

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C022

An Exploratory Study of Designing and Developing Core Physiology Curriculum for Pre-registration Nursing Education

A.F. Wood¹, C. Chandler⁴, S. Connolly⁴, C. Davis², G. Finn⁷, A. Grant³, J. Jolly⁵, A. Powell⁶ and C. Redmond²

¹School of Health and Social Care, Edinburgh Napier University, Edinburgh, UK,

²University College Dublin, Dublin, Ireland, ³Glasgow Caledonian University, Glasgow, UK, ⁴University of Edinburgh, Edinburgh, UK, ⁵University of Leeds, Leeds, UK,

⁶Birmingham City University, Birmingham, UK and ⁷Hull York Medical School, York, UK

Physiology is considered a core component for all nursing and midwifery courses, however there is a lack of clarity on content across higher education on what is included in this core component. Physiology is part of 'bioscience' education, within pre-registration nursing (and midwifery) programmes, but the content and delivery is highly varied, lacking consistency and parity across different institutions and education programmes in the UK (McVicar et al 2015). The bio-scientific nursing community have warned that nurses pre-and post-registration lack the confidence to apply and articulate biological knowledge to patients and to other health-care professionals respectively. Despite evidence highlighting concerns over nurses lacking bioscience knowledge and unsafe practice (Taylor et al 2015), there is no universal agreed curriculum for the detailed outcomes. This study aimed to inform the creation of a discipline specific physiology core curriculum that is agreed by stakeholder groups vested in nursing and physiology education to

clarify relevant physiological topics required in pre-registration nursing. Initially, 360 learning outcomes were identified from textbooks, online sources and the Bioscience in Nurse Education group (BiNE). Using a modified Delphi approach, an expert panel of 8 from BiNE (all academics who teach nursing and/or physiology) reviewed and modified the list resulting in 195 proposed outcomes. The outcomes were grouped using a systems approach for circulation through an online survey. The first round was circulated to Universities in the UK who teach Nursing (n=65). It allowed for all outcomes which had 80% to be automatically included to the next round, and comments could be made on each outcome. At this point 35 outcomes were to be modified, one to be added and 14 rejected (response rate 21%). The panel reviewed the modifications prior to the second round survey. The second round allowed either accept or reject, and comments could be added at the end of each system. From the 182 outcomes circulated (response rate 23%), 177 outcomes were accepted, with 4 were rejected and one was removed by the panel consensus due to replication. The majority, 106 of these 177 outcomes reached 100% consensus from both the expert panel and the second round Delphi survey. Only 4 outcomes were rejected (less than 80% consensus) through the second round of the survey suggesting high consensus from the participants who are experts in this subject group. This work provides guidance for higher education institutions to deliver a comprehensive education for student nurses to support fit for purpose graduates. This core curriculum does not suggest how it is delivered but gives the basic level required for qualification as a nurse to commensurate with the Nursing and Midwifery Council (NMC)(2018) new standards for the 'future nurse'.

McVicar A., Andrew S., Kemble R. (2015) The 'bioscience problem' for nursing students: an integrative review of published evaluations of year 1 bioscience, and proposed directions for curriculum development. *Nurse Education Today*. 35, 500-509

Taylor V., Ashelford S., Fell P., Goacher P.J., (2015) Biosciences in nurse education: is the curriculum fit for practice? Lecturers' views and recommendations from across the UK. *Journal of Clinical Nursing*. 24, 2797-2806.

Nursing and Midwifery Council (NMC) (2018) *Future nurse: Standards of proficiency for registered nurses*. UK: NMC. Retrieved from:

<https://www.nmc.org.uk/globalassets/sitedocuments/education-standards/future-nurse-proficiencies.pdf>

This study was funded by the Physiological Society and the David Jordan Teaching Award Scheme.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Student perceptions of gamified learning activities.

D. Bovell, S. Holroyd, A. Sultan, S. Latifi, M. Healy and J. Gray

Medical Education, Weill Cornell Medicine in Qatar, Doha, Qatar

Background: Modern learners find classic didactic lectures ineffective¹ and favour more interactive teaching modalities². In response to requests for more active learning sessions, we incorporated flipped classroom (FCR) activities with quiz games into subject reviews. Here we present continuing data from undergraduate students (Ug) and between 1styr (M1) and 2ndyr medical students (M2). The data allows us to examine whether the incorporation of gaming into the student's learning has continued to have a beneficial effect.

Activity: We described previously where FCR sessions for Ug, M1 and M2 students, were augmented with a review activity conducted using gaming and students surveyed about the usefulness of the gaming activity. In this study, we added two further quiz gaming formats to the FCR review sessions and compared the effectiveness of using polleverywhere® (PEW) and Kahoot!® (KT) for Ug students, and for previous M1 students who had progressed to M2 level, we compared the effectiveness of using PEW and Double Jeopardy® (DJ) formats.

Results: Preferred modality for Ug students- A comparison of PEW (n= 31) and KT (n= 32) modalities, showed that both were very effective in delivering review content and making the students feel engaged. A Mann-Whitney test indicated that KT (Median_{KT} = 4.5) was preferable to PEW (Median_{PEW} = 4.0), by students for delivery of materials, U= 226, p< 0.01. A similar preference was observed for the students' engagement (Median_{KT} = 5.0, Median_{PEW} = 4.0; U= 175, p< 0.01).

Does preference change over time- A comparison of student responses M1 (n= 28) and M2 (n = 47) showed that the level of effectiveness and engagement remained high and that there was no significant difference between the M1 and M2 years using PEW to deliver content reviews (Median_{M1-PEW} = Median_{M2-PEW} = 4.0, U = 604.5, p = 0.531).

The findings suggest that DJ was very effective for engagement in M2 class review sessions, and while there was no significant difference in the levels of engagement between PEW (n=47) and DJ (n=19); students did show a slight preference for the DJ activity (Median_{PEW} = 4, Median_{DJ} = 5, U= 379.5, p = 0.363).

Discussion: The results show that students find the inclusion of gaming activities very effective both for reviews and engagement level and that the level of effectiveness does not diminish between years. The introduction of different modalities throughout the course, was also well received. However, KT has limited use, as there are character limits for questions and answer options, however Ug students viewed it as great fun. Additional student comments included: more time to read questions; harder questions; more discussion of answers; slower pace.

Conclusion: Students continue to find the use of gaming to be very engaging and effective in reviewing course materials and that using different modality can be effectively used without loss of perceived benefits.

Boeker M, *et al.* Game-based e-learning is more effective than a conventional instructional method: a randomized controlled trial with third-year medical students. *PLoS One.* 2013 Dec 5;8(12):e82328

Akl EA, *et al.* The effect of educational games on medical students' learning outcomes: a systematic review: BEME Guide No 14. *Med Teach.* 2010 Jan;32(1):16-27

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C024

Impact of Year 1 Attendance on Exam Performance throughout Medical School

C. Torrens and A.P. Sampson

Medicine, University of Southampton, Southampton, UK

The impact of student attendance at learning sessions on their exam performance is controversial, with reports of declining attendance associated with technologies such as lecture capture (Edwards & Clinton, 2019). Students may prefer to access material online rather than engage with academic staff (O'Malley *et al.* 2018), but poor attendance may have a detrimental effect on performance (Mills *et al.* 2009). Relatively little is known about this relationship in medical students, but we hypothesised that attendance in Year 1 would predict medical student performance in assessments throughout their 5-year programme.

The study was approved by the Faculty Ethics Board (ERGO II #9191). The Medicine programme in Southampton had an annual entry of approximately 200 students over the course of the study. Attendance of Year 1 students was monitored by signing in at random lectures one or more times a week and in the majority of small-group sessions (tutorials, workshops & practical classes). The number of sessions attended by each student was expressed as a percentage of the total number of monitored sessions. Using a simple regression model, Year 1 attendance scores were correlated with student performance in each decile in the end-of-year assessments in Year 1, in Year 2 (another preclinical year) and in the written papers of Finals examinations (in Year 5). Comparisons between groups were made using the Mann-Whitney U test. Significance was accepted at $p < 0.05$.

Attendance was monitored in 33 lectures and small-group sessions across Year 1, producing an attendance score for 197 students who sat the assessments at the end of year. Linear regression showed a highly significant correlation between attendance and Year 1 assessment decile ($r^2 = 0.74$, $p < 0.005$). The same cohort's Year 1 attendance also correlated significantly with their performance in Year 2 assessments ($r^2 = 0.84$, $p < 0.001$). A positive relationship was further seen between Year 1 attendance and Final Year assessment performance in the 57% of students

who progressed to Final Year on schedule ($r^2 = 0.37$, $p = 0.06$, $n = 112$). Students with attendance scores above 80% in Year 1 were significantly more likely to progress to Final Year on schedule compared to those with lower Year 1 attendance ($p < 0.05$). Our 5-year study at a UK medical school shows that good student attendance in Year 1 not only correlates with assessment performance in the pre-clinical years of the programme but also predicts whether students will progress on schedule to Year 5 and correlates with their performance in Finals exams. This initial analysis suggests that encouraging good pre-clinical attendance in order to fulfil professionalism requirements for medical students may also improve their academic and clinical performance across the Medicine programme.

Edwards MR & Clinton ME. 2019, *High Educ.* 77: 403

Mills RM, Dyson S & Cannon D. 2009, *Adv Physiol Educ* 33 139-143

O'Malley D, Barry DS & Rae MG. 2018, *Proc Physiol Soc* 41, C114

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C025

Transforming physiology tutorials for medical students through transdisciplinary collaboration with drama studies; taking the physiology tutorial case from the page to real life

S.M. Roe¹, M. McGahon¹, S. Parkinson¹, E. Tansey¹ and P. Murphy²

¹Centre for Biomedical Sciences Education, The Queen's University of Belfast, Belfast, UK and ²Drama Department, School of Arts, English and Languages, The Queen's University of Belfast, Belfast, UK

There has been a recent drive from the General Medical Council to embed what have hitherto been known as “soft” skills of communication and empathy more deeply within curricula. In the 2018 “Outcomes for Graduates” document, these are cited in the “communication and interpersonal skills” section where “sharing”, “empathy” and “compassion” are referenced (General Medical Council, 2018). This drive is reflected in the medical education literature where replacing the terms “non-technical skills” with “behavioural” skills has been advocated (Nestel *et al.* 2011). Behavioral skills training underlies recent work from Queens University Belfast involving drama and final year medicine students during which they collaborate in realistic and emotionally charged simulations of doctor patient interactions (Walsh & Murphy, 2017). This received positive feedback from both cohorts and improved clinical team performance scores. We adapted this transdisciplinary, high-fidelity simulation approach to second year physiology tutorials. These lend themselves well to the application of basic science skills to clinical (and in this case behavioural) contexts (Michael, 2006). Benefits are manifold: emphasising the holistic importance of physiology to patient care, while also embedding “human factors” skills throughout the entire medical curriculum.

To prepare, academics and students of the 2nd year medical physiology module attended acting classes based on aspects of Konstantin Stanislavski's "System" (Stanislavski & Benedetti, 2016) as part of a "Healtheatre" club. This culminated in March 2019 when drama students participated in a "physiology of hypofertility" tutorial, playing a 34 year old woman with premature menopause. The drama students analysed the data and details of the clinical scenario to develop the "given circumstances" of the role, enabling them to effectively become the female in the scenario. There were two drama students for every group of 23 medical students. Debriefing sessions were held afterwards, and all involved were invited to email their impressions to the module coordinator.

Drama students noted the medical students' professionalism at all times, a difficulty with "personal" terms and their struggle to balance truth-telling and compassion in the tough diagnosis. Medical students gave mixed responses, some deeming the tutorial more engaging, authentic and unpredictable than even general practice family attachments with genuine but "ideal" patients. Others felt, however, that it encroached on "real" science skills. Academics also highlighted efforts to be factual but compassionate among medical students and noted the awkwardness they had with the more "personal" interactions. All emphasized the need for more academic scaffolding and preparation for the tutorial, along with a desire for more drama students.

These are considerations that will factor in ongoing collaborations between Drama Studies and Physiology that will continue next year.

General Medical Council (2018). In *Outcomes for Graduates 2018* London: General Medical Council.

Michael, J. (2006). Wheres the evidence that active learning works? *Advances in Physiology Education* 30, 159-167

Nestel, D., Walker, K., Simon, R. Aggarawal, R. & Andreatta, P (2011). Nontechnical skills: An inaccurate and unhelpful descriptor? *Simulation in Healthcare* 6 (1), 2-3

Stanislavski, K. & Benedetti, J. (2016). In *An Actor's Work*, 1st ed.; Oxon, NY, USA: Routledge.

Walsh, I. & Murphy, P. (2017). Healtheatre: Drama and medicine in concert *Healthcare* 5, 37-43

The authors acknowledge the contribution of Ms. Rosie Pelan of QUB Drama Department to the drama training of staff and students

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Image fraud and confidentiality as vehicles for learning about ethical issues in medical science teaching, a pilot study

J.S. Gregory and D. Scott

University of Aberdeen, Aberdeen, UK

Graduate attributes are widely used by Universities to identify the qualities and skills developed by their students. Though definitions vary between universities, ethics, professionalism and citizenship are included by the majority of UK institutions (Lipan et al. 2019). Biomedical imaging has been a growth area with advances in technology with computing power massively increasing the scope, speed and scale of what can be achieved. Alongside this arise ethical issues particularly in the areas of security, anonymity and fraud. Tackling these issues alongside methods of analysis provided an opportunity to directly address “Active Citizenship” graduate attributes alongside academic knowledge.

The graduate attributes chosen (www.abdn.ac.uk/graduateattributes/) were from the Active Citizenship area, specifically 1) an awareness and appreciation of ethical and moral issues and 2) an understanding of social and civic responsibilities, and of the rights of individuals and groups. These areas were specifically highlighted to the 20 students in an undergraduate anatomy course. The first topic covered was the risks and benefits of harnessing big data. Then, during a practical workshop, the controversial area of imaging fraud in research publications, focussing on published work (Bik, Casadevall and Fang, 2016) but also a related Twitter feed. This showed that whilst image fraud was straightforward (and tempting) to commit, it was easy to detect and had severe consequences, completely undermining the credibility of the research and researchers. The final area was that of data privacy. Examples were shown of the information hidden within image headers, including medical images (Dicom files), where personal information including a patient’s name, date of birth and hospital ID are easily accessible. This was followed by instructions for securely and automatically anonymising these files and a discussion about research study design and the importance of considering the risk of de-anonymization. Annual student feedback was used to audit whether student’s reflections of the course showed an increase in awareness of graduate attributes. The question “the course improved your graduate attributes employability” showed that this year 80% of the students agreed (score 3/5 and above) which was marginally (but not significantly) higher than the previous two years (67% and 75%). These results may not truly reflect the graduate attributes alone as the question compounded graduate attributes and employability.

Identification of problems or controversial areas in the field can provide a good platform for discussion within a course and also help to build graduate attributes alongside academic knowledge. More work may however need to be done to highlight these areas to the students as they arise and in student feedback, it may

be beneficial to separate graduate attributes from employment to identify the success of this approach.

Bik, EM., Casadevall, A and Fang, FC (2016) *mBio*, 7(3) e00809-16

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C027

Are Human Volunteer Practicals Essential for First Year Undergraduates Studying Human Physiology?

E. Sheader, T. Pocock, M. Keown and T. Speake

Faculty of Biology Medicine and Health, The University of Manchester, Manchester, UK

Human volunteer practicals are an integral component of our undergraduate bioscience programmes at the University of Manchester. These practicals are always highly rated by students across the bioscience programmes (rated best year 1 practicals 2010-2019) and have survived many revisions of the curriculum over the last 20 years. However, as student numbers continue to rise and the administrative burden (of satisfying) and obtaining approval from the University Research Ethics Committee increases, the logistics of running these practicals are now being questioned. This study examined student perceptions of these practicals across the 1st year bioscience courses, and tried to support or dispute the notion that these practicals are essential for undergraduate science students.

Currently, all bioscience students take 15 practicals in year 1 of which 7 are human volunteer practicals. These include two human volunteer practicals (Haematology and BP and Pulses) in semester 1 which are taken by all 650 year 1 students within the School of Biological Sciences and five semester 2 practicals (Drug action on the Eye, Asthma treatment, Lung Mechanics, EMG and Nerve Conduction, and ECG) which are taken by approximately 350 students.

Students completed a survey on their perceptions of the validity of these practicals to their degree programme and the skills acquired. Data from our initial evaluation shows that all students found these human volunteer practicals useful in supporting physiological knowledge and core degree programme units (60% extremely useful, 27% fairly useful, 13% somewhat useful). When asked what skills they felt these practicals had enhanced, responses included: measurement of physiological parameters (79%), accuracy and precision (64%) and data handling (52%). Furthermore, 75% of students stated that these practicals improved their communication skills and 79% felt that these practicals had enhanced their capacity for teamwork. This perhaps suggests student's value human interaction over simulation. In addition, 91% students highlighted that these practicals raised their awareness of the ethics of research involving human volunteers.

This initial evaluation suggests that students feel these practicals are still valid for enhancing and developing generic skills. Moreover, they feel these practicals

improve communication and teamwork, which is of interest in our advancing technological age. In addition, they serve to support and reinforce our core bioscience course units and they still hold great value to students. This study supports the inclusion of human volunteer practicals in our core curriculum.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C028

Increasing public engagement opportunities for final year physiology focused science communication projects

M. Keown and E. Sheader

Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

All University of Manchester (UoM) SBS Honours undergraduate students complete a final year research project. There are a number of different project types ranging from the traditional laboratory to science communication, including education and science media projects. These science communication projects often provide students with an opportunity to engage with people within the local community in various settings. As our student numbers rise, we are under increasing pressure to identify more opportunities for students to carry out these projects with. UoM hosts a public engagement schools fair annually during British Science Week (BSW). For the last 3 years, we have utilised this event for our students to deliver their physiology focused education projects. Each year, up to four students have delivered their projects in this setting. This study aims to evaluate this involvement and experience for our project students as well as the benefits to the attendees. Across the week, approximately one thousand 11-14 year olds from local secondary schools and two hundred 10-11 year olds from local primary schools attend.

These projects therefore must map onto areas of either the KS2 or KS3 curriculum related to human biology. Example projects include: an interactive resource and game on how the heart works; what is blood made of using a model and a life size interactive GI tract model to show how the body handles water.

Through delivery of these projects, our students gain an appreciation of the social responsibility aspect of UoM as well as exposure to importance of widening participation in HE. Preliminary results show this experience helps our project students improve their communication of science to a unique audience. Furthermore, it is an opportunity to use their interactive physiology focused teaching tool that they have designed and developed. This also helped develop their project management skills and improve their ability to troubleshoot. Individual comments included: 'although it was based around education and communication it was extremely beneficial for my current job which is based around building medical education materials (from Pharma companies) for HCPs' and 'It was essential in showing me that I could still work in science without being in a lab'

For the attendees, it offers an opportunity for exposure to HE as well introducing them to different scientific subject areas in an interactive education setting outside the school classroom. All children attending our physiology stand commented positively. Some common words in their feedback on the learning experience included 'fun', 'creative', 'making', 'explained' and 'clear'. In addition, these science communication projects in combination with the public engagement setting are vital in increasing awareness of physiology as a subject to the next generation of scientists.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C029

Do pre-clinical medical students prefer summative or formative evaluations in physiology?

M.G. Rae and M.H. Abdulla

Physiology, University College Cork, Cork, Ireland

The perception of some students that physiology is a “hard” subject to learn has been well documented¹. As such, numerous strategies have been employed by educators to assist students with their understanding and acquisition of physiological concepts. One such strategy is to provide students with regular evaluations to allow both student and educator to gauge the level of understanding of particular topics. However, it remains unclear what form of evaluation students prefer and, empirically, which best facilitates student learning and understanding. In this particular study we sought to determine whether students in the first year graduate entry to medicine (GEM) programme at University College Cork preferred to sit either summative individual evaluations in physiology for a certain percentage of marks, or formative, no stakes assessments, individually or in a group, with immediate feedback from the questioner.

The study was conducted over the first two semesters of the GEM programme. In semester 1 students each sat 5 separate summative 20 question evaluations on computers, with each test worth 0.2% of their final end of semester physiology mark (*i.e.* 1% in total), and feedback on the questions provided at a later date. In semester 2, students sat 4 entirely formative evaluations, with each conducted using the cloud-based student response system, Socrative. For these tests, students could participate either individually or in collaborative groups (to facilitate peer instruction). At the end of the study period, 54 students (66.7% of class; 27 male, 27 female) completed, or partially completed, an anonymised survey, collecting information on both student demographics and their views on the summative and formative evaluations performed in semesters 1 and 2.

Survey responses revealed that students found both forms of evaluation almost equally useful both for providing feedback about their understanding of physiology material (86.5% strongly agreed (SA) or agreed (A) for summative vs 88.5%

SA or A for formative) and for identifying deficits in their physiology knowledge (75% SA or A summative vs 78.8% SA or A, formative). However, a significantly larger number of students indicated that they felt that the summative evaluations provided a greater motivation to study and keep up with taught material than the formative style of evaluations (82.7% SA or A, summative vs 63.5% SA or A, formative; $p=0.015$, unpaired t -test, $n=51$). In spite of this finding however, slightly more students indicated that they would favour the formative (31.4%) to the summative (29.4%) evaluations if they were given a choice, although this difference was not significant.

Our findings indicate that pre-clinical medical GEM students value both summative and formative evaluation roughly equally to facilitate their learning and understanding of physiology, although for slightly differing reasons.

Slominski T. *et al.* (2019) Physiology is hard: a replication study of students' perceived learning difficulties. *Advances in Physiology Education*, 43, 121-127.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C030

A Video on the Pathophysiology of Heart Failure for Reinforcing First Year Medical Students' Learning: Do Video Techniques that Break from Medical Education Tradition Undermine the Video

H.J. Witchel

Neuroscience, Brighton and Sussex Medical School, Brighton, UK

Heart failure is conceptually more difficult to learn than myocardial infarction, and many students who are taught about both at the same time will later remember and understand MI while retaining misconceptions and uncertainties about heart failure. The fact that heart failure is a physiological and functional description, rather than an anatomical/structural description of a specific injury that can be pinpointed, and that heart failure has many diverse causes (including thyrotoxicosis) underscore the potentials for confusion that bedevil first year undergraduate students. While there are a growing number of educational videos on heart failure, most of them take the form of a talking head or an animated whiteboard. In our partially-animated video we take three approaches that violate traditional medical educational video-making to determine whether any or all of them are truly sacred: 1) to have a dialogue (physiologist to actress/patient) rather than a monologue voice-over, 2) to include upbeat music to lighten the atmosphere, and 3) to use humorous metaphors to explain and reinforce difficult concepts (Figure 1). In addition the video ends with an in-video quiz, to take advantage of the known effects of intercalated formative testing on computer-based learning [Szpunar *et al.* 2013]. Before the video is generally released, it is being laboratory-tested on students in its current form. Meanwhile, we seek feedback from our teaching colleagues at

Physoc on ways to improve or reformulate this kind of video. This video results from the David Jordan Teaching Award from the Physiological Society (2017). To watch with music: <https://youtu.be/PycKqILSXB4>
Without music: <https://youtu.be/bXLoGgu1y50>

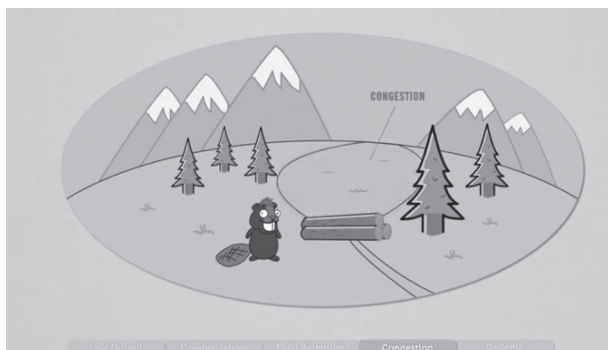


Figure 1. A still from the video. To explain congestion, the metaphor of a dam is used.

Szpunar, K. K., Khan, N. Y., & Schacter, D. L. (2013). Interpolated memory tests reduce mind wandering and improve learning of online lectures. *Proceedings of the National Academy of Sciences*, 110(16), 6313-6317.

We gratefully acknowledge the Physiological Society for the David Jordan Teaching Award (2017), Brighton and Sussex Medical School, and the students of our Student Selected Component who contributed to this project.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C031

A proposal concerning what to do about p values

D. Colquhoun

NPP, UCL, Kings Langley, UK

The reproducibility of results in both clinical and preclinical science is lower than we had supposed. This is serious because every false positive result that is published provides ammunition for those who oppose science. There are many reasons for irreproducibility, not least the perverse incentives imposed on scientists to publish too much. But one of the less-appreciated reasons lies in the misinterpretation of tests of statistical significance. Tests of significance give you a p value. If you ask what a p value means, the most common answer you'll get is that it is "the probability that your results occurred by chance" (1). This is plain wrong (2). I take it that what we want to know is the probability that one will claim that there is an effect when in fact the observations arose by chance only. This quantity may

appropriately be called the false positive risk (FPR) (3-5) and it's not the same thing as the p value. Here's why. It would obviously be a mistake to confuse the probability that you have four legs given that you are a cow, with the probability that you are a cow given that you have four legs. The latter is obviously much smaller than the former because there are many non-bovine creatures that have four legs. The former is large because there aren't many three-legged cows. Confusion of FPR with the p value is to make an exactly analogous mistake. The p value is the probability that you make your observations (or more extreme ones) given that the null hypothesis is true. The FPR is the probability that the null hypothesis is true given our observations. These two quantities are quite different, and, under almost all circumstances, the FPR is bigger than the p value. Suppose that you observe $p = 0.047$. That implies, for a well-powered experiment, a FPR of at least 20% (there are various ways of calculating this number but most of them give a value of between 20% and 30% (5)). Your chance of making a fool of yourself is not 5%, as most people still seem to think. It's at least 26%. That means that the term "statistically significant" tells you remarkably little about the truth of a hypothesis. I propose that journals should require not only a p value and confidence interval, but also one more number that gives a realistic idea about what really matters, the FPR (3-5). The simplest way to do this would be to give the FPR that is calculated on the assumption that there was a 50:50 chance of there being a real effect before the experiment was done, which may be dubbed the minimum FPR. This is the easiest solution to understand, because it tells you much the same thing as people mistakenly think the p value tells you. It would be overoptimistic for implausible hypotheses, but it would be a great improvement on the present convention. The term "statistically significant" should never be used. Just give the numbers.

Gigerenzer G, Krauss S, Vitouch O. The Null Ritual. What You Always Wanted to Know About Significance Testing but Were Afraid to Ask. In: Kaplan D, editor. *The Sage handbook of quantitative methodology for the social sciences*. Thousand Oaks, CA: Sage Publishing; 2004. p. 391-408. Available at https://library.mpib-berlin.mpg.de/ft/gg/GC_Null_2004.pdf

Wasserstein RL, Lazar NA. (2017) The ASA's Statement on p -Values: Context, Process, and Purpose. *American Statistician* 2017;70(2):129-33. Available at <https://amstat.tandfonline.com/doi/abs/10.1080/00031305.2016.1154108>

Colquhoun D. (2017) The reproducibility of research and the misinterpretation of P values. *Royal Society Open Science*, 4: 171085.<http://dx.doi.org/10.1098/rsos.17108>

Colquhoun D. (2018). The false positive risk: a proposal concerning what to do about p -values (version 2). <https://www.youtube.com/watch?v=jZWgijUnxl>

Colquhoun, D. (2019). The false positive risk: a proposal concerning what to do about p values. *American Statistician*. In the press. Available at <https://arxiv.org/abs/1802.04888>

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Development of flexible experiential learning exercises to deliver online practical teaching

S.J. Tucker

School of Medicine, Medical Science and Nutrition, University of Aberdeen, Aberdeen, UK

Introduction

With increasing student numbers and timetable constraints, there is a limited capacity to engage students in laboratory exercises. Furthermore, certain key protocols are not feasible due to costs and the length of time they take to complete. Most practical classes are procedural in nature, with a strong decision making element woven through. These decisions at key points in the protocol influence the outcome and so provide directed stages for assessment and feedback. This project showcases the development and use of visual, interactive practical simulations to address these challenges.

Process

Using an online assessment software package, practical procedures were mapped as a series of steps, instructions and questions where students move through the protocol, making decisions and answering questions learning how their actions influence the the experiment. Each decision is linked to feedback branches and loops to develop an understanding of the procedure. These approaches have been used to equip students with working experience of Western blotting, standard curves, serial dilution and cell culture in a safe, cost free environment, where learning and experience is enriched in a risk free, sustainable way.

Results

The extremely high level of student engagement (when exercises available as a non-compulsory link in the virtual learning environment) is presented in Table 1 demonstrating that these different exercises were engaged with multiple times by individuals across the whole cohort. The impact on the student experience was evaluated in the student feedback survey exercise and Table 2 shows the overwhelmingly positive reception across the cohort for all of these exercises.

Flexibility of these tools is evidenced by embedding them in:

- UG courses (preparation around core laboratory skills)
- PGT courses (transitional support for students with varying practical skills)
- PGR training prior to work with specific protocols
- online courses and distance learning modules

Discussion

The design is simple, adaptable and innovative. It creates solutions for many educational challenges, and has been very positively received by the students. Furthermore, students across the breadth of the test cohort engaged with these simulations despite them not being a compulsory part of the taught materials. Finally, the flexibility means this approach can be readily used across a range of subject areas in life science.

Table 1: Student engagement measures

Non-compulsory cell culture simulation: PGT cohort (n = 93)	
Total number of accesses	387
% students completing at least once	95.7%
Mean number of completions/student	3.1 ± 0.9
Non-compulsory mole calculation/serial dilution simulation: PGT cohort (n = 93)	
Total number of accesses	303
% students completing at least once	85.7%
Mean number of completions/student	2.5 ± 1.2
Non-compulsory standard curve simulation: PGT cohort (n = 93)	
Total number of accesses	311
% students completing at least once	89.3%
Mean number of completions/student	2.9 ± 0.7

Table 2: Student survey results

Simulation survey: PGT cohort (n = 93)	
Survey question	% ± SD who strongly or generally agree
Simulation developed understanding of the procedure	93.2 ± 7.2%
Simulation enhanced confidence in ability to conduct the procedure	83.7 ± 5.7%
Simulation provided skills necessary for the procedure	71.9 ± 10.7%

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C033

Physiology for all: Increasing undergraduate student choice increases engagement with physiology

S. Hall, S. Amici-Dargan and S. Rutherford

School of Biosciences, Cardiff University, Cardiff, UK

Student choice has now become central to the design and delivery of many university curricula, allowing undergraduate students to tailor their degree to reflect their own specific interests. Over the last decade, the suite of Bioscience degree programmes at Cardiff University has been restructured and developed to offer students more choice of modules and more opportunity to switch degree programmes as their degree progresses. All of our students now complete a common core first year diet comprising six modules spanning the spectrum of biosciences, from molecular biology to ecology. From 2013-14, all students have been exposed to physiology in their first year (20 credit module, Level 4), regardless of their initial entry degree, and are free to change into the BSc (Hons) Biomedical degree programmes (including Physiology) after this core year. The impact of this change has been reflected as a clear increase in the proportion of students electing to study advanced physiology modules in the second year of their degree (Level 5), from 18% ($n=68/374$) in 2013-14 to 37% ($n=168/450$) in 2014-15. The proportion of students on physiology modules has remained high in subsequent years, with almost half of the entire Biosciences cohort selecting at

least one physiology module each year; in 2018-19, 43% of students ($n=202/466$) have chosen to complete the 40-credit Physiology module in the second year of their degree.

Increased exposure to physiology content in the early years of the degree has also led to a large increase in the number of students electing to graduate with a Physiology degree. In 2014-15 (the last cohort before introduction of the core curriculum in year 1), there were 14 graduates (15% of total from Biomedical Sciences degree programmes and 5% of all Bioscience graduates); in 2015-16 (the first cohort to complete the common core), this number had increased to 38 (27% of all Biomedical Sciences graduates and 8% of all graduates from the School of Biosciences). The proportion of Physiology graduates each year has subsequently remained stable at this higher level.

Our data suggest that wider inclusion of physiology components in the early years of related degree schemes allows students to make more informed choices about their degree discipline, and that inclusion of physiology in the core curriculum facilitates selection of Physiology as an exit degree. Furthermore, our BSc (Hons) degree programme now benefits from the contributions of colleagues across the School of Biosciences, reflecting the integrated, interdisciplinary nature of modern physiology.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C034

Are learning technologies a barrier to education? – The results of a survey investigating engagement with an online development portfolio for bioscience students.

M.E. HARDY, H.A. Khan and S. Howells

School of Pharmacy and Medical Sciences, University of Bradford, Bradford, UK

Portfolios allow staff/students to reflect upon their personal and professional development and to monitor their skill-set. The University of Bradford recently adapted the personal and professional development portfolio for Clinical Sciences Foundation students from a word document into an online assessment utilising PebblePad software. The portfolio assessed students on a number of tasks, including: a self-assessed skills audit; reflection upon prior and current learning; SMART action plans, to identify barriers to learning and implementing strategies to overcome them; communication via a blog and the development of a plan to improve presentation skills; and also students' ability to respond to feedback. The role of the personal tutor was to provide guidance during meetings and both oral and written feedback. We utilised the recent change to an online format to investigate whether technology is a barrier to students' engagement with the portfolio.

Two weeks prior to portfolio submission, students were surveyed with 20 questions to identify characteristics regarding background, use of computer applications and engagement with the portfolio. Responses to the survey were anonymous and ethics approval was granted by the Chair of the Biomedical, Natural, Physical and Health Sciences Research Ethics Panel at the University of Bradford.

97 out of 188 students responded to the survey. Of these, 78% identified as British/British with dual nationality. 51% identified as Asian, 19% as Caucasian, 17% as black, and 5% as mixed/multiple ethnic groups. The demographic of students surveyed is dissimilar to the national enrolment statistics in Higher Education in the UK (77% White, 10% Asian, 7% Black, 5% Other in 2016-7)^[1].

Most students agreed or strongly agreed that they had engaged with the portfolio (75:12:11, % strongly agreed/agreed: neither agreed nor disagreed: disagreed/strongly disagreed). The majority of students agreed that they were: confident in using MS Word (94:5:1); MS Excel (64:18:20); would prefer to use computer/tablet-based applications to undertake academic work (57:29:14); and perceived themselves as confident in adapting to software that was new to them (55:29:16). Of the 40% students who considered PebblePad to be a barrier to portfolio engagement (40:31:27), over half were confident in adapting to new software (See Fig. 1) and/or had a preference for using a tablet/computer.

These data demonstrate that students' confidence in using new technologies is not representative of their perceptions of technology as a barrier to learning. However, engagement with the portfolio remained comparatively high.

This study is limited by the single cohort surveyed; further study of multiple groups with different demographics may be beneficial.

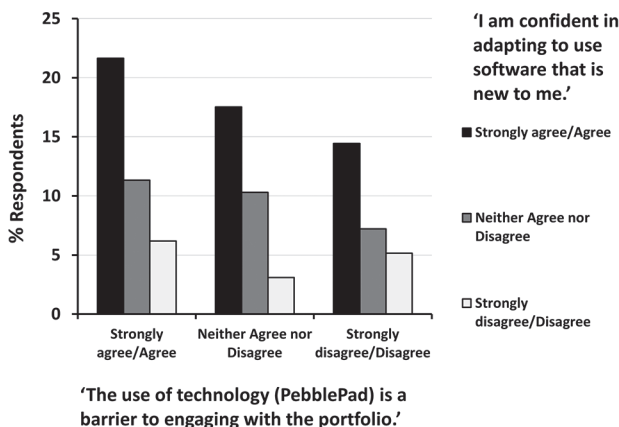


Fig. 1. Confidence in adapting to new technology vs. technology as a barrier to engagement. Student responses to a survey indicate that more than half those who consider themselves confident in adapting to use novel software, also consider the use of technology (Pebblepad) to be a barrier to engagement (n = 97).

Hesa.ac.uk. (2019). *Higher Education Student Statistics: UK, 2016/17 - Student numbers and characteristics/HESA*. [online] Available at: <https://www.hesa.ac.uk/news/11-01-2018/sfr247-higher-education-student-statistics/numbers> [Accessed 15 Apr. 2019].

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C035

Student-created video resources can enhance medical science practical class assessment.

C. Malcolm², J. Kirkman¹, J. Hislop², F. Murray², A. Jenkinson¹ and D. Scott¹

¹*Institute of Education for Medical & Dental Sciences, University of Aberdeen, Aberdeen, UK* and ²*Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK*

Increasing academic diversity and student numbers has challenged traditional delivery of core practical skills. We have pioneered the use of Objective Structured Practical Examinations in medical science teaching and previously reported successful delivery of theoretical, practical and problem-solving skills at multiple stations to formally examine a wide range of communication and science laboratory practical skills. However, teaching delivery had the potential to be variable at times and some students required more practice time. Student-led development of video resources which were released to students through the VLE, with instructions to prepare prior to the practical class, enabled smaller groups of students to be allocated to 2 hour practicals with focussed practice time and further instruction available. These training videos included topics such as ECG measurement, acid-base balance urinalysis, serial dilutions, spirometry and were created on mobile phones using free recording/editing software. Videos were no longer than 5 mins duration and were designed to help students understand how best to complete the tasks at each assessment station.

There was no significant reduction in mean score achieved by physiology, anatomy and sport science students (2017-18 cohort, 20.25 ± 2.01 , $n = 71$) compared with students who did not have access to video resources (2016-17 cohort, 19.82 ± 1.54 , $n = 67$) (Data represents mean score $\pm$ stdev). However, there was a small but significant improvement in outcomes when comparing the 2016-17 cohort with 2018-19 (20.54 ± 1.89 , $p < 0.05$; $n = 70$). This suggests that student performance did not suffer from reduced practice time before the assessment and the availability of the video resource may have contributed to enhanced assessment preparation. The 2018-19 VLE showed regular use of the videos throughout the practical delivery schedule with videos accessed 2697 times in total (an average of 6.4 ± 0.03 per student). There was also a surge in video access the day before the practice and formal assessment days ($36.7 \pm 0.18\%$ of students). Feedback recorded in University central course evaluation forms in 2018-19 reflected previous years with 86.34% of students positively rating the practical at 4 or 5 on a 1-5 satisfaction scale. They also cited the OSPE assessment and videos as being one of the best

things about their respective courses. Staff also reported that students appeared better prepared when attending practice sessions and during the examination following use of the video resources.

These results suggest that student-created video resources can contribute to delivering larger, more diverse practical classes in a consistent and time effective manner. Future work will involve the development of further videos for a wider range of practical skills and adapting this method to other traditional practicals within the curriculum.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C036

Data capture, analysis and assessment in physiology education

J.E. Clark

Cardiology, King's College London, London, UK

Physiology as a discipline benefits from practical classes to bolster theoretical teaching. However, with ever increasing class sizes, budget considerations and the limitation of laboratory space in a central London University we face a growing challenge to deliver high quality physiology teaching in our undergraduate and postgraduate courses. In this paper, we address some of the specific solutions we have reached in the Physiology department at King's College London at to deliver and assess an engaging, integrative and manageable human physiology practical programme. We introduce the use of web-based tutorials to aid students' understanding of data capture, analysis and presentation and interactive physiology practical classes which re-enforce the classroom-based theoretical learning and allow students to progress at their own rate and in their own time. Overall, we believe we have demonstrated that the use of these resources enhance student engagement and increase satisfaction among undergraduate and postgraduate physiology students.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C037

The effect of cystic fibrosis sputum on cystic fibrosis transmembrane regulator mediated Cl^- transport and airway-surface-liquid height. Is gene editing sufficient to restore function in the cystic fibrosis environment?

M. Woodall¹, R. Tarran² and D. Baines¹

¹*Infection and immunity, St. George's University, London, London, UK and* ²*Marsico Lung Institute, University of North Carolina, Chapel Hill, Chapel Hill, NC, USA*

Cystic Fibrosis, a recessive genetic disease. Mutations within the cystic fibrosis transmembrane regulator (CFTR) gene can impair the function of the CFTR anion channel and disrupt the mucociliary escalator; the first line of defense against lung infection. Due to the monogenetic nature of the disease and the accessibility of the airways to nebulizers, CF lung disease is an obvious candidate for gene therapy. It is unclear how many cells expressing functional CFTR are required for restoration of ion channel activity in CF models whilst considering the downregulatory effect of the abnormal sputum produced in the CF lung (including upregulated cytokines, bacterial toxins, altered pH). With gene editing technology continually improving, we want to identify how much functional CFTR is required to restore activity of CF cultures in the presence of CF sputum.

To answer this question we used two models: human bronchiolar epithelial cell cultures of mixed CF (CFBE):normal (NHBE; a model for gene editing) from alternate sexes and CFBE transduced with CFTR under the expression of a high activity promoter (a model for gene therapy). Cells were differentiated at airway-liquid interface. Cells were incubated with apically applied normal or CF induced sputum pooled from 9 donors before functional analysis. Digital droplet PCR (ddPCR) and immunohistochemistry were performed for characterisation. Short circuit current (I_{sc}) and airway surface liquid (ASL) height were measured as functional read-outs. We demonstrated a 41% decrease ($n=5$, $p<0.001$) in CFTR-mediated I_{sc} and a loss of vasoactive intestinal peptide (VIP, activator of CFTR) stimulated ASL secretion ($n=5$, $p<0.001$) after incubation with CF sputum compared to normal sputum. Using ddPCR analysis of AMEL-X/Y to accurately determine the number of CFBE vs NHBE in the mixed cell cultures, we observed that CFTR-mediated I_{sc} increased in a linear fashion with the percentage NHBE cells ($R^2=0.91$, $n=28$). The VIP-stimulated increase in ASL height followed a similar trend. In comparison, ~16% transduction of CF cultures with CFTR was sufficient to fully restore Cl^- secretion and increase ASL height ($n=6$) in the presence of CF sputum.

Our data implies that overexpression rather than endogenous expression of CFTR is more effective at restoring function in a CF culture. However, this technology is limited by its transient and immunoreactive nature. Currently, permanent restoration of function in the CF lung would require an unachievable level of gene editing events. Thus, we propose the use of an enhanced CFTR gene we have developed for gene editing. Codon optimization and a substitution mutation in cytosolic loop 3 produced ~7 fold greater processing in to protein and dramatically increased

anion transport activity as shown by a YFP quenching assay in Hek293T cells (n=3). It is our hope that this enhanced gene could be used to reduce the editing events necessary to restore function in the CF lung.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C038

Calcium entry through TRPC1 and ORAI1 regulates activated pancreatic stellate cells proliferation

S. RADOSLAVOVA^{2,1}, T. LEFEBVRE², M. Telliez², A. VANLAEYS², I. DHENNIN-DUTHILLE², M. GAUTIER², N. PREVARSKAYA¹ and H. OUADID-AHIDOUCH²

¹INSERM U1003, Université de Lille, LILLE, France and ²LPCM-EA46-67, Université Picardie Jules Vernes, AMIENS, France

Pancreatic ductal adenocarcinoma (PDAC) is currently the 4th leading cause of cancer related deaths in the world. One of the principal characteristics of PDAC is the presence of a dense “desmoplastic reaction”, mainly constituted of pancreatic activated stellate cells (PSCs), that often accounts for more than half of the tumor volume. However, the cellular and molecular mechanisms regulating their activation remains little known. Calcium acts as a universal and versatile second messenger in the regulation of cellular processes including cancer. The store operated calcium channels (SOCs) represent one of the major calcium entry pathways in non-excitabile cells. Recently, we showed the involvement of ORAI1 and STIM1 in calcium entry (SOCE) and survival in PDAC cell lines (Kondratska *et al.* 2014). Here, we aimed to assess the role of ORAI1 and TRPC1, two of the main SOC actors, in the human activated PSC cell line PS-1.

ORAI1 and TRPC1 expression was detected in both mRNA level by qPCR and in protein level by Western Blot, in PS-1 cell line. The silencing of ORAI1 and TRPC1 by siRNA transfection resulted to a significant decrease (siCtrl: 100±1.99%, siORAI1: 66.93±1.45%, siTRPC1: 37.72±5.09%; N=3; P<0.001) of SOCE induced by thapsigargin (measured by calcium imaging). Moreover, ORAI1 and TRPC1 knockdown strongly decreased PS-1 proliferation (siCtrl: 100±5.79%, siORAI1: 36.70±3.78%, siTRPC1: 50.68±5.03%; N=3; P<0.001) without affecting their mortality (evaluated by MTT assays). These results were confirmed by using the pharmacological SOCs inhibitors SKF96365 at 5µM (DMSO: 100±5.01% vs SKF96365: 50.87±2.80%; N=3; P<0.001) and 2APB at 50µM (DMSO: 100±5.89% vs 2APB: 43.23±3.12%; N=3; P<0.001). Furthermore, calcium also regulates the α-SMA expression, the principal marker of stellate cell activation, in hepatic stellate cells (Iyer SC *et al.* 2015). We then investigate the impact of ORAI1 and TRPC1 silencing on α-SMA protein expression in pancreatic stellate cells. We found that silencing of TRPC1 but not of ORAI1 induced 58.36% decrease of α-SMA protein expression (N=4, P<0.01). Interestingly, α-SMA knockdown induced 60.92% decrease of TRPC1

protein expression (N=3, P<0.05) and decrease of PS-1 cell proliferation (siCtrl: 100±6.89% vs si α -SMA: 45.81±4.43%; N=4; P<0.001).

In conclusion, we demonstrate that the human activated PSCs are characterized by a SOCE which is ORAI1 and TRPC1 dependent. Both ORAI1 and TRPC1 are required for activated PSC proliferation, probably by maintaining a calcium entry. Moreover, it seems that a feedback regulation exists between TRPC1 and α -SMA expression that regulates cell proliferation. However, more experiments are needed to determine the mechanism by which ORAI1 and TRPC1 regulate PSC proliferation, as well as the link between TRPC1 and α -SMA expression.

Prevarskaya, Ouadid-Ahidouch *et al.* (2014). *Philos Trans R Soc Lond B Biol Sci.* 2014 Feb 3;369(1638):20130097.

Kondratska *et al.* (2014). *Biochim Biophys Acta.* 2014 Oct;1843(10):2263-9

Iyer SC *et al.* (2015). *Exp Cell Res.* 2015 Aug 1;336(1):66-75

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C039

Intracellular pH modulation in human umbilical vein endothelial cells requires sodium/proton exchangers activity in gestational diabetes but sodium/proton exchanger-1 activity in gestational diabetes with maternal pre-gestational normal weight or overweight

G. Fuentes^{1,4}, P. Valero¹, M.A. Ramírez⁴ and L. Sobrevia^{1,2,3}

¹Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile, ²Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Seville, Spain, ³University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, Herston, Queensland, University of Queensland, Brisbane, QLD, Australia and ⁴Facultad de Ciencias de la Salud, Universidad de Antofagasta, Antofagasta, Chile

Gestational diabetes is a metabolic condition where the mother shows pre-gestational obesity and develop gestational diabetes mellitus (GDM) (Cabalín *et al.*, 2019). Human umbilical vein endothelial cells (HUVECs) from GDM pregnancies show Na⁺/H⁺ exchanger 1 (NHE1)-dependent alkaline intracellular pH (pHi). However, it is unknown whether gestational diabetes results in altered NHE1-dependent pHi modulation in HUVECs. Methods: HUVECs were isolated (collagenase digestion) from full-term pregnancies collected at the Clinical Hospital CHRISTUS-UC (Chile). The study conformed to the principles outlined in the Declaration of Helsinki. HUVECs were isolated from the following experimental groups: Normal pregnancies with maternal pre-pregnancy normal weight (Nnw, body mass index (BMI) 20-24.9 kg/m², n = 5 or overweight (Now, BMI 25-29.9 kg/m², n = 3), GDM pregnancies with maternal pre-pregnancy normal weight (GDMnw, n = 3),

overweight (GDMow, $n = 3$) or obesity (GDMob or gestational diabetes, BMI >30 kg/m², $n = 3$). Cells were cultured in medium 199 plus sera (20%) up to passage 2. The pHi was measured in cells loaded with the fluorescent pH-sensitive probe BCECF-AM (12 μ M, 10 min) and exposed to NH₄Cl (20 mM). Basal and pHi recovery rate ($dpHi/dt$) were estimated (up to 360 s) in cells exposed to 5 μ M 5-N, N-hexamethylene-amiloride (HMA, NHE general inhibitor), 0.1 μ M zoniporide (Zn, NHE1 inhibitor), 0.1 μ M concanamycin A (CoA, V-ATPases inhibitor), or 10 μ M Schering (Sch, H⁺/K⁺-ATPase inhibitor). Results: The basal pHi was higher ($P < 0.05$, unpaired ANOVA) in HUVECs from GDMnw (7.73 ± 0.03 , mean $\pm$ S.E.M.), GDMow (7.53 ± 0.04), and GDMob (7.35 ± 0.03) compared with Nnw (7.08 ± 0.02) or Now (7.04 ± 0.02). The $dpHi/dt$ was higher ($P < 0.05$) in Now (2.82 $\pm$ 0.22 fold), GDMnw (4.01 $\pm$ 0.21), GDMow (4.33 $\pm$ 0.33), and GDMob (1.97 $\pm$ 0.06) compared with Nnw (0.0043 pHi units/10 s). The $dpHi/dt$ in GDMob was lower than GDMnw and GDMow. Zn and HMA reduced the $dpHi/dt$ in Nnw (18 $\pm$ 6 and 59 $\pm$ 9%, respectively), GDMnw (98 $\pm$ 2 and 95 $\pm$ 4%) GDMow (98 $\pm$ 2 and 94 $\pm$ 5%), and GDMob (58 $\pm$ 5 and 98 $\pm$ 2%); however, these inhibitors were not effective in cells from Now. Incubation of with CoA or Sch did not alter the $dpHi/dt$, but it was blocked in cells exposed to CoA or Sch in the presence of Zn, HMA, or both, in cells from GDMob. Conclusion: The pHi recovery in HUVECs from normal pregnancies is partially dependent on NHEs activity. However, in HUVECs from gestational diabetes the pHi recovery may require NHE1 along with other NHEs isoforms.

Cabalin C et al. (2019). *Mol Aspects Med* (doi:10.1016/j.mam.2019.01.001).

FONDECYT 1190316 (Chile), GF holds MSc fellowship from the Universidad de Antofagasta, Chile.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C040

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

J.S. Smyth¹, A. Bussayajirapong¹, N.K. Lajczak-McGinley¹, J.D. Foulke-Abel², M. Donowitz² and S. Keely¹

¹Department of Molecular Medicine, Royal College of Surgeons in Ireland, Dublin 9, Ireland and ²Departments of Physiology and Medicine, Gastroenterology Division, The Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction and Aims: The intestinal epithelium functions to transport nutrients, fluid and electrolytes, while at the same time acting as a barrier to the entry of harmful substances. CFTR is a transmembrane Cl⁻ channel important in regulating intestinal fluid transport and is implicated in the pathogenesis of a number of intestinal diseases. Bile acids, classically known for their roles in lipid digestion, are now also recognised as important enteric hormones that regulate many aspects

of epithelial function. Indeed, it is thought that bile acid metabolism is a primary mechanism by which the microbiota communicates with its human host. Here, we set out to investigate the role of the nuclear bile acid receptor, farnesoid x receptor (FXR), in regulating epithelial CFTR expression.

Methods: T84 human colonic epithelial cells were cultured as polarised monolayers on permeable supports and treated bilaterally with the FXR agonist, GW4064 (5 μ M), over a range of times. Levels of FGF19, an index of FXR activation, CFTR, FXR, FOXA1, and miR-494 were measured by qRT-PCR, western blotting, or ELISA. **Results:** Treatment of the cells with GW4064 significantly increased FGF19 mRNA expression by 1235 ± 237.3 fold ($n = 8$; $p < 0.05$) and protein by 719.6 ± 92.0 pg/mL ($n = 5$; $p < 0.01$) after 48 hrs. Moreover, GW4064 downregulated CFTR mRNA to 0.47 ± 0.1 fold after 12 hrs ($n = 8$; $p < 0.01$) and protein levels to 0.36 ± 0.1 fold after 48 hrs, compared to vehicle-treated controls ($n = 4$; $p < 0.05$). Transcriptomic analysis confirmed FXR-induced downregulation of CFTR in primary human colonic enteroids. Expression of miR-494, which is known to target CFTR, was not increased by GW4064 treatment in T84 cells. However, mRNA expression of FOXA1, a transcription factor that regulates CFTR, was inhibited by $33.2 \pm 5.2\%$ after 3 hrs ($n = 4$; $p < 0.05$).

Conclusion: The nuclear bile acid receptor, FXR, regulates colonic epithelial CFTR expression. Such actions are likely to be important in the setting of microbial regulation of host intestinal physiology in health and disease. As such, FXR represents an excellent target for development of new drugs and nutraceuticals to treat intestinal diseases associated with dysregulated CFTR function.

Funded by Science Foundation Ireland

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C041

Exploring the mechanism of inactivation of Kir7.1 K⁺ channel by snowflake vitreoretinopathy-associated mutation R162W

E. Vera, I. Cornejo, M. Niemeyer, F.V. Sepulveda and L. Cid

Centro de Estudios Científicos (CECs), Valdivia, Chile

Kir7.1 is a potassium channel expressed in a variety of epithelial tissues prominent among which is the retinal pigment epithelium of the eye. Mutations in KCNJ13, the gene coding Kir7.1, cause eye diseases in humans. An example is snowflake vitreoretinal degeneration (SVD), an autosomal dominant inherited pathology characterized by retinal and vitreous deterioration associated to an R162 to W mutation in Kir7.1 (Hejtmancik et al., 2008). The mechanism by which this mutation produces its dominant negative effect is not clear. This C-terminus located arginine is speculated to be part of a ligand site for PIP2 which would be necessary for stabilizing the open state of the channel and its neutralization in the R162W

mutant would lead to channel silencing (Pattnaik et al., 2013; Zhang et al., 2013). We are now using site directed mutagenesis and functional analysis after transfection into HEK-293 cells to test this hypothesis.

Cells transfected with the R162W mutant of Kir7.1 do not express any specific current as expected for an inactivating mutation and consistent with the loss of charge and PIP2 interaction hypothesis. We then assayed an R162C mutant of Kir7.1, that given the neutral nature of the cysteine side-chain was expected to behave as Kir7.1-R162W. Kir7.1-R162C, however, showed robust activity measured as inward Rb^+ whole cell current at -160 mV (-8.8 ± 1.5 nA, compared to -3.6 ± 0.7 nA in WT Kir7.1). Experiments using Kir7.1-R162Q gave a current of -2.0 ± 0.6 nA whilst the R162F mutant of Kir7.1 completely lacked channel activity. These data suggest a relation between the volume of the amino acid side chain (Cys 39.7, Gln 85.6, Phe 129.7, Trp 167.9 Å³) and the current through the channel.

To emulate a large neutral amino acid side chain in the background of the Kir7.1-R162C mutant we used BMTS benzylmethanethiosulfonate (10 µM) to attempt to modify C162. These experiments were conducted in excised inside-out patches. BMTS had no effect on WT Kir7.1 activity but elicited a rapid and complete suppression of current in the Kir7.1-R162C mutant. The smaller MMTS methylmethanethiosulfonate also inhibited the current through Kir7.1-R162C but to a lesser extent. These results suggest that the volume of the side chain at position 162 of Kir7.1 is an important determinant of activity. The increase in side chain volume that occurs when replacing arginine by a tryptophan residue in SVD might be at the root of the dominant-negative effect behind the pathology.

Hejtmancik JF, Jiao X, Li A, Sergeev YV, Ding X, Sharma AK, Chan CC, Medina I & Edwards AO. (2008). Mutations in KCNJ13 cause autosomal-dominant snowflake vitreoretinal degeneration. *Am J Hum Genet* 82, 174-180.

Pattnaik BR, Tokarz S, Asuma MP, Schroeder T, Sharma A, Mitchell JC, Edwards AO & Pillers DA. (2013). Snowflake vitreoretinal degeneration (SVD) mutation R162W provides new insights into Kir7.1 ion channel structure and function. *PLoS One* 8, e71744.

Zhang W, Zhang X, Wang H, Sharma AK, Edwards AO & Hughes BA. (2013). Characterization of the R162W Kir7.1 mutation associated with snowflake vitreoretinopathy. *Am J Physiol Cell Physiol* 304, C440-449.

Supported by Proyecto Fondecyt grant 1150735.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Nox-derived ROS in bladder urothelium – effect of inflammatory mediators and pathological significance

J. Amosah¹, M. Roberts¹, S. Archer¹, M. Ruggieri² and C. Wu¹

¹University of Surrey, Guildford, UK and ²Temple University, Philadelphia, PA, USA

Overactive bladder syndrome and interstitial cystitis are age-related disorders affecting millions worldwide but the factors that predispose aging bladders to these chronic bladder diseases are poorly understood. The role of oxidative stress and reactive oxygen species (ROS) have been postulated to an underlying mechanism for aging and many age-associated disorders. NADPH oxidases (Nox enzymes) are professional ROS-generating enzymes in the body that produce superoxide as their sole function and thus serve as specific ROS-controlling therapeutic target with little side effect on physiological oxidation. Nox-derived ROS have been shown to contribute to many age-related chronic conditions, but little is known about its role in the bladder, in particular in the inner-lining epithelial layer – the urothelium. Urothelium has been recognised as a new sensory structure and its dysfunction may hold key to understanding age-related bladder disorders. The purpose of this work was to determine Nox-dependent superoxide generation in bladder tissue in response to bladder pathology-related inflammatory mediators. Wild-type C57BL/6 J mice were maintained under standard feeding conditions and euthanized in accordance with UK and EU regulations. Bladders were isolated, bladder mucosa and smooth muscle layer were dissected under microscopic guidance. Nox proteins were determined by Western blot with Nox subtype-specific antibodies. NADPH-dependent superoxide production was measured by lucigenin-enhanced luminance. ATP release, a sensory transduction process of the urothelium, was quantified by luciferin-luciferase assay. Data are expressed as mean±SEM. Two group means were analysed by t-test (paired or non-paired). Multiple means were tested by ANOVA and pairwise comparisons. Major Nox subtypes (Nox 2, Nox1 and Nox4) were shown to be expressed within the bladder, specifically within the bladder mucosa, a structure containing the urothelium and the lamina propria (n=5-8 bladders). The superoxide generation was significantly higher in bladder mucosa than in smooth muscle layer (relative light unit [RLU]/mg tissue: 201±23 vs 37±4 for mucosa and smooth muscle respectively, n=45, p<0.01). The bladder inflammation-associated mediator bradykinin (10µM) increased the superoxide production in bladder mucosa to 250±34% of control (n=11, p<0.05). Bladder obstruction and hypoxia-related inflammatory mediator endothelin-1 enhanced the superoxide production to 231±43% of control (n=12, p<0.05). Further experiments showed that these inflammatory mediators also augmented ATP release from the urothelium. These results demonstrate a potential role for Nox-derived ROS in chronic inflammation in response to inflammatory mediators encountered in bladder pathologies and suggest pathological significance. The effect of these mediators on urothelial ATP release supports functional relevance.

BBSRC BB/P004695/1; NIA 1R01AG049321-01A1

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C043

GR activation stimulates NCC and determines diurnal rhythm of its phosphorylation

J.R. Ivy, M.K. Mansley, T. Peltz, P.W. Flatman and M.A. Bailey

University of Edinburgh, Edinburgh, UK

The distal convoluted tubule (DCT) reabsorbs ~7% of the filtered sodium load and contributes to blood pressure regulation. Sodium entry here is through the thiazide-sensitive Na-Cl co-transporter (NCC). Both aldosterone and glucocorticoids (GC) can activate NCC. However, we find that the GC-deactivating enzyme, 11 β -hydroxysteroid dehydrogenase (11 β HSD2), rarely colocalises with NCC¹, leaving the transporter vulnerable to activation by GC. NCC has a diurnal rhythm of activation, which is attenuated by chronic GC treatment². Here, we investigate the role of the mineralocorticoid receptor (MR) and GC receptor (GR) in NCC activation and rhythmicity. Male C57BL6 mice were implanted with pellets containing no drug, spironolactone (50 mg, chronic MR blockade) or RU486 (60 mg, chronic GR blockade). After 5 days, corticosterone (cort, 6 mg/kg, s.c.) or vehicle (veh, 2% DMSO, s.c.) was injected at 8am (nadir of endogenous corticosterone rhythm). 4 hrs later kidneys were taken. In a separate cohort treated as above with RU486 or blank pellets, kidneys were taken every 6 hrs starting at 7am local time (ZT0). NCC threonine phosphorylation (on T53, pT53-NCC) was measured by western analysis as an index of transporter activation. Data are mean $\pm$ SD. In control mice, acute cort doubled pT53-NCC abundance (1.0 $\pm$ 0.49 AU (veh) vs 1.9 $\pm$ 0.28 AU (cort), $p=0.004$, Student's t test, $n=6$). MR blockade reduced total NCC protein (0.39 $\pm$ 0.21 AU (spironolactone; $n=5$) vs 0.80 $\pm$ 0.18 AU (control; $n=6$), $p=0.004$, by post hoc Tukey tests) but acute cort still substantially increased pT53-NCC abundance (1.0 $\pm$ 0.46 AU (veh; $n=5$) vs 3.7 $\pm$ 1.4 AU (cort; $n=7$), $p=0.0018$, Student's t test). GR blockade did not affect total NCC abundance (0.59 $\pm$ 0.15 AU (RU486) vs 0.80 $\pm$ 0.18 AU (control) $p=0.15$, Student's t test, $n=6$ in each group) but did prevent cort-induced phosphorylation (1.0 $\pm$ 0.19 AU (veh) vs 1.0 $\pm$ 0.15 (cort), $p=0.51$, Student's t -test $n=6$ in each group). GR, MR and NCC mRNA transcript levels were unchanged by either acute cort treatment or chronic RU486 or spironolactone treatment. Sgk1 and per1 transcripts were increased by cort treatment in control mice (0.36 $\pm$ 0.07 versus 0.80 $\pm$ 0.34 AU, $p=0.0029$, post hoc Sidak tests, $n=5-6$); spironolactone and RU486 blocked this. Chronic RU486 treatment dampened the diurnal rhythm of pT53-NCC with elevated levels at ZT0 and ZT 6 (Treatment $p=0.049$, time $p=0.0004$, interaction $p=0.16$, twoway ANOVA, $n=5-6$). Total NCC protein was unchanged at any time point.

These data suggest MR activation is required for maintenance of tonic levels of NCC protein but GC can regulate NCC function by acutely increasing NCC

phosphorylation through GR activation. The data further suggest that GR is required for the NCC's diurnal rhythm. This has implications for blood pressure control particularly in individuals with altered GC levels, such in Cushing's disease, chronic stress or corticosteroid therapy.

1. Hunter RW, Ivy JR, Flatman PW, Kenyon CJ, Craigie E, Mullins LJ, Bailey MA and Mullins JJ. Hypertrophy in the Distal Convoluted Tubule of an 11 β -Hydroxysteroid Dehydrogenase Type 2 Knockout Model. *Journal of the American Society of Nephrology* : JASN. 2015;26:1537-48.

2. Ivy JR, Oosthuyzen W, Peltz TS, Howarth AR, Hunter RW, Dhaun N, Al-Dujaili EA, Webb DJ, Dear JW, Flatman PW and Bailey MA. Glucocorticoids Induce Nondipping Blood Pressure by Activating the Thiazide-Sensitive Cotransporter. *Hypertension (Dallas, Tex : 1979)*. 2016;67:1029-37.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C044

Ionic modulation of immune checkpoint proteins

M.A. Shandell¹, S.M. Lawrence², W.J. Brackenbury¹ and D. Lagos¹

¹Biology, University of York, Heslington, UK and ²Faculty of Biological Sciences, University of Leeds, Leeds, UK

Despite extensive basic and clinical research on immune checkpoint regulatory pathways, little is known about the effects of the ionic tumour microenvironment on immune checkpoint expression and function. Here, we describe a mechanistic link between Na⁺/K⁺ ATPase inhibition and activity of indoleamine-2',3'-dioxygenase 1 (IDO1), a well-characterised immune checkpoint. IDO1 catalyses the rate-limiting step of tryptophan catabolism and inhibits the immune response to the tumour by local depletion of tryptophan, an amino acid essential for anabolic functions in cancer cells and T cells (Brochez, Chevolet and Kruse, 2017). To assess IDO1 function, we employed a medium-high throughput screening assay based on indirect spectrophotometric detection of kynurenine, a downstream tryptophan metabolite (Takikawa *et al.*, 1988). Interferon- γ /tumour necrosis factor- α (IFN- γ /TNF- α) are used to stimulate kynurenine production in many human cell lines (Takikawa *et al.*, 1988). Using this assay, we tested a library of 31 model ion channel targeting compounds for their effect on kynurenine production by IFN- γ /TNF- α stimulated breast cancer (MDA-MB-231) and lung cancer (A549) cells. We discovered that the cardiac glycosides ouabain and digoxin (Clark, Swanson and Stahl, 1975) inhibited kynurenine production in both MDA-MB-231 and A549 cancer cells. An initial screen of MDA-MB-231 resulted in decreased kynurenine (mean $\pm$ SD) upon treatment with 50 μ M ouabain: 3.8 ± 1.3 μ M, n=3, vs. 1% DMSO control: 27.5 ± 4.1 μ M, n=6 (p<0.0001, one-way ANOVA Bonferroni post-hoc). An initial screen of A549 resulted in decreased kynurenine upon treatment with 50 μ M ouabain: 1.5 ± 1.6 μ M (conservatively, <3.1 μ M), n=3, vs. 1% DMSO control: 15 ± 2.9 μ M, n=6 (p<0.001, one-way ANOVA Bonferroni post-hoc). Titration of ouabain in the kynurenine assay resulted in

half-maximal inhibitory concentrations (IC50s) of 89 nM for MDA-MB-231 and 17 nM for A549 cells. Titration of the related cardiac glycoside, digoxin, resulted in IC50s of 164 nM for MDA-MB-231 and 40 nM for A549 cells. Decreased cancer cell survival due to treatment with cardiac glycosides was measured by Alamar Blue absorbance ($\text{Abs}_{570 \text{ nm}} - \text{Abs}_{600 \text{ nm}}$) compared to 0.05% DMSO control (O'Brien *et al.*, 2000). MDA-MB-231 cells decreased in number by 56% upon treatment with 500 nM ouabain and 33% upon treatment with 500 nM digoxin. A549 cells decreased in number by 39% upon treatment with 50 nM ouabain and 16% upon treatment with 50 nM digoxin. Thus, inhibition of kynurenine production may not be only due to cell death. Preliminary studies suggested that Na^+/K^+ ATPase inhibition by ouabain, but not digoxin, downregulated IDO1 expression in both cell lines (western blot, $n=1$), implying that the two inhibitors might alter tryptophan catabolism through distinct mechanisms. Ionic modulation via the Na^+/K^+ ATPase is a novel regulatory pathway of the immune checkpoint protein IDO1, with both mechanistic and clinical implications.

Brochez, L., Chevolet, I. and Kruse, V. (2017) 'The rationale of indoleamine 2,3-dioxygenase inhibition for cancer therapy', *European Journal of Cancer*, 76, pp. 167–182.

Clark, A. F., Swanson, P. D. and Stahl, W. L. (1975) 'Increase in dissociation rate constants of cardiotonic steroid-brain (Na^+/K^+)-ATPase complexes by reduction of the unsaturated lactone.', *Journal of Biological Chemistry*, 250(24), pp. 9355–9359.

O'Brien, J. *et al.* (2000) 'Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity', *European Journal of Biochemistry*. John Wiley & Sons, Ltd (10.1111), 267(17), pp. 5421–5426.

Takikawa, O. *et al.* (1988) 'Mechanism of interferon-gamma action. Characterization of indoleamine 2,3-dioxygenase in cultured human cells induced by interferon-gamma and evaluation of the enzyme-mediated tryptophan degradation in its anticellular activity', *Journal of Biological Chemistry*, 263(4), pp. 2041–2048.

The work was funded by a Medical Research Council institutional Confidence in Concept Award, reference MC_PC_16064.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C045

Targeting proton secretion in CF airway epithelial cells to help restore airway pH homeostasis

V. Saint-Criq, L. Delpiano, M. Karavadra, L. Gleghorn and M. Gray

Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle-upon-Tyne, UK

Background. Targeting the airway surface liquid (ASL) pH in Cystic Fibrosis (CF) could potentially restore bacterial killing, ASL hydration and mucociliary clearance. CFTR and the non-gastric H^+/K^+ -ATPase ATP12A have been implicated in ASL pH regulation but other transporters and channels might also be involved. We have shown that the Proton Pump Inhibitor (PPI), esomeprazole (Eso), increased ASL pH

in CF cells via the downregulation of ATP12A but that it also acutely induced an intracellular acidification that was ATP12A-independent¹. Our current work focuses on identifying the H⁺ transporter acutely targeted by Eso and testing other PPIs as well as potassium-competitive acid blockers (P-CABs) on pH homeostasis.

Methods. Primary CF human airway epithelial cells (hAECs) were grown on semi-permeable supports under thin-film condition. ASL pH was measured using a mixture of dextran-coupled pHrodo and AlexaFluor488, in the presence of vehicle control (DMSO) or test compounds; the PPI omeprazole (OME, 300 μ M), the P-CAB TAK-438 (TAK, 100 μ M) or ZnCl₂ (300 μ M). Moreover the activity of H⁺-ATPases, H⁺ exchangers or H⁺ channels were assessed using intracellular pH (pH_i) measurements after loading the cells with BCECF-AM (5 μ M, 60 min).

Results. Neither the Na⁺-H⁺ Exchanger (NHE, inhibited by EIPA) nor the V-ATPase (inhibited by Bafilomycin A1) were active in the apical membrane of CF hAECs. Although the putative H⁺ channel blocker Diphenhydramine hydrochloride (DPH) had no effect on pH_i (Δ pH_i = 0.009 $\pm$ 0.002, n=3), ZnCl₂ induced a dose-dependent acidification, suggesting that the voltage gated H⁺ channel HVCN1² was active under resting conditions. Although not significant, inhibition of the H⁺ channel by overnight apical exposure to ZnCl₂ induced an increase in ASL pH of CF hAECs (CTL: 7.08 $\pm$ 0.08 vs. ZnCl₂: 7.26 $\pm$ 0.09, n=4, p=0.07). OME induced a decrease in pH_i (Δ pH_i = -0.16 $\pm$ 0.04, n=3) that was not significantly different from the one observed after apical exposure to Eso (Δ pH_i = -0.12 $\pm$ 0.01, n=3). However, the P-CAB TAK showed no effect on pH_i (Δ pH_i = 0.03 $\pm$ 0.02, n=3). Preliminary results using these molecules on ASL pH confirmed the effect of OME and the lack of effect of TAK.

Conclusion. Considering the reversibility of Eso and OME on pH_i, the absence of an appropriate acidic environment required for their activity and the lack of effect of the TAK on pH_i, the gastric H⁺/K⁺-ATPase is unlikely to be a target for the modulation of H⁺ secretion in primary CF hAECs. However, this study has identified HVCN1 as a potential H⁺ transporter at the surface of CF airway cells that is targeted by PPIs. Considering the demonstrated benefits of PPIs against bacterial and viral infections, oxidative stress and inflammatory processes in other chronic airway diseases, we propose that the inhibition of multiple H⁺ transporters by the use of PPIs (potentially combined with future identified inhibitors) in CF airways might help alleviate CF lung pathophysiology.

¹Delpiano L et al *Frontiers in Pharmacology* 2018

²Cho DY et al *Am J Rhinol Allergy*. 2009

Supported by the CF Trust SRC grants SRC003 and SRC013 and the Medical Research Council (MRC) Confidence in Concept grant (MC_PC_15030).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Epithelial sodium channels containing the δ -subunit operate under high extracellular sodium concentrationsS.M. Gettings^{1,2}, G. Vande Velde², M. Schoenberger¹ and M. Althaus¹¹School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK and ²Imaging and Pathology, Faculty of Medicine, KU Leuven, University of Leuven, Leuven, Belgium

The epithelial sodium channel (ENaC) mediates transepithelial sodium absorption and is important for the control of vertebrate sodium and water balance. Canonical ENaC is composed of the α -, β - and γ -subunits, but the physiology of ENaCs composed of the δ -, β - and γ -subunits is poorly understood. We have previously shown that $\delta\beta\gamma$ -ENaC has a higher open probability than $\alpha\beta\gamma$ -ENaC and generates larger currents when expressed in *Xenopus* oocytes. We hypothesise that $\delta\beta\gamma$ -ENaC provides a route for maximised sodium absorption and studied the activity of ENaC isoforms in response to changes in the extracellular sodium concentration ($[Na]_e$). Guinea pig $\alpha\beta\gamma$ - and $\delta\beta\gamma$ -ENaC were heterologously expressed in *Xenopus* oocytes and channel activity was measured by the two-electrode voltage-clamp technique at a holding potential of -60 mV. ENaC-mediated current fractions were determined using the inhibitor amiloride (100 μ M). Currents were measured under increasing $[Na]_e$ from 1 – 300 mM. N-methyl-D-glucamine chloride was used to maintain osmolarity. The allosteric inhibition of ENaC by extracellular sodium (sodium self-inhibition, SSI) was determined as the percentage of current decline within 3 min after rapidly changing $[Na]_e$ from 1 to 90 mM. Delivery of ENaC to the plasma membrane was blocked by brefeldin A (BFA, 5 μ M) and the rate of retrieval (in %) of functional ENaC from the plasma membrane under 1 mM and 90 mM $[Na]_e$ was determined by monitoring ENaC currents 5 h after application of BFA. Data are reported as means $\pm$ standard error.

Amiloride-sensitive currents generated by $\delta\beta\gamma$ -ENaC were 6.03 ± 0.79 μ A ($n = 20$) and were significantly larger than those generated by $\alpha\beta\gamma$ -ENaC (2.1 ± 0.21 μ A, $n = 19$; $p < 0.0001$, Mann-Whitney U-test). Currents triggered by increasing $[Na]_e$ followed Michaelis-Menten kinetics and had a V_{max} of 3.44 ± 0.79 μ A ($\alpha\beta\gamma$ -ENaC, $n = 12$) and 7.035 ± 0.68 μ A ($\delta\beta\gamma$ -ENaC, $n = 12$; $p = 0.0005$; Mann-Whitney U-test). K_M values were 26.78 ± 4.09 mM sodium for $\alpha\beta\gamma$ -ENaC ($n = 12$, $p < 0.0001$, Mann-Whitney U-test), and 76.86 ± 4.63 mM sodium for $\delta\beta\gamma$ -ENaC expressing oocytes ($n = 12$). SSI of $\delta\beta\gamma$ -ENaC expressing oocytes was 16.09 ± 2.49 % ($n = 17$) and significantly smaller than that of $\alpha\beta\gamma$ -ENaC (46.52 ± 2.71 %, $n = 18$; $p < 0.0001$, Student's unpaired t-test). In the presence of BFA and 1 mM $[Na]_e$, currents generated by $\alpha\beta\gamma$ -ENaC decreased by 23.74 ± 8.35 % ($n = 14$) and those generated by $\delta\beta\gamma$ -ENaC by 28.84 ± 5.65 % ($n = 14$; $p = 0.62$, Student's unpaired t-test). In the presence of BFA and 90 mM $[Na]_e$, the current decrease was 16.76 ± 13.11 % ($n = 10$) for $\alpha\beta\gamma$ -ENaC and 4.72 ± 17.16 % ($n = 10$) for $\delta\beta\gamma$ -ENaC ($p = 0.58$, Student's unpaired t-test).

In conclusion, there is no difference in membrane residency between ENaC isoforms but $\delta\beta\gamma$ -ENaC has a decreased SSI and generates larger currents over a wider range of $[\text{Na}]_e$. This suggests that $\delta\beta\gamma$ -ENaC might provide a route for efficient transepithelial sodium absorption in the presence of high $[\text{Na}]_e$.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C047

Localization of UT-B and AQP3 in developing bovine rumen epithelium

C. Zhong, A. Gohl, O. Heussaff, O. Phelan, D. Ronan, T. Lyons, E. Doyle and G. Stewart

School of Biology and Environmental Science, University College Dublin, Dublin, Ireland

The rumen ecosystem represents a classic example of a mutualistic relationship between host animals and microbial populations. Urea nitrogen secreted from the blood to the rumen is a crucial factor shaping this relationship. The transport of urea across rumen epithelium is facilitated by urea channels, namely UT-B and possibly AQP3. However, how ruminants regulate nitrogen availability to microbes through these transport proteins remain unclear. Given that calves are the stage at which of rumen microbe colonization begins, the developing rumen is an excellent model to observe this regulation. This study investigated the localization of urea channels (UT-B and AQP3) during rumen development. Using rumen epithelium samples of calves aged from birth to three months old (0, 7, 14, 21, 28, and 96 days old; weaned at 56 days old; $n = 4-8$ at each age), immunolocalization of UT-B and AQP3 protein was performed. Initial results showed that, although abundance varied between the individual animals within each age groups, UT-B protein was found expressed at all six stages ($n = 9/14$). The immunostaining started from the cells of stratum basale at all stages ($n = 9/14$) and progressively extended to the adjacent cell layers toward the rumen lumen as the rumen papillae developed ($n = 3/14$). The strongest staining appeared to be found in stratum basale cells ($n = 9/14$), especially those surrounding large blood vessels ($n = 3/14$). The staining in stratum spinosum was mainly present in well-developed papillae at day 96 and was weak ($n = 3/4$), while stratum granulosum and stratum corneum were mainly negative throughout the six stages ($n = 12/14$). Occasionally, strong staining was found in balloon cells of stratum corneum at age day 96 ($n = 2/4$). The subcellular staining was clearly seen in the cells of stratum basale at the age of day 96, with the strongest density at the basolateral side of plasma membrane facing blood vessels ($n = 3/4$). In contrast to UT-B, the staining of AQP3 was only found in cells of stratum corneum at stages of day 28 ($n=1/2$) and day 96 ($n = 2/3$). Overall, the localization pattern of UT-B is consistent with the hypothesised functional organization of transporting urea nitrogen from blood to rumen. The localization of AQP3 in stratum corneum is unexpected and the physiological meaning is unclear.

In on-going studies, the staining of UT-B and AQP3 will be quantified between the six stages. Given that papillae development is a key reflection of rumen function, precise measurement of papillae development will be also undertaken. Overall, these results give insight into how ruminant host animals regulate nitrogen availability, and hence commensal microbial populations, through altering the permeability of the rumen.

Keywords: developing rumen, UT-B, AQP3, calves

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C048

Alterations in the colonic microbiome and age-dependent changes in stool water content, mucus production, mucosal inflammation and survival in *Slc26a3* (DRA)-deficient mice and wt-littermates

A. Kini¹, M. Basic³, D. Roemermann¹, B. Zhao², A. Bleich³, T. Strowig² and U. Seidler¹

¹Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany, ²Helmholtz Center for Infection Research, Braunschweig, Germany and

³Institute of Laboratory Animal Science, Hannover Medical School, Hannover, Germany

Background: Mutations in the *dra* gene result in congenital chloride diarrhoea (CLD) which is characterized by secretory diarrhoea, loss of Cl⁻ in the stool, dehydration and metabolic alkalosis. These patients also have a higher risk of incidence of acute as well chronic intestinal inflammation. The *slc26a3* (*dra*) deficient mice mimic the features and symptoms observed in CLD patients, but it is unknown whether they also display the propensity for intestinal inflammation.

Aim: The present study was undertaken to explore whether the *slc26a3*^{-/-} mice develop spontaneous intestinal inflammation, and if an altered epithelial barrier function and/or microbiome composition may be the underlying molecular mechanism for inflammation.

Methods: *Slc26a3*^{-/-} and wt littermates were studied from week 5-20 after birth. Stool water and Lipocalin2 (intestinal inflammation marker) were assayed weekly. After sacrifice at the designated time points, the inflammatory state of the mucosa was assessed by qPCR and immunohistochemistry. Mucus production and composition was assessed by histochemical staining of the mucins. The luminal and the mucosa-adherent colonic microbiome was assessed by 16S rRNA sequencing, and the effect of the microbiome of the *slc26a3*^{-/-} colon on the development of inflammation in germ free mice by fecal crossing experiments.

Results: In the *slc26a3*^{-/-} mice, stool water content was increased (Weeks 4-7; n=9 pairs; p<0.0037, weeks 8-11; n=9 pairs; p<0.0003, weeks 12-15; n=9 pairs; p<0.0009, weeks 16-20; n=7 pairs; p<0.0002). Qualitatively, immunohistochemistry of *muc2* revealed a significant reduction in the goblet cells with a shift being observed towards the acidic mucins (n=3 pairs/time point). The luminal

microbiome was dysbiotic from the earliest measurable time point with dramatically reduced microbiome diversity (n=5 pairs/time point). Over time, the *slc26a3*^{-/-} mice developed mild colonic inflammation as evidenced by an increase in the stool lipocalin2 levels (n=5 pairs, p<0.05) and an increase in the mRNA levels of *Ly6g*, a neutrophil marker (Weeks 11-15, n= 16 pairs; p = 0.0070, weeks 17- 21, n= 13 pairs, p = 0.0003). The differences in microbiome composition partially persisted after transfer into germfree mice. However they did not develop intestinal inflammation and displayed an increase in the microbiome diversity.

Conclusions: *Slc26a3*^{-/-} mice display very low colonic pH microclimate, disturbances in mucin production and mucus barrier properties, strongly reduced microbiome diversity, and some of the *slc26a3*^{-/-} mice develop overt colonic inflammation. Similar events may occur in the gut of CLD patients.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C049

Neutraceutical targeting of the bile acid receptor, farnesoid X receptor, for intestinal disease.

C.M. Fallon¹, O. Edupuganti², N.K. Lajczak-McGinley¹, H. Sheridan² and S. Keely¹

¹*Molecular Medicine, The Royal College of Surgeons in Ireland, Dublin, Ireland and*
²*School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Dublin, Ireland*

INTRODUCTION: Bile acids, classically known for their roles in fat digestion, are now recognised as important regulators of many aspects of intestinal physiology, including mucosal immune responses, epithelial proliferation/apoptosis, and transepithelial transport and barrier function. Therefore, the nuclear bile acid receptor, farnesoid X receptor (FXR), represents a promising therapeutic target for intestinal disorders, such as inflammatory bowel disease and colorectal cancer. Previous studies suggest that a particular class of plant-derived phytochemicals modulates FXR activity.

AIMS AND METHODS: Here we set out to investigate the effects of a phytochemical, denoted KFS1, on FXR signalling in colonic epithelia. T₈₄ colonic epithelial cells, grown as polarised monolayers, were treated bilaterally with the FXR agonist, GW4064 (5 µM) and KFS1 (1 – 100 µM) for 24 hrs. Expression of FXR and FGF-19, an index of FXR activation, were measured by qRT-PCR and ELISA. Transepithelial electrical resistance (TEER) was measured as an index of epithelial integrity. Lactate dehydrogenase (LDH) release was used as a measure of cytotoxicity. Data are expressed as the mean ± SEM for a series of *n* experiments.

RESULTS: GW4064 (5 µM) induced a 719.8 ± 183.3 fold increase in FGF-19 mRNA expression (n = 20; p<0.001) and a 100 ± 24.6 pg/ml increase in FGF-19 protein expression (n = 5; p<0.01). KFS1 (1 – 100 µM) did not significantly increase FGF-19

protein expression and at higher concentrations (50 – 100 μ M) reduced TEER. Lower concentrations of KFS1 (5 – 10 μ M) did not alter TEER but significantly increased FXR mRNA expression by 4.16 ± 0.4 and 3.3 ± 0.4 fold, respectively ($p < 0.05$; $n = 4$). Moreover, pre-treatment of T₈₄ cells with KFS1 (1, 5, or 10 μ M) for 1 hr prior to treatment with GW4064 (5 μ M; 24 hrs) potentiated GW4064-induced FGF-19 mRNA expression by 2.5 ± 0.8 , 2.9 ± 0.2 , and 4.6 ± 0.5 fold, respectively ($p < 0.01$; $n = 6$) and FGF-19 protein expression by 292.4 ± 52.7 , 477.3 ± 66.5 , and 530.3 ± 61.8 pg/ml, respectively ($p < 0.05$; $n = 7$). Combined treatment with KFS1 (1, 5, 10 μ M and 50 μ M) and GW4064 (5 μ M) for 24 hrs also reduced TEER to $49.8 \pm 7.4\%$, $50.6 \pm 8.3\%$, $40.6 \pm 6.6\%$ and $37.2 \pm 10\%$ of control values, respectively ($n = 7$). Only at the highest concentration of KFS1 tested (50 μ M), did co-treatment with GW4064 increase LDH release ($p < 0.01$; $n = 5$). Finally, a methanolic extract of plant material known to be rich in KFS1 (denoted QE1) was prepared. Similar to KFS1, QE1 (100 μ g/ml) increased GW4064-induced FGF-19 release from T₈₄ cells to 309.3 ± 18.9 pg/ml compared to 171.1 ± 34.1 pg/ml in controls ($n = 6$; $p < 0.01$). CONCLUSION: Our data suggest that KFS1, a common dietary phytochemical, increases FXR expression in colonic epithelial cells, an effect which may prime cells for agonist-induced FXR activation. These findings suggest that foods, or food supplements, rich in such plant-derived phytochemicals have potential for development as FXR-targeted nutraceuticals.

Funded by The Royal College of Surgeons in Ireland, StAR PhD Programme & Science Foundation Ireland.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C050

Differential effects of ceramide on permeability in human airway epithelial cells

K.K. Kalsi¹, S. Jackson² and D. Baines¹

¹Infection and Immunity, St George's University of London, London, UK and ²Respiratory, Inflammation & Autoimmune Innovative Medicine, Lung Immunity, AstraZeneca, Mölndal, Sweden

Maintaining the integrity of the airway epithelial barrier is essential in preventing increased permeability to harmful molecules and pathogens, which may lead to increased risk of developing respiratory infection and disease. We have previously shown that in human airway epithelial cells metformin increases tight junction (TJ) assembly after pathogen disruption via an AMPK-PKC ζ pathway^{1,2}. The sphingo-myelinase/ceramide system can cleave membrane proteins releasing ceramide to form ceramide rich domains, signaling the reorganization of receptors and activation of protein kinase C isoforms such as PKC ζ ³. This study aimed to investigate the effect of *N*-Palmitoyl-d-erythro-sphingosine (C16:0 ceramide), a PKC ζ activator on

epithelial integrity of human airway epithelial cells (H441 cells). We investigated the effect of ceramide on claudin-3 which can alter ion selectivity⁴.

H441 cells were grown on permeable supports at air-liquid interface, TJ were disrupted by depleting the calcium, complete media with 0.1 μM or 1 μM ceramide was then added to measure re-assembly of TJ. After 24h the transepithelial electrical resistance (TEER) was measured and apical to basolateral permeability of sodium-fluorescein (MW 376 Da) which is used as an index of permeability (P_{app}). With 0.1 μM ceramide TEER was $138 \pm 15 \Omega \cdot \text{cm}^2$ compared to control $125 \pm 14 \Omega \cdot \text{cm}^2$ ($p < 0.01$, $n = 4$). P_{app} was 16 ± 6 vs $18 \pm 7 \text{ cm/s} \times 10^{-6}$, $p = 0.19$; $n = 3$, 0.1 μM ceramide vs control respectively. No changes were observed with 1 μM ceramide. Immunofluorescence staining for occludin, ZO-1 or claudin-3 did not show a difference in localisation with ceramide treatment compared to control cells. TJ were also disrupted by adding pro-inflammatory mediators (PIMS) -100 ng/ml TNF- α , 100 ng/ml IFN- γ and 10 $\mu\text{g/ml}$ LPS, in the presence of ceramide. TEER, P_{app} and TJ immunofluorescence was measured after 24h, 48h and 72h. TEER was significantly reduced by PIMS at 24h and 48h but not at 72h ($205 \pm 31 \Omega \cdot \text{cm}^2$ vs $401 \pm 95 \Omega \cdot \text{cm}^2$, $p < 0.01$, $n = 4$) and ($188 \pm 51 \Omega \cdot \text{cm}^2$ vs $413 \pm 49 \Omega \cdot \text{cm}^2$, $p < 0.01$, $n = 4$) compared to control. Ceramide did not however increase PIMS induced decrease in TEER. Permeability with PIMS was significantly increased after 72h from $5.9 \pm 2.5 \text{ cm/s} \times 10^{-6}$ to $13.4 \pm 1.9 \text{ cm/s} \times 10^{-6}$, $p < 0.001$, $n = 3$. Ceramide + PIMS at 0.1 μM decreased P_{app} to 9.0 ± 2.2 ($p < 0.05$, $n = 3$) and at 1 μM to 6.8 ± 1.3 ($p < 0.01$, $n = 3$) both vs PIMS. Immunofluorescence staining for ZO-1 and claudin-3 was decreased with PIMS and restored with ceramide. These results indicate that in airway epithelial cells activation of PKC ζ by ceramide only evokes a minor effect on tight junction reassembly or permeability. In the presence of PIMS ceramide does not change ionic resistance but does change permeability. This could be mediated through claudin-3 which can alter TJ meshwork and seals paracellular pathways against small ions and solutes⁵. Kalsi K *et al.* (2019). *J Cell Mol Med*, 23, 317-327.

Patkee W *et al.* (2016). *J Cell Mol Med*, 20, 758-64

Wang G *et al.* (2009) *JBC*, 280, 26415-24.

Mitchell L *et al.* (2011). *AJP*, 301, L40-L49

Milatz S *et al.* (2010). *BBA*, 1798, 2048-57.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Bioelectrical control of axial regeneration in planaria flatworms

A.M. Rajnicek¹, S. Ihalainen¹, S. Dulajova¹ and I. Rowe²

¹School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK and ²School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, UK

Planaria flatworms regenerate new heads and tails after amputation by differentiation of stem cells (neoblasts), but the cues that drive spatially appropriate differentiation (head versus tail) are not clear. Previous work identified a voltage gradient inside planaria, with the tail mesenchyme tissue positive relative to the head (Annand, 2014). A membrane potential gradient also exists in planarian epithelial cells, which is relatively more depolarised at the head (Beane et al., 2011). We therefore tested the hypothesis that a bioelectrical gradient and voltage-gated chloride ion transport contribute to axial regeneration polarity.

Dugesia japonica (4-6 mm) were amputated on ice into equal head, trunk and tail fragments. Trunk fragments were immobilised in 7% methylcellulose and exposed to a DC electric field of 150 mV/mm for 1h with the polarity either parallel or antiparallel to the fragment's endogenous voltage gradient. After treatment fragments were transferred to planaria water for 2 weeks to regenerate. Appearance of eyespots was used to score head regeneration in trunks of 5 worms in 3 independent experiments. Frequencies were compared with a D test (Bailey, 1981); significance at $P < 0.05$.

Eye regeneration was first apparent on day 4 (20% had eyes) in control trunks and on day 5 (50% with eyes) when the EF was parallel to the endogenous internal EF (anterior facing the cathode) but it was delayed until day 7 (51% had eyes) in trunks exposed to an antiparallel external EF. 100% of regenerating fragments had eyes by day 6 for control and parallel EF conditions but eye appearance was delayed until day 8 for the antiparallel EF group. Fragments survived better (93%) in control and parallel EF groups than the antiparallel EF group (60%; $P < 0.001$), in which ectopic eyes were observed.

The role of voltage-gated chloride transport was tested by incubating trunk and tail fragments in planaria water with 10 μ M 4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) or 0.1% DMSO (vehicle) throughout the whole 2 week regeneration period. Trunk and tail fragments were pooled from 4 control experiments and 5 DIDS and DMSO experiments run in parallel; 5 worms each. Head regeneration was delayed by DIDS; 7 days after amputation 100% of controls and 89% of DMSO controls (not statistically significant) had eyes, but only 58% ($P < 0.001$ compared to DMSO) of the DIDS group had eyes. By day 10, 100% of the control and DMSO groups had eyes and 90% of the DIDS group had eyes and some of those were ectopic.

We conclude that the inherent bioelectrical gradient and epithelial chloride transport each contribute to axial regeneration polarity in planaria. The ability to drive neoblasts selectively down distinct lineages may have clinical relevance for

mammalian regeneration, suggesting strategies that target ionic flux and electrical properties specific to regenerating tissues.

Annard S (2014). PhD thesis, University of Aberdeen.

Bailey NT (1981). Statistical Methods in Biology, pp38-39. Hodder and Stoughton, London

Beane WS et al. (2011). Chem Biol. 18,77-89.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

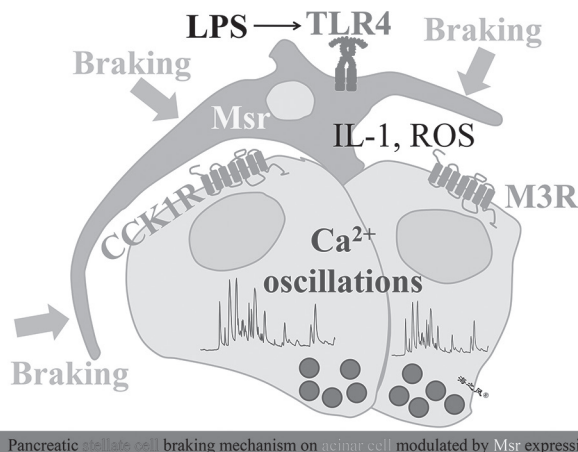
C052

Pancreatic stellate cells serve as a brake mechanism on pancreatic acinar cell calcium signaling modulated by methionine sulfoxide reductase expression

J. Liu and Z. Cui

Institute of Cell Biology, Beijing Normal University, Beijing, China

Although methionine sulfoxide reductase (Msr) is known to modulate activity of multiple functional proteins, roles of Msr in pancreatic stellate cell physiology have not been reported. In the present work we investigated expression and function of Msr in freshly isolated and cultured rat pancreatic stellate cells. Msr expression was determined by RT-PCR, Western blot and immunocytochemistry. Msr over-expression was achieved by transfection with adenovirus vectors (10^{12} vg.mL⁻¹ x 5 microL, to each well of 6-well plates). Pancreatic stellate cells were co-cultured with pancreatic acinar cells AR4-2J in monolayer culture. Pancreatic stellate and acinar cell function was monitored by Fura-2 calcium imaging. Rat pancreatic stellate cells were found to express MsrA, B1, B2, their expressions diminished in culture ($P < 0.05$, $N = 3-4$, by Student's T test). Over-expressions of MsrA, B1, or B2 were found to enhance ATP (10 microM)-stimulated calcium increase ($P < 0.05$, $N = 3$) but decreased reactive oxygen species production ($P < 0.05$, $N = 3$) and lipopolysaccharide-elicited IL-1 production (from 1067 ± 24 to 537 ± 72 , 211 ± 41 , 286 ± 35 ng.L⁻¹ respectively, in mean $\pm$ SEM, with $P < 0.05$, $N = 3$). Pancreatic stellate cell-co-culture with AR4-2J blunted cholecystokinin (CCK 20, 60, 200 pM)- and acetylcholine (ACh 20, 60, 200 nM)-stimulated calcium increases in AR4-2J ($P < 0.05$, $N = 3$), depending on acinar / stellate cell ratio (2, 10, 50) ($P < 0.05$, $N = 3$), this inhibition was reversed by MsrA, B1 over-expression in stellate cells ($P < 0.05$, $N = 3$) or by Met supplementation (2, 10, 50 mM) in the co-culture medium ($P < 0.05$, $N = 3$). These data together suggest that Msr play important roles in pancreatic stellate cell function and the stellate cells may serve as a brake mechanism on pancreatic acinar cell calcium signaling subject to modulation by stellate cell Msr expression.



CCK1 or M3 receptor-mediated calcium oscillations in rat pancreatic acinar cells are blocked by surrounding pancreatic stellate cells. LPS stimulation of stellate cell surface TLR4 triggers reactive oxygen species (ROS) generation and IL-1 production, leading to activated state of stellate cells. Stellate cell activation is inhibited by over-expression of Msr to decelerate the braking mechanism. Both IL-1 and ROS could readily diffuse from stellate to acinar cells.

Funding: Work was funded by The Natural Science Foundation of China (31670856, 31270892) and The Ministry of Science and Technology of China (2011CB809101).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C053

Modulation of ion transport and pro-inflammatory cytokine release by HO-1/CO in cultured human airway epithelial cells

R. Zhang², C. Yip¹, K. Pan¹ and W. KO¹

¹School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong and ²Department of Physiology, Guangdong Medical University, Guangdong, China

Carbon monoxide (CO) is an important gas produced endogenously in humans by heme oxygenase (HO) enzymes (HO-1 and HO-2). CO is known to function as an anti-inflammatory agent, but its effects on airway inflammation and ion transport remain unclear. The effects of CO on cell damage- and nucleotide-induced pro-inflammatory cytokine release were investigated in cultured primary human bronchial epithelial (HBE) cells (ScienCell Research Laboratories, San Diego, CA, USA) and the 16HBE14o- HBE cell line. Its effects on calcium- and cAMP-dependent chloride (Cl⁻) secretion were examined using a technique that allowed

the simultaneous measurement and quantification of real-time changes in signalling molecules (cAMP and Ca^{2+} levels) and ion transport in a polarised epithelium (1, 2). Changes in Fura-2 ratios and short circuit current (I_{SC} ; an index of electrogenic ion transport) were measured concurrently in polarised epithelial cells mounted in a miniature Ussing chamber. Real-time changes in cAMP levels were detected using an EPAC-based fluorescence resonance energy transfer (FRET) sensor. In this study, using RT-PCR and western blot analysis, we demonstrated that both HO-1 and HO-2 mRNAs and proteins were expressed in primary HBE and 16HBE14o- cells, respectively (n = 3-4). The upregulation of HO-1 protein expression by hemin (10 μM), a well-known HO-1 inducer, inhibited the cationic protein poly-L-arginine (1 μM ; PLA)- or $\text{ATP}\gamma\text{S}$ (10 μM)-induced IL-6 and IL-8 release in primary HBE and 16HBE14o- cells, as quantified by ELISA (n = 5-10). CORM-3 (100 μM ; a CO-releasing molecule) inhibited the $\text{ATP}\gamma\text{S}$ -induced polarised secretion of IL-6 and IL-8 in 16HBE14o- cells (n = 5-7). Furthermore, CORM-3 (100 μM) inhibited UTP (100 μM)-induced increases in calcium (Control: change in the Fura-2 ratio = 0.052 ± 0.007 ; +CORM-3: change in the Fura-2 ratio = 0.032 ± 0.003 ; $p < 0.05$; values are means $\pm$ S.E.M., compared using ANOVA) and I_{SC} (Control: change in I_{SC} = $26.79 \pm 1.73 \mu\text{Acm}^{-2}$; +CORM-3: change in I_{SC} = $12.45 \pm 2.14 \mu\text{Acm}^{-2}$; $p < 0.05$; n = 8). CORM-3 (100 μM) also inhibited the forskolin (10 μM)-induced increase in the FRET ratio (Control: change in the ratio = 0.09 ± 0.01 ; +CORM-3: change in the ratio = 0.05 ± 0.01 ; $p < 0.05$) and subsequent increase in I_{SC} (Control: change in I_{SC} = $47.35 \pm 2.80 \mu\text{Acm}^{-2}$; +CORM-3: change in I_{SC} = $26.56 \pm 2.59 \mu\text{Acm}^{-2}$; $p < 0.05$, n = 4-7). Overall, our data suggest that CO modulates the pro-inflammatory state of the human airway epithelia by interfering with the P2Y receptor-mediated signalling pathway. CO also modulates the Ca^{2+} - and cAMP-dependent secretion of Cl^- in the human airway epithelia. Thus, therapeutic strategies using HO-1-inducing agents or CORMs may be developed for treating airway-related inflammatory disorders, such as asthma.

Zhang RG, Yip CY, Ko WH. Carbon Monoxide Inhibits Cytokine and Chloride Secretion in Human Bronchial Epithelia. *Cell Physiol Biochem*. 2018;49(2):626-37.

Hao Y, Liang JF, Chow AW, Cheung WT, Ko WH. P2Y₆ Receptor-Mediated Proinflammatory Signaling in Human Bronchial Epithelia. *PLoS One*. 2014;9(9):e106235.

Supported by an RGC GRF grant (#466913)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Artificial sweeteners increase the pathogenic potential of model gut bacteria on the intestinal epithelium

A. Shil¹, L. King¹, B. Evans² and H. Chichger¹

¹Department of Biomedical Science, Anglia Ruskin University, Cambridge, UK and

²University of East Anglia, Norwich, UK

Artificial sweeteners (AS) are synthetic sugar substitutes that gained popularity for their low-price and for no-calorie gain. However recent studies have demonstrated considerable health risks since AS consumption has been linked to metabolic derangements and gut microflora perturbations (Suez et al., 2014). Several studies have represented AS consumption-related intestinal barrier impairment and gut bacteria dysbiosis. But, how AS affect the intestinal epithelial cell function or change the commensal bacteria to pathogenic has not yet been defined. The current *in vitro* study investigated the effect of physiologically-achievable concentrations of three commercially-available AS on the monolayer permeability of intestinal epithelial cell model, Caco-2 cells and metabolism of model gut bacteria, *E. coli* NCTC 10418. Also, the pathogenic potential of the bacterium in terms of biofilm formation and haemolysing red blood cells was studied. In addition, AS (100 μ M) effect on the bacterial adhesion and invasion ability to the intestinal epithelial cells was assessed. Data were analyzed using GraphPad Prism (v.8), compared by ANOVA and values are presented as mean $\pm$ S.E.M. Saccharin and sucralose at physiological concentration (1 mM) significantly increased intestinal epithelial cell monolayer permeability percentage (195 ± 34 and 164 ± 18 , respectively) whilst aspartame was found to increase leak (377 ± 90) at sub-physiological concentration (100 μ M) ($p < 0.05$, $n = 5$). There observed no effect of the three sweeteners (100 μ M) on the model bacteria growth and no change in its characteristic α -hemolysis pattern. However, all the tested sweeteners significantly increased (almost doubled) the biofilm formation ($n = 5$) and inhibiting the sweet taste sensing reduced the effect. *E. coli* exposed to sucralose and aspartame showed a significant increase in their adhesion/invasion percentage (204 ± 26 / 267 ± 61 and 286 ± 66 / 249 ± 58 , respectively) to Caco-2 cells, however, saccharin exposure significantly increased adherence (226 ± 41) but not invasion (107 ± 26) ($p < 0.05$, $n = 4$). Similar to biofilm formation, exposure in the presence of zinc sulfate attenuated the bacterial pathogenic effect. These data suggest that AS causes a leak in the intestinal epithelium, which can lead to inflammatory responses. Also, AS exposure induces bacterial pathogenic factor and promotes their adherence and intrusion to intestinal epithelial cells. Further investigations on the mechanisms through which AS affect gut bacteria and bacteria-epithelium interactions will extend our understanding of sweetener-induced dysbiosis formation. Also, the mechanism of how zinc sulfate exerts an inhibitory effect on model bacterial pathogenicity needs to be investigated. Since artificial sweetener consumption in the diet continues to increase, it is vital to understand how this food additive affects microflora and gut health.

Keywords: Artificial sweetener; microflora; dysbiosis; epithelial cells.

Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C.A., Maza, O., Israeli, D., Zmora, N., Gilad, S. & Weinberger, A. 2014, "Artificial sweeteners induce glucose intolerance by altering the gut microbiota", *Nature*, vol. 514, no. 7521, pp. 181-186.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C055

Do the peripheral chemoreceptors contribute to exercise blood pressure in people with a family history of hypertension?

A. Smets, P.E. Ramasawmy, K. Hope, Z. Adams, T. Hinton, R. Baker, A. Nightingale and E.C. Hart

Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK

People with a family history of hypertension (FH+) have a greater rise in blood pressure (BP) during exercise compared to people without FH (FH-). The peripheral chemoreceptors, particularly the carotid body, play a role in the pressor response to exercise [1]. The carotid bodies have been implicated in the aetiology of hypertension, thus, it is possible the carotid bodies play a role in the excessive BP response to exercise in people with FH+. Therefore, we aimed to establish whether inhibiting the carotid body during submaximal steady state exercise (via hyperoxia), could blunt the rise in systolic BP (SBP) in people with FH+ (n=9, age; 23 ± 1 years, body mass index (BMI); 21.4 ± 1.6 kg/m²) vs. people with FH- (n=9, age; 21 ± 1 years ($P=0.004$), BMI; 21.9 ± 2.1 kg/m² ($P=0.59$). FH+ was defined as people with 1° or 2° relatives with a diagnosis of hypertension. People were excluded if they could not find out their family history. Chemoreflex sensitivity (CRS) at rest was measured using poikilopapnic intermittent hypoxia. This involved measuring the minute ventilation response to desaturation via short bursts of nitrogen [2]. An incremental exercise test was completed on a bike ergometer to measure peak oxygen consumption (VO₂ peak). On a separate day, participants were asked to complete two 5 min bouts of exercise at 60% of their VO₂ peak. One was completed under normoxic conditions (room air) and the other was completed during hyperoxic conditions (0.80 fraction of inspired O₂ to inhibit the carotid body). The order was counterbalanced and separated by a 20 min rest. BP was measured using an automated cuff at baseline, 3 and 5 mins during each exercise bout. Group difference in CRS was assessed using an unpaired Student's T-test and differences in SBP between FH+ and FH- during submaximal exercise in normoxia and hyperoxia were measured using a two-way ANOVA. Data are mean $\pm$ SD. We found that there was no difference in CRS between the groups (FH+; -0.41 ± 0.85 L/min/% vs. FH-; -0.35 ± 0.37 L/min%, $P=0.84$). However, during normoxic submaximal exercise there was a greater change in SBP in FH+ vs. the FH- group (56 ± 21 mmHg vs. 35 ± 19 mmHg; $P=0.05$), but there was no group interaction effect by gas on the absolute SBP

during exercise ($P=0.07$), although there was a moderate effect size ($\eta^2=0.59$). In summary, resting CRS is not different in people with FH+ vs FH-. The change in SBP during exercise is higher in people with FH+, however, it is not clear whether the carotid body plays a role in this.

Stickland MK, Fuhr DP, Haykowsky MJ, Jones KE, Paterson DI, Ezekowitz JA, McMurtry MS. Carotid chemoreceptor modulation of blood flow during exercise in healthy humans. *J Physiol*. 2011. 589: 6219-6230.

Chua TP, Coats AJ. The reproducibility and comparability of tests of the peripheral chemoreflex: comparing the transient hypoxic ventilatory drive test and the single-breath carbon dioxide response test in healthy subjects. *Eur J Clin Invest*. 1995. 25:887-892.

Thank you to the participants and our expert Research Nurses for supporting this study.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C056

Longer Home-based HIIT intervals elicit a greater improvement in aerobic capacity – a 6-week intervention study.

H.L. Church, M. Cocks, K. Hesketh, J. Strauss, S. Shepherd and A. Wagenmakers

Sport and Exercise Science, Liverpool John Moores University, Liverpool, UK

Currently 40% of the UK population do not meet physical activity guidelines with 'lack of time' cited as the most common barrier to physical activity. High intensity interval training (HIIT) is an effective time-efficient alternative to moderate intensity continuous training (MICT). However, public health researchers have criticised current HIIT research as exercise is often supervised and many protocols employ expensive equipment available only in laboratories or gyms, creating additional barriers to exercise. As such, HIIT protocols using simple body weight exercises that can be performed in one's home (home-based HIIT) are becoming more popular. However, the effect of interval duration has not been investigated using home-based HIIT protocols. Therefore our aim was to investigate if two popular HIIT protocols (30 or 60 second interval durations (30HIIT; 60HIIT)) induce similar improvements in aerobic capacity, arterial stiffness, and body composition when performed using body weight exercises. Twenty-six, previously sedentary men ($n=9$) and women ($n=17$) were randomised to complete either 6 weeks of 30HIIT ($n=15$; $29\pm 3y$, BMI $25\pm 0.8\text{kg.m}^{-2}$) (4-8 30s intervals with 120s rest) or 60HIIT ($n=11$; $28\pm 4y$, BMI $26\pm 1.4\text{kg.m}^{-2}$) (6-10 60s intervals with 60s rest). Both training protocols used body weight exercises, and training sessions were completed without supervision in a place of the participants choosing, 3 times per week. Training adherence and intensity (Heart rate (HR)) were monitored using the Polar Beat mobile app. $\text{VO}_{2\text{peak}}$, body composition (Bio-impedance), and arterial stiffness (aortic pulse wave velocity (aPWV)) were assessed pre and post training. There

was no difference between interventions in the peak HR (60HIIT 178 ± 5 bpm; 30HIIT 173 ± 4 bpm) or mean heart rate (60HIIT 145 ± 4 bpm; 30HIIT 132 ± 2 bpm) recorded during training sessions ($P > 0.05$). There was a 7% increase in VO_{2peak} following 6 weeks of 60HIIT (33 ± 7 to 35 ± 7 ml.min⁻¹.kg⁻¹; $P < 0.05$), but VO_{2peak} was not increased by 30HIIT (32 ± 3 to 33 ± 2 ml.min⁻¹.kg⁻¹; $P = 0.51$). Neither 60HIIT or 30HIIT caused significant improvements in any measure of body composition (body fat % and visceral adipose tissue area; $P > 0.05$). There was also no significant difference in aPWV post intervention in either 60HIIT or 30HIIT ($P > 0.05$). This research confirms recent observations that 60HIIT, using body weight exercises, increases aerobic capacity in previously sedentary individuals. However, unlike 60HIIT and previous laboratory based research using cycle ergometers, 30s intervals of body weight exercises did not induce a significant improvement in aerobic capacity. This suggests that the physiological responses to home-based HIIT may differ from traditional protocols using laboratory equipment and, therefore, future work should investigate the optimal work to rest ratio for different HIIT protocols when body weight exercises are used.

Bhf.org.uk. (2019). *Physical Inactivity Report 2017*. [online] Available at: <https://www.bhf.org.uk/informationsupport/publications/statistics/physical-inactivity-report-2017>

I wish to acknowledge my supervisors Matthew Cocks and Katie Hesketh for their continuous support and guidance. Thank you for all the help throughout this year. Also thank you to the extremely helpful technicians Gemma Miller and Dean Morrey.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C057

Four-weeks, equipment-free, high-intensity interval training (HIIT) elicits improvements in the cardiorespiratory fitness and body composition of older adults irrespective of exercise supervision

T. Sian^{1,2}, A. Kwakye¹, I. Kotler¹, J. Lund^{1,2} and B. Phillips¹

¹MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Derby, UK and ²Division of Surgery and Anaesthetics, Royal Derby Hospital, Derby, UK

Introduction

Inactivity is a major risk factor for premature mortality [1], as is reduced cardiorespiratory fitness (CRF) [2] and low levels of lean muscle mass (LMM) [3]. Despite this, less than one third of adults in Europe achieve the World Health Organisation (WHO) minimum weekly exercise targets [4], with “lack of time” a commonly cited barrier. High-intensity interval training (HIIT) is a proven time-efficient, feasible method to improve the CRF of older adults [5]. However, the majority of studies have employed HIIT in a supervised laboratory setting using specialised equipment. In order to assess the efficacy of HIIT for improving the CRF and LMM of

older adults, with two known barriers to exercise (equipment and supervision) removed, we investigated the efficacy of 4-weeks supervised and unsupervised, equipment-free HIIT.

Methods

Twenty-one healthy older adults, median age 70 (range 61-81) years were randomised to complete 4-weeks laboratory HIIT (L-HIIT; supervised), home-based HIIT (H-HIIT; unsupervised) or a non-exercise control period. HIIT sessions lasted 15-minutes and comprised a brief warm-up, five 1-minute bouts of equipment-free high intensity efforts (star-jumps (2), static sprints (1) and squats (2)) interspersed with 90-seconds recovery and a final recovery period. The HIIT groups completed 12 sessions over 4-weeks. CRF, determined by VO_2 peak and anaerobic threshold (AT), was assessed before and after the intervention period by a ramped cardiopulmonary exercise test (CPET), with LMM assessed by dual-energy X-ray absorptiometry (DXA).

Results

Both the L-HIIT (26.5 ± 7.8 vs 28.3 ± 8.6 ml/kg/min; $p=0.04$) and H-HIIT (21.2 ± 3.3 vs 23.5 ± 4.2 ml/kg/min; $p=0.008$) groups significantly improved their VO_2 peak after HIIT, with no significant change in the control group (27.0 ± 4.4 vs 26.8 ± 3.8 ml/kg/min; $p=0.99$). Despite numerical increases in AT in both HIIT groups these increases failed to reach statistical significance (L-HIIT: 12.5 ± 3.0 vs 13.7 ± 3.0 ml/kg/min; $p=0.13$, H-HIIT: 11.8 ± 1.1 vs 13.2 ± 2.3 ml/kg/min; $p=0.07$), with no significant change in the control group (12.7 ± 2.1 vs 12.2 ± 1.4 ml/kg/min; $p=0.77$). Whole-body LMM significantly increased in both of the HIIT groups (L-HIIT: 48.9 ± 9 vs 49.6 ± 10 kg; $p=0.05$, H-HIIT: 39.2 ± 7 vs 40 ± 6 kg; $p=0.03$), with no significant change in the control group (46.2 ± 7.7 vs 46.5 ± 7.9 kg; $p=0.69$).

Conclusion

A 4-week, time-efficient, equipment-free HIIT programme is able to elicit improvements in the CRF and LMM of older adults. Furthermore, this programme is equally successful when performed unsupervised at home and as such may be useful in combating the rise of inactivity and associated poor health outcomes in adults. Larger studies are required to confirm these findings.

Lee I-M, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, *et al.* Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet (London, England)* 2012; 380:219–29.

Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, *et al.* Cardiorespiratory Fitness as a Quantitative Predictor of All-Cause Mortality and Cardiovascular Events in Healthy Men and Women. *JAMA* 2009; 301:2024.

Lee DH, Keum N, Hu FB, Orav EJ, Rimm EB, Willett WC, *et al.* Predicted lean body mass, fat mass, and all cause and cause specific mortality in men: prospective US cohort study. *BMJ* 2018; 362:k2575.

World Health Organisation. Physical Activity. 2018.<http://www.who.int/news-room/fact-sheets/detail/physical-activity>

Boereboom CL, Phillips BE, Williams JP, Lund JN. A 31-day time to surgery compliant exercise training programme improves aerobic health in the elderly. *Tech Coloproctol* 2016; 20:375–382.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C058

During maximal exercise frontal lobe oxygenation decreases despite elevation in middle cerebral artery flow velocity

R. Hansen¹, P. Nielsen¹, M. Schelske¹, N. Secher² and S. Volianitis¹

¹Aalborg University, Aalborg, Denmark and ²Dept. of Anaesthesia, The Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen Ø, Denmark

This study evaluated the influence of cerebral blood flow on the decrease in frontal lobe oxygenation (ScO_2) during maximal exercise by clamping end-tidal CO_2 tension (PetCO_2). Twelve endurance-trained males (age: 25 ± 3 years, height: 183 ± 8 cm, weight: 75 ± 9 kg) performed, in a cross-over design, isocapnic (PetCO_2 clamped at 40 ± 1 mmHg) and poikilocapnic (ambient air) high-intensity constant load ergometer cycling to exhaustion. Mean blood flow velocity in the middle cerebral artery ($\text{MCA } V_{\text{mean}}$; transcranial Doppler ultrasound) and ScO_2 (near-infrared spectroscopy) were assessed. Values are means $\pm$ SD, compared by ANOVA. Exercise duration ($12 \text{ min } 24.9 \pm 1 \text{ min } 18 \text{ s}$) was matched in the two trials, while pulmonary oxygen uptake (VO_2) was not quite equal ($90 \pm 6\%$ vs. $93 \pm 5\%$ of the maximal value; $P=0.012$) and PetCO_2 was 40 ± 1 and 34 ± 4 mmHg, respectively ($P<0.05$). During the isocapnic trial $\text{MCA } V_{\text{mean}}$ increased by $16 \pm 13\%$ from the resting value until the time that clamping was applied and then increased further by $14 \pm 28\%$ ($P=0.017$) until the end of exercise, while $\text{MCA } V_{\text{mean}}$ did not change significantly during the poikilocapnic trial ($P=0.071$). In contrast, ScO_2 decreased similarly (by $-3.2 \pm 5.1\%$ and $-4.1 \pm 9.6\%$; $P<0.001$) at the end of both trials. During maximal exercise, ScO_2 is reduced despite an increase in $\text{MCA } V_{\text{mean}}$ suggesting dissociation between frontal lobe oxygenation and cerebral blood flow.

Olin JT et al. (2012) *Eur J Appl Physiol* 112, 3439–3444.

Olin JT et al. (2011). *Respir Physiol Neurobiol* 175, 176–80.

The study was funded by Kultur Ministeriets Forskningsudvalg (FPK.2016-006). Special thanks to Niels Olesen for valuable guidance and assistance during data collection.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Exogenous testosterone therapy overcomes anabolic blunting to resistance-type exercise in older men

N. Gharahdaghi, S. Rudrappa, M.S. Brook, I. Idris, M. Aziz, B. Phillips, D.J. Wilkinson, N.J. Szewczyk, K. Smith and P.J. Atherton

Medical Sciences & Graduate Entry Medicine, University of Nottingham, Derby, UK

The andropause is associated with declines in serum testosterone (T), loss of muscle mass (sarcopenia) and frailty. Conversely, two major interventions purported to offset sarcopenia are anabolic steroid therapies and resistance exercise training (RET). Given the andropause and blunted hypertrophic responses to RET with advancing age¹, the efficacy, and physiological and molecular impacts of T therapy adjuvant to *short-term* RET remain poorly defined.

18 non-hypogonadic healthy older men, 65-75y, BMI≤30kg.m⁻² (serum T>230 ng.dl⁻¹) were assigned in a random double-blinded fashion to receive bi-weekly: placebo (P, saline, n=9) or T (Sustanon 250-mg, n=9) injections over 6-weeks of whole-body RET (6 exercises, 3-sets, 8-10reps at 80% 1-RM). Subjects underwent Dual-energy X-ray Absorptiometry, ultrasound of m.vastus lateralis (VL) architecture and Oral Glucose Tolerance Testing; finally, m.vastus biopsies were taken to quantify myogenic gene expression and activation of insulin/anabolic pathways, and muscle protein synthesis (D₂O) and breakdown (extrapolated).

T adjuvant to RET, augmented whole-body (53.0±1.7 to 56.0±5.2, P<0.0001 vs. 54.1±6.3 to 54.8±5.8 kg, P>0.05) and appendicular lean mass gains (7.8±0.3 to 8.3±0.3 vs. 7.9±0.3 to 8.0±0.3 kg.m², P<0.0001) while decreasing body fat (22.5±2.0 to 21.3±1.8 vs. 24.7±2.8 to 24.5±2.7kg, P=0.01). T also augmented VL thickness gains (2.3±0.07 to 2.6±0.04 vs. 2.3±0.1 to 2.4±0.09 cm, P<0.0001) and fascicle-length (7.1±0.2 to 7.9±0.2, P<0.0001 vs. 7.7±0.1 to 8.1±0.1 cm, P<0.0001) in addition to dynamic strength (T:+60.8±3.6 vs. P:+43.25±2.4%, P=0.0009). Mechanistically, both muscle protein synthesis rates (T: 2.13±0.21 vs. P: 1.34±0.13%. day⁻¹, P=0.0009) and absolute breakdown rates (T:140.2±15.8 vs. P:90.2±11.7g. day⁻¹, P=0.02) were elevated with T therapy, which led to higher net turnover and protein accretion (T:8.3±1.4 vs. P:1.9±1.2 g.day⁻¹, P=0.004). Increases in ribosomal biogenesis (RNA:DNA ratio); mRNA expression relating to T metabolism (*Androgen Receptor*: 1.4-fold; *Srd5a1*: 1.6-fold; *AKR1C3*: 2.1-fold); IGF-1-signalling (*IGF-1Ea* (3.5-fold), *IGF-1Ec* (3-fold) and myogenic regulatory factors (MRF); and the activity of anabolic signalling (e.g. mTOR, AKT, RPS6; P<0.05) were all upregulated with T therapy. Only T up-regulated mitochondrial citrate synthase activity (P=0.03) and transcription factor A (*Tfam*) (1.41±0.2-fold, P=0.0002), in addition to PGC1-α mRNA (1.19±0.21-fold, P=0.037). Finally, T augmented insulin sensitivity (e.g. Cederholm index: T: 57.3±5 to 68.6±5.3 vs. P: 49.1±3.7 to 57.1±5.1 mg.L².mmol. L⁻¹.mU⁻¹.min⁻¹, P=0.02)

Administration of T adjuvant to RET enhanced skeletal muscle mass and performance, while upregulating myogenic gene programming, myocellular translational

efficiency and capacity - collectively resulting in higher protein turnover, and net protein accretion. Thus, T coupled with RET is an effective short-term intervention to improve muscle mass/ function in older non-hypogonadal men.

¹ Phillips BE et al. (2017). JCI insight 2(17), 1-16.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C060

Carbohydrate ingestion is more beneficial for time trial performance in cycling than running.

A.D. Black¹, R. King¹, O. Wilson¹, M. Lees¹, D. Morrison², T. Preston², J. Rowe¹ and J. O'Hara¹

¹Institute for Sport, Physical Activity and Leisure, Leeds Beckett University, Leeds, UK and ²Scottish Universities Environmental Research Centre, East Kilbride, UK

The ergogenic benefits of carbohydrate (CHO) ingestion in cycling and running have been widely reported. Studies directly comparing the effect of CHO ingestion on CHO oxidation rates in cycling and running suggest no difference in exogenous CHO oxidation between these exercise modes. Potential differences in endogenous fuel use between cycling and running when ingesting CHO may lead to a greater benefit in cycling rather than running time trial (TT) performance. Direct comparisons of the effect of CHO ingestion during endurance exercise on subsequent cycling or running TT performance are limited. This study tested the hypothesis that ingesting CHO during 120 min of constant intensity exercise would benefit subsequent TT performance more in cycling than running. Methods: In a randomised, placebo-controlled, double blind crossover trial, 10 male triathletes (VO_{2max} cycle 51.65 ± 5.53 , run 59.07 ± 6.14 mL·kg⁻¹·min⁻¹) completed 4 separate exercise trials. Each trial consisted of cycling or running at 70% of mode-specific VO_{2max} for 120-min whilst ingesting either a CHO drink (2:1 glucose: fructose, 90g·h⁻¹) or an equal volume of taste-matched 0% CHO placebo (PLA) (750mL·h⁻¹). After 10-min recovery, participants completed a TT in the same mode of exercise, running 6km or cycling 16km. The CHO drink was enriched with uniformly labelled ¹³C glucose and ¹³C fructose isotopes to quantify exogenous and endogenous CHO oxidation. Due to limitations of the tracer methods used in the first hour, only data for the final 60-min of exercise are presented. Total CHO and fat oxidation rates were calculated from expired air using stoichiometric equations. Data were analysed using ANOVA, values are means±SD. Results: From 60-120 min of exercise mean fat oxidation rates did not differ between exercise modes (PLA cycle 0.52 ± 0.17 , run 0.56 ± 0.22 g·min⁻¹ $p=0.86$; CHO cycle 0.37 ± 0.12 run 0.40 ± 0.17 g·min⁻¹, $p=0.82$). CHO ingestion reduced fat oxidation within cycling ($p<0.01$) and running trials ($p=0.02$) compared to PLA. Mean total CHO oxidation rates did not differ between trials (PLA cycle 2.52 ± 0.74 , run 2.21 ± 0.46 g·min⁻¹, $p=0.23$, CHO cycle

2.95±0.40, run 2.77±0.55g·min⁻¹, p=0.47). Mean exogenous CHO oxidation did not differ with CHO ingestion (cycle 0.87±0.22 run 0.75±0.31g·min⁻¹, p=0.31). CHO ingestion reduced endogenous CHO oxidation in cycling (PLA 2.52±0.74, CHO 2.10±0.41g·min⁻¹, p=0.03) but not running (PLA 2.21±0.46, CHO 2.00±0.61g·min⁻¹, p=0.44). TT performance improved 10% in cycling and 3% in running after CHO ingestion (p<0.01). TT performance improvement was greater in cycling than running (p=0.04, ES=1.45). Conclusions: This study demonstrates a greater improvement in cycling versus running TT performance after CHO ingestion. This may relate to the greater sparing of endogenous CHO in cycling versus running during the previous exercise period when CHO is ingested.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C061

Untargeted metabolomics using a novel bioinformatics approach for classification of human skeletal muscle ageing

D.J. Wilkinson^{1,2}, W.B. Dunn³, I.J. Gallagher⁴, B. Phillips^{1,2}, J.P. Williams¹, P.L. Greenhaff^{1,2}, K. Smith^{1,2} and P. Atherton^{1,2}

¹MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Nottingham, UK, ²National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Nottingham, UK, ³School of Biosciences and Phenome Centre Birmingham, University of Birmingham, Birmingham, UK and ⁴Faculty of Health Sciences & Sport, University of Stirling, Stirling, UK

When skeletal muscle mass is lost in ageing and disease, health outcomes can be compromised. Identification of biological signatures that are able to stratify ageing tissue may not only assist to predict those at risk of health decline, but also identify novel preventative measures for a number of age associated diseases. To date, metabolomic approaches have yielded insight into changes in the muscle metabolome with age (1), and how changes in the plasma metabolome relate to age-associated co-morbidities and health (2). What is still not known, is whether changes in the muscle metabolome associated with ageing relate to health, and whether the muscle metabolome could be predictive of ageing. Here we take a unique bioinformatic approach utilising untargeted metabolomics alongside machine learning, in an attempt to identify metabolites associated with chronological ageing and health biomarkers. Fasted muscle biopsies were collected from young ($n=10, 25\pm 4y$), middle-aged ($n=18, 50\pm 4y$), and older ($n=18, 70\pm 3y$) men and women, and untargeted metabolomics data were then generated using UHPLC-MS. Variable importance measures, derived from the Random Forest (RF) algorithm, were used to determine metabolite features most informative in stratifying older age. Potential biological context that may regulate muscle ageing phenotype was examined using the PUIMet algorithm (3). Linear regression was then used to

determine any relationship of candidate metabolites to clinical variables commonly associated with age-related health decline. The RF algorithm was able to filter a large dataset of metabolites to those capable of predicting older age. Following putative identification of each metabolite, key subnetworks associated with human muscle ageing included phosphocreatine, androgen metabolism, histamine and phospholipid metabolism. Following FDR corrected linear regression, the phospholipid metabolite LysoPE(20:5) showed a significant negative relationship with leg lean mass (LLM), strength and resting heart rate (RHR), and LysoPE(22:6) showed a positive relationship to %Body Fat. Expected relationships were observed between dehydroepiandrosterone sulfate and total cholesterol, and the fatty acid metabolite capryloylglycine with HDL. However, others such as spermine with HDL and RHR, histamine with mean arterial pressure (MAP), dihydrothymine with insulin, and 4-imidazolone-5-propionic acid with HOMA-IR, showed potential novel metabolite links to age associated disease factors. This study reveals that a novel bioinformatics based metabolomics approach is potentially useful to classify human tissue ageing, and intriguingly, that a number of the age predictive metabolites show associations with common health biomarkers, and therefore could be used to assist towards the identification of novel preventative measures for a number of age associated diseases.

1. Fazelzadeh P, Hangelbroek RWJ, Tieland M, de Groot LCPGM, Verdijk LB, van Loon LJC, et al. The Muscle Metabolome Differs between Healthy and Frail Older Adults. *J Proteome Res* 2016;15(2):499–509.

2. Johnson LC, Martens CR, Santos-Parker JR, Bassett CJ, Strahler TR, Cruickshank-Quinn C, et al. Amino acid and lipid associated plasma metabolomic patterns are related to healthspan indicators with ageing. *Clin Sci*. 2018;132(16):1765–77.

3. Pirhaji L, Milani P, Leidl M, Curran T, Avila-Pacheco J, Clish CB, et al. Revealing disease-associated pathways by network integration of untargeted metabolomics. *Nat Methods*. 2016;13(9):770–6.

This work was funded by the Biotechnology and Biological Sciences Research Council (BB/C516779/1), and the MRC-ARUK Centre for Musculoskeletal Ageing Research.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Effect of type 2 diabetes in muscle deoxygenation during ramp incremental exercise in older individuals.

A.D. McDermott¹, N. Gildea¹, A. Nevin¹, J. Rocha², D. O'Shea³, S. green⁴ and M. Egana¹

¹Physiology, Trinity College Dublin, Dublin, Ireland, ²Abertay University, Dundee, UK, ³St.Columcille's Hospital, Dublin, UK and ⁴Western Sydney University, Sydney, NSW, Australia

Peak oxygen uptake (VO_{2peak}) in people with type 2 diabetes (T2D) of all ages is significantly lower than in healthy individuals (Green *et al.* 2015). In middle-aged individuals with T2D (< 55yrs) there appears to be an over reliance in fractional oxygen extraction during maximal ramp incremental exercise (RI), reflected by an increased rate of muscle deoxygenation (i.e., deoxygenated haemoglobin and myoglobin, [HHb+Mb]) (Gildea *et al.* 2016). To better understand the reduced VO_{2peak} responses observed in older individuals (>60 yrs) with uncomplicated T2D the present study investigated whether T2D also alters the profile of muscle fractional O_2 extraction in older individuals with uncomplicated T2D during incremental cycling exercise. Twelve older participants (9 men, 3 women) with T2D (age: 63.0 ± 2.9 yrs range: 59.8 – 67.4 yrs; BMI: 29.2 ± 4.1 kg.m⁻²) and twelve older healthy controls (9 men, 3 women) (age: 64.5 ± 3.9 yrs range: 59.3 – 68.2 yrs; BMI: 27.5 ± 2.8 kg.m⁻²) took part in the study. Each participant completed a ramp incremental cycling test to volitional exhaustion in an upright position followed by a verification test. Exercise was performed on an electrically braked cycle ergometer with participants initially exercising for 2-min at 10W before the intensity increased by 10-15W.min⁻¹ for women and 15-20W.min⁻¹ for men. Pulmonary oxygen uptake (VO_2) was measured on a breath-by-breath basis with an online metabolic system while muscle deoxygenation was assessed using near-infrared spectroscopy (NIRS) at the vastus lateralis muscle. Normalised [HHb+Mb] profiles ([HHb+Mb]%) were plotted as a function of relative work rate (%POpeak) using a bilinear model. Physiological variables were compared using unpaired t-tests or Mann-Whitney U tests with statistical significance set at $P < 0.05$. Values are means $\pm$ SD. Normalised VO_{2peak} was significantly ($P < 0.05$) reduced in the older individuals with T2D compared with the healthy older controls (23.9 ± 4.1 mL.kg.min⁻¹ vs 27.2 ± 5.3 mL.kg⁻¹.min⁻¹), representing a 12% reduction in peak exercise capacity. There was no difference in the slope of the first linear component of the delta[HHb+Mb]% vs delta%POpeak profile (S_1) between the 2 groups (Controls: 1.82 ± 0.8 a.u vs T2D: 1.81 ± 0.6 a.u.). Such findings are suggestive that in contrast to middle-aged individuals with T2D, a greater rate of oxygen extraction for a given increase in VO_2 , indicative of reduced O_2 delivery, is not a contributing factor to the reduction in VO_{2peak} observed in older individuals with T2D compared to healthy controls.

Gildea N, Rocha J, O'Shea D, Green S & Egaña M (2016). The effect of type 2 diabetes in muscle deoxygenation during ramp incremental exercise. Poster communication, Biomedical Basis of Elite Performance, East Midlands Conference Centre, Nottingham, UK.

Green S, Egana M, Baldi C, Lamberts R & Regensteiner J (2015). Cardiovascular control during exercise in type 2 diabetes mellitus. *J Diabetes Res* 2015, 1-11.

The authors would like to acknowledge the study participants for their time

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C063

Effect of age on ankle proprioception

Z.J. Djajadikarta¹, S.C. Gandevia¹ and J.L. Taylor²

¹Neuroscience Research Australia, Randwick, NSW, Australia and ²Edith Cowan University, Jundaloo, WA, Australia

Falls in older adults are a significant contributor to mortality and morbidity and may become increasingly common as the world's population ages ¹. Worsening proprioception at the ankle may contribute to mobility and balance deficits. The effect of age on proprioceptive acuity has been investigated but with opposing conclusions and variable methods ^{2,3}. Dependence on the history of muscle contraction and length changes is one influence on proprioception that has not been taken into account. The current study aimed to determine the effect of age on proprioceptive performance at the ankle with the knee straight, as this is relevant for standing, and controlled the history of muscle length changes during testing.

Studies were performed in 80 healthy people (42 female, age range 19 – 80 years) to see the effect of age on proprioceptive ability at the ankle joint. Participants sat with their leg extended and rested their foot on a motorised foot plate which rotated through the plantarflexor/dorsiflexor plane. The following proprioceptive outcomes were measured; 1) threshold of detection to passive movement at the ankle, 2) proprioceptive reaction time 3) ankle joint position reproduction. Prior to each trial for each test the ankle was moved backwards and forwards several times to present a constant history of muscle length changes. Reliability of this new methodology and equipment was also studied across multiple test sessions. Linear regression of age against the outcome for each task showed proprioceptive acuity was not affected by age (detection threshold $R^2 = 0.01$, $P = 0.42$; reaction time, $R^2 = 0.003$, $P = 0.65$; position reproduction [absolute mean error], $R^2 = 0.02$, $P = 0.17$). Older people (66-80 years; $n=20$) had the same ability as younger people (18-35 years; $n=20$) in all tasks, respective detection thresholds of passive movement (mean $\pm$ SD) were $0.14\pm0.07^\circ$ and $0.14\pm0.14^\circ$; reaction times to movement were 0.25 ± 0.06 s vs 0.25 ± 0.07 s, respectively; and absolute mean error for joint position reproduction, $16.6\pm10.1^\circ$ vs $12.4\pm8.4^\circ$, respectively. All measures ranked as good to excellent in reliability: detection threshold ICC = 0.89

(CI = 0.78 -0.95), reaction time ICC = 0.89 (0.77 – 0.95), and joint position sense ICC = 0.93 (0.85 – 0.97).

This study is not alone in finding no difference in proprioceptive performance between young and old people^{2,4}. Exercise has been implicated in preserving proprioceptive ability of older people to the same level as young people⁵ and it may be that the older cohort in this study participated in enough activity to retain ankle proprioceptive function. Future age-related proprioceptive investigations should also ascertain participant activity levels.

1. WHO. (2007) *Geneva: World Health Organization*

2. Boisgontier MP *et al.* (2012) *Age* 34, 1179-94

3. Gilsing MG *et al.* (1995) *Age and Ageing* 24, 58-66

4. Contu S. *et al.* (2016) *IEEE 38th Ann Inter Conf of IEEE*, 4614-4617

5. Tsang WWN & Hui-Chan CWY. (2004) *Medicine & Science in Sports & Exercise* 36, 658-67

I would like to thank my supervisors Simon Gandevia and Janet Taylor and NHMRC for providing the Motor Impairment Program Grant which funded this project.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C064

Morphological alterations of mouse skeletal muscle during late maturation and early ageing are muscle specific

G.M. Messa¹, M. Piasecki², J. Hurst³, J. McPhee⁴, J. Tallis⁵ and H. Degens^{1,5,6}

¹Healthcare Science, Manchester Metropolitan University, Manchester, UK, ²MRC-ARUK Centre for Musculoskeletal Ageing Research and National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Nottingham, UK, ³Center for Sport, Exercise and Life Sciences, Coventry University, Coventry, UK, ⁴Department of Sport and Exercise Sciences, Research Centre for Musculoskeletal Science & Sports Medicine, Manchester Metropolitan University, Manchester, UK, ⁵Institute of Sport Science and Innovations, Lithuanian Sports University, Lithuania, Lithuania and ⁶University of Medicine and Pharmacy of Targu Mures, Targu Mures, Romania

Abstract

Maturation and ageing are associated with muscle growth and wasting, respectively. Comparing partially matured animals with those in early ageing may mask age effects on muscle morphology. To investigate this, we compared capillarisation, fibre type composition, fibre size (FCSA) and oxidative capacity of individual fibres of the soleus (SOL), extensor digitorum longus (EDL) and diaphragm (DIA) muscles in 20- (adult) and 79-week-old (early ageing) CD-1 female mice. The FCSA was larger and the capillary density (CD) lower at 79 than 20 weeks in the DIA, while in the EDL the opposite was found ($p < 0.02$). There were no significant age-related differences in FCSA and CD in the SOL. The heterogeneity in capillary spacing was

highest in muscles from 20-week-old mice, irrespective of muscle ($p \leq 0.011$). Succinate dehydrogenase activity (SDH-OD), an indicator of oxidative capacity, progressively declined with age in the DIA only ($p < 0.010$). At all ages, the capillary supply to a fibre was positively related to FCSA in each muscle. We conclude that in mice late maturation (20 weeks) and early ageing (79 weeks) differ, and patterns of changes in FCSA and CD differ between the DIA, EDL and SOL. The relationship between the capillary supply to a fibre with fibre size is similar throughout maturation and ageing, and is independent of muscle of origin and oxidative capacity. Keywords: Ageing, Maturation, Capillarisation, Oxidative capacity, Skeletal muscle

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C065

Post-exercise hypotension and skeletal muscle oxygenation is modulated by nitrate-reducing activity of oral bacteria in healthy individuals

R. Bescos, C. Cutler and M. Hickson

University of Plymouth, Plymouth, UK

Post-Exercise Hypotension (PEH) is a common physiological response leading to lower blood pressure during the recovery period after an acute bout of exercise. However, the mechanism triggering PEH is still not fully understood. Here, we investigated whether oral nitrite synthesis during the recovery period is a key mechanism inducing PEH in healthy individuals.

Twenty-three individuals participated in this randomized, double-blind and cross-over study. They performed two treadmill trials, separated by a week running four sets of 7 min at 65% of VO_{2peak} interspersed with 3 minutes of passive recovery. At 1, 30, 60 and 90 minutes after exercise, participants rinsed their mouth for 1 minute with antibacterial mouthwash to inhibit activity of oral bacteria, or a placebo mouthwash. Blood pressure was measured before, 1 and 2 hours after exercise. The microvascular response was assessed measuring the tissue oxygen index (TOI) in the forearm (extensor digitorum) before and 2 hours post-exercise as determined by near-infrared spectroscopy (NIRS). TOI levels from the rectus femoris were also measured at the same time using a second NIRS channel. Saliva and blood samples were also taken before and 2 hours after exercise.

Exercise induced a significant reduction of systolic blood pressure (SBP) (1h: -5.2 ± 5.0 mmHg, $P < 0.001$; 2h: -3.8 ± 5.3 mmHg, $P = 0.005$) compared to baseline in the placebo condition. Similarly, diastolic blood pressure (DBP) fall in the first hour of recovery (1h: -2.4 ± 3.4 mmHg; $P = 0.004$), but not at 2 hours in the placebo treatment (2h: -0.9 ± 3.3 mmHg; $P = 0.167$) compared to baseline levels. These changes were associated with higher plasma nitrite availability (pre-exercise: 59 ± 34.3 nM; 2h post-exercise: 100 ± 50 nM, $P = 0.013$) and a greater peak of TOI following the occlusion period of reactive hyperamia ($+1.2 \pm 2.0\%$, $P = 0.010$) after

exercise in the placebo condition. Average TOI values from the rectus femoris were also higher after exercise ($77.7 \pm 5.8\%$) compared to baseline ($74.2 \pm 5.6\%$, $P < 0.001$). Antibacterial mouthwash reduced significantly nitrite availability in saliva ($P < 0.001$) and plasma ($P < 0.001$) compared to placebo. Consequently, reduction of SBP was attenuated by 61% at 1h ($+ 2.4 \pm 4.6$ mmHg, $P = 0.021$) and fully inhibited at 2h ($+ 4.1 \pm 4.6$ mmHg, $P = 0.026$) after exercise. A similar response was observed in DBP as its reduction was attenuated by 29% at 1h and fully inhibited at 2h after exercise in the antibacterial mouthwash treatment compared to placebo, but differences between treatments were not statistically different ($P > 0.05$). TOI levels in the forearm (reactive hyperamia test) and leg did not differ ($P > 0.05$) after exercise compared to baseline when oral bacteria was inhibited with antibacterial mouthwash.

Nitrate-reducing activity of oral bacteria is a key regulator of PEH and skeletal muscle oxygenation, and this response is disabled by using antibacterial mouthwash in healthy individuals.

Halliwill JR, Buck TM, Laceywell AN, Romero SA. Postexercise hypotension and sustained postexercise vasodilatation: what happens after we exercise? *Experim Physiol.* 2013;98(1):7-18.

Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008;7(2):156-67.

Bryan NS, Tribble G, Angelov N. Oral Microbiome and Nitric Oxide: the Missing Link in the Management of Blood Pressure. *Curr Hypert Rep.* 2017;19(4):33.

Kapil V, Haydar SMA, Pearl V, Lundberg JO, Weitzberg E, Ahluwalia A. Physiological role for nitrate-reducing oral bacteria in blood pressure control. *Free Rad Biol Med.* 2013;55:93-100.

This study was supported by research grants from the Oral and Dental Research Trust (UK) and the Institute of Health & Community (University of Plymouth, UK).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C066

21-day 'dry' immersion of healthy people leads to reduction of baroreflex synchronization of heart rate and arterial pressure during orthostasis, similar to observed in patients with vasovagal syncope

A. Borovik¹, E. Orlova¹, V. Negulyaev^{1,2}, O. Tarasova^{1,2} and O. Vinogradova¹

¹Institute for Biomedical Problems, Moscow, Russian Federation and ²Lomonosov Moscow State University, Moscow, Russian Federation

'Dry' immersion is a commonly accepted simulation of microgravity conditions (Tomilovskaya et al., 2019). Investigation of disorders in hemodynamic regulation, caused by immersion, can furnish insights into the nature of orthostatic intolerance observed in astronauts upon return to earth.

8 healthy young men were in «dry» immersion for 21 days. The day before, on 7th, 14th and 19th day of immersion, as well as on 1st and 5th day after, all participants

performed head-up tilt test (TT): 15 min in supine position (0°) then 15 min orthostasis (60°). During the test ECG (PneumoCard, MCS, Russia), arterial pressure, stroke volume (Finometer, Finapres Medical System, The Netherlands) and respiration rate (nasal thermistor sensor) were recorded. Index of phase synchronization (PSI) of beat-to-beat mean arterial pressure (MAP) and heart rate (HR) at frequency range of baroreflex waves (~0.1 Hz) was used as a measure of baroreflex activity (Borovik et al.,2014). Similar measurements during prolonged (up to 40 min) TT were performed on a group of patients with diagnosed vasovagal syncope.

Long stay in dry immersion leads to drastically alterations in hemodynamic response to the change of body position (Table 1). In immersion, participants had tachycardia and reduction in SV even at a horizontal position of the body, there was also a more pronounced increase of HR during orthostasis. Slight decrease in pressure during orthostasis can be noted also. Before immersion, a transition from supine position to orthostasis was accompanied by an increase in PSI, in contrary during immersion PSI did not change. All measured parameters returned to initial values at 5th day after immersion.

Patients were divided into 2 groups: who did not show any evidence of syncope during orthostasis (TT- group) and who showed signs of pre-fainting (TT+ group). In TT- group (N=13), as well as in control group (N=13), a transition from supine position to orthostasis increased PSI (from 0.18 ± 0.05 to $0.28 \pm 0.09^*$, respectively, $p < 0.05$) in baroreflex frequency range, in contrast to TT+ group (N=11) in which PSI did not change.

CONCLUSION: The lack of increase of phase synchronization of MAP and HR at the frequency range of baroreflex waves during TT indicates disorders of baroreflex regulation of hemodynamics and, as a consequence, the inability to maintain blood pressure at the level necessary to support the permissible level of cerebral perfusion.

Table 1.

	Day before immersion		19th day immersion	
	supine	orthostasis	supine	orthostasis
HR, bpm	68±9	85±9*	83±13#	137±13*
MAP, mmHg	95±6	91±6	98±9	90±9*
SV, ml	88±16	67±17*	62±9#	42±9*
PSI	0.18±0.06	0.30±0.09*	0.22±0.06	0.22±0.05

* $p < 0.05$ vs respective values in supine position; # $p < 0.05$ vs respective values measured before immersion (Wilcoxon matched-pairs signed rank test). Values are presented as Mean±S.D.

Borovik AS *et al.* (2014). IEEE Xplore Digital Library, 8th Conference of ESGCO, 217-218.

Tomilovskaya E *et al.* (2019). Front Physiol 10,284-301.

The work is performed according to the Plan for Fundamental research of SRC RF Institute of Biomedical Problems RAS and with partial financial support of the RFBR (N17-04-01943).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Human disuse atrophy may be wholly explained by sustained declines in muscle protein synthesis in the absence of increased muscle protein breakdown

M.S. Brook¹, T. Stokes², S. Gorissen³, J.J. Bass¹, J. Cegielski¹, D.J. Wilkinson¹, B. Phillips¹, K. Smith¹, S. Phillips² and P.J. Atherton¹

¹University of Nottingham, Nottingham, UK, ²McMaster University, Hamilton, ON, Canada and ³Maastricht University, Maastricht, Netherlands

Introduction: Skeletal muscle mass is maintained by the balance between muscle protein synthesis (MPS) and breakdown (MPB), with chronic imbalances resulting in atrophy or hypertrophy. Immobilization (e.g. due to casting, bed rest) results in deleterious atrophy. However, the primary driver (i.e. MPS vs. MPB) of disuse atrophy remains poorly defined - primarily because methods to quantify MPB have remained technologically difficult, while quantification of MPS has been historically limited by duration. However, recent developments in D2O stable isotope tracer methods could help resolve these questions **Aim:** To determine the effect of short-term immobilization on integrative MPS and extrapolated MPB. **Methods:** Nine healthy young men (22±2y, BMI 24±1) undertook 4 days unilateral lower limb suspension, in which after randomization, one leg remained ambulatory (control) and the other immobilized (immob). Subjects underwent baseline (d0) measures of ultrasound-derived *vastus lateralis* (VL) muscle thickness (MT), maximal voluntary contraction (MVC), and leg fat free mass (LFFM) via DXA; all measures were repeated in both legs 4 days (d4) after immobilization. In addition, after a baseline blood sample on d0, subjects consumed 3ml/kg of 70% D2O with VL muscle biopsies taken from each leg after immobilization. MPS was calculated using GC-Pyrolysis-IRMS methods $MPS = -\ln((1 - [APE \text{ Ala} / APE \text{ P}]) / t) \times 100$. Fractional breakdown rate (FBR) was estimated as fractional synthetic rate (FSR) – the fractional growth rate (FGR), with FGR calculated as the change in DXA mass. **Results:** After immobilization, MVC remained stable in control legs (253±16Nm to 264±23Nm), whilst immob legs declined (260±14Nm to 230±12Nm (P<0.05)) at d0 and d4, respectively. In control legs, LFFM remained unchanged (7476±474g to 7501±477g) whilst the immob legs declined (7477±423g to 7365±420g (P<0.05)) at d0 and d4, respectively. This was consistent with VL MT remaining stable in control legs (2.7±0.2cm to 2.7±0.2cm) whilst declining in immob legs (2.7±0.2cm to 2.6±0.2cm (P<0.05)) at d0 and d4, respectively. Reflecting declines in muscle mass, MPS decreased during immob (1.29±0.2%.d⁻¹ vs. 1.54±0.2%.d⁻¹ (P<0.01)) in immob versus control legs, respectively; further, decreases in MPS correlated with decreased VL MT (P<0.05 r²=0.5). FGR decreased in immob vs. control (-0.37±0.15%.d⁻¹ and 0.08±0.12%.d⁻¹ respectively). As such, FBR in control was 1.44±0.2%.d⁻¹ being in overall balance with FSR (P=0.7 vs. FSR). Similarly, FBR was unchanged with immob (1.67±0.1%.d⁻¹ (P=0.3 vs. control)). **Conclusion:** Declines in muscle mass are reflected by, and correlated with, sustained declines in MPS. Extrapolated FBR further supports the notion that declines in MPS are the primary

driver of disuse atrophy. These results have major implications in the hunt for therapeutics.

The authors would like to acknowledge the MRC-ARUK Centre for Musculoskeletal Ageing Research and the National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C068

Compromised skeletal muscle stem cell function in a mouse model of hyperlipidaemia and atherosclerosis

J. Barlow, P. Sfyri, S. Verpoorten, D. Scully and A. Matsakas

Hull York Medical School, Hull, UK

Background: Skeletal muscle stem cells are responsible for the regenerative capacity of skeletal muscle and undergo activation, proliferation, and differentiation upon injury, in order to support muscle repair. Apolipoprotein E deficient (ApoE KO) mice develop hyperlipidaemia and atherosclerosis and exhibit increased oxidative stress and triglyceride contents in skeletal muscle. Experimental evidence suggests that ApoE KO mice have impaired skeletal muscle regeneration after injury. However, the skeletal muscle stem cell function of ApoE KO mice, remains largely unknown. We hypothesised that the attenuated regenerative capacity of the ApoE KO mice derives from deficits in skeletal muscle stem cell function. Therefore, we investigated the impact of ApoE deficiency on the proliferation and differentiation profiles of muscle stem cells.

Methods: Muscle stem cells from the extensor digitorum longus and biceps brachii were isolated from ApoE KO and wild type mice for single fibre culture experiments *ex vivo* and isolated stem cell cultures *in vitro*. Isolated stem cell proliferation and differentiation, morphology, immunohistochemistry and gene expression analysis were performed. Cultures were treated with 0.5mM palmitate for 24h in relevant experiments. Cell culture experiments were conducted with n=6-9 technical replicates and n=2-3 independent experiments. Significant differences were detected by the Mann-Whitney U test for $p < 0.05$.

Results: No significant differences in myogenic proliferation were observed between wild type and ApoE KO mice in single fibres after 48h in culture. However, impaired differentiation of ApoE KO mouse muscle stem cells was evident on single fibres *ex vivo* after 72 hours, based on expression patterns of myogenin. Furthermore, palmitate treatment resulted in loss of stemness and increased commitment to differentiation (i.e. Pax7^{-ve}/MyoD^{+ve} cells). Isolated muscle stem cells from ApoE KO mouse displayed impaired proliferation as well as differentiation, with morphological studies resulting in reduced myotube width and length and fusion-index, an indicator of reduced myotube fusion. These results were exacerbated by

application of palmitate in both wild type and ApoE KO. These findings are strengthened by reduced gene expression for key factors regulating skeletal muscle fusion of myogenic progenitors, cell fate and contractility (i.e. MyoD, Scrib1, myogenin, Bex1, tmem8c, srf, mhc1 and acta1).

Conclusion: We report for the first time impaired skeletal muscle stem cell function in hyperlipidaemic ApoE KO mice, which may account for the impaired muscle regenerative capacity of this model. These data establish a link between muscle stem biology and impaired myogenesis in an experimental model of atherosclerosis and systemic hyperlipidaemia with implications for the myopathies seen in cardiovascular disorders.

Sfyri PP et al. (2018). *Free radical biology & medicine*. 129:504-19.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C069

Transcriptional pathways of skeletal muscle protein synthesis and turnover are associated with the post-exercise protein and polyphenol supplementation mediated recovery from maximal eccentric contractions.

G.F. Pavis¹, T.S. Jameson¹, M.L. Dirks¹, B.T. Wall¹, N. Alamdari², C. Mikus² and F. Stephens¹

¹University of Exeter, Exeter, UK and ²Beachbody LLC, Santa Monica, CA, USA

Consumption of a post-exercise protein-polyphenol supplement accelerates recovery of muscle function after maximal eccentric contractions (EC), likely due to increased muscle protein turnover and/or reduced inflammation. Here we investigate the differences in transcriptional events that occur during recovery with protein-polyphenol supplementation to provide novel understanding in to the mechanisms by which this is mediated.

Eighteen young, recreationally active participants (age: 22 ± 1 y; BMI: 24.3 ± 0.8 kg·m⁻²) performed 300 maximal unilateral quadriceps EC on day 7 of a 14-day protocol, followed by daily assessment of isokinetic concentric work (IC). Supplements providing 20 g protein from a blend of whey, casein, and pea, and 650 mg pomegranate extract (Beachbody LLC, USA; PRO; n = 9; 3 females) or an isocaloric carbohydrate placebo (PLA; n = 9; 4 females) were consumed daily, alongside full dietary control providing 1.2 g·kg⁻¹ of protein. RNA was isolated from muscle biopsy samples obtained at 24, 27, 36, 72 and 168 h post EC and reverse transcribed into cDNA. Transcriptional expression of 224 genes associated with skeletal muscle metabolism were assessed by RT-PCR. IC data were measured relative to the contralateral leg (%con). Gene expression data were normalised to α -actin and β 2-microglobulin, and to the control leg of a PLA subject, using the $2^{-(\Delta\Delta Ct)}$ method. Two-way ANOVAs were used to analyse the data, with the alpha level for transcriptional changes informed by a prior Significance Analysis for Microarrays calculation (FDR<1%). Gene ontology

analysis of differentially expressed genes was performed against the KEGG pathway (DAVID 6.8, NIH). The original gene list was used as a background for the enrichment analysis to eliminate bias from the selection of genes.

Eccentric exercise reduced IC to $67.6 \pm 5.9\%$ con at 48 h in PLA, which remained suppressed for 120 h ($P < 0.05$). Conversely in PRO, IC dropped to $78.3 \pm 7.2\%$ con at 24 h ($P < 0.01$) and had recovered by 48 h ($P > 0.05$). Expression of 68 genes changed over time following EC ($n = 7$ per group; time effect; $P < 0.01$), with mean expression reducing linearly from 19.3 ± 5.4 -fold at 24 h to 3.3 ± 0.4 -fold 168 h post-EC. Between PLA and PRO, 112 genes were differentially expressed (group effect; $P < 0.01$), with 'mTOR signaling' the most enriched pathway in gene ontology analysis. Over time, 26 genes were expressed divergently between PLA and PRO (interaction effect; $P < 0.01$), with 'Hippo signaling' pathway the most enriched. EC induces a rapid, robust change in skeletal muscle gene expression that is associated with reduced muscle function. Consumption of a protein-polyphenol supplement following EC alters this response and is associated with improved recovery, likely via protein synthesis (mTOR) and regulation of turnover (Hippo) pathways.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C070

Post-Exercise and Pre-Sleep Protein-Polyphenol Supplementation Attenuates NF- κ B Related Signalling and Improves Recovery following Muscle-Damaging Eccentric Exercise.

T.S. Jameson¹, G.F. Pavis¹, M.L. Dirks¹, B.T. Wall¹, N. Alamdari², C. Mikus² and F. Stephens¹

¹University of Exeter, Exeter, UK and ²Beachbody, Los Angeles, CA, USA

We have previously shown protein-polyphenol drinks consumed post-exercise and before bed accelerate functional recovery following eccentric contractions (EC). Microarray analysis has identified the inflammatory NF- κ B signalling pathway as important 3 h after EC, but its involvement beyond 3 h and association with functional recovery of skeletal muscle is unclear. Here we investigate changes in NF- κ B associated transcripts and muscle force production for 168h following EC and the effect of protein-polyphenol drink consumption.

In a randomised, parallel groups, placebo controlled double blind design, eighteen healthy males and females (22 ± 1 y; BMI: 24.0 ± 0.9 kg/m² ($\pm$ SEM)) consumed a controlled isocaloric diet (1.2 g/kg protein) for 6 days before and 7 after a single bout of 300 maximal unilateral quadriceps EC, in combination with either daily post-exercise (20 g whey, casein and pea protein blend and 650 mg pomegranate extract) and pre-bed (20 g casein protein and 480 mg tart cherry extract) drinks (Beachbody LLC) (PRO; $n = 9$; 4 females), or isocaloric maltodextrin placebos (PLA; $n = 9$; 3 females). Total isokinetic work over 30 maximal knee extensions (TW) was

measured relative to the contralateral control leg (%con) before and every 24 h for 7 d following EC. Muscle biopsies from the vastus lateralis were taken 24, 27, 36, 72 and 168 h post EC. RT-PCR of 21 NF- κ B related transcripts was performed using OpenArray plates on a QuantStudio 12K Flex System. Data were normalised to α -actin and β 2-microglobulin, and the control leg of a PLA subject, using the $2^{-\Delta\Delta Ct}$ method. Muscle function and gene expression data were analysed using two- (group*time) and three-way (leg*group*time) ANOVAs, respectively. EC caused a maximum decline in TW after 48 h in PLA to 68 ± 6 %con ($P < 0.001$), which remained impaired until 120 h. Conversely, TW in PRO was impaired at 24 h only (89 ± 5 %con; $P < 0.05$). We identified 10 differentially expressed NF- κ B associated transcripts in the eccentric leg 24 – 168 h post EC ($P < 0.05$). Of these, 8 showed an increase in expression at 24 h in both groups, 2 in PLA only (IL1R1, IL1RL1) and 1 in PRO only (NFKB1) ($P < 0.05$). Interaction effects show the expression of two cytokine receptors, IL1R1 and IL1RL1 peaked at 24 h in PLA (16.8 ± 4.7 fold and 216.0 ± 96.3 fold, respectively. $P < 0.01$) and declined linearly thereafter. Expression of both IL1R1 and IL1RL1 was higher in PLA than PRO at 24 h (PRO = 5.5 ± 8.6 and 38.4 ± 23.5 fold, respectively. $P < 0.05$). IL1R1 and IL1RL1 expression in PRO did not change throughout the time course. Increased expression of some NF- κ B related transcripts precedes the functional decline of skeletal muscle following EC. Post-exercise and before bed protein-polyphenol beverages attenuate this response suggesting inflammatory NF- κ B signalling may negatively impact recovery following EC.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C071

The effect of sprint interval training in fasted and carbohydrate-fed states on exercise performance including inter-individual adaptive responses in recreationally active males

T.P. Aird^{1,2} and B.P. Carson^{1,2}

¹Physical Education and Sport Sciences, University of Limerick, Limerick, Ireland and

²Health Research Institute, University of Limerick, Limerick, Ireland

Sprint interval training (SIT) has emerged as a time efficient alternative to conventional endurance exercise, inducing beneficial physiological adaptations. Nutritional support, including exercise in the fasted state, to optimise the response to SIT remains poorly characterised. The purpose of this study was to compare the effects of fasted (FAST) and CHO-fed (FED) SIT on exercise performance in recreationally active males and to describe the inter-individual variation in response to SIT. This study received local ethical approval and was conducted in line with the principles of the World Medical Association's Declaration of Helsinki. Healthy, untrained, recreationally active ($\text{VO}_2 \text{ max} < 50 \text{ ml.kg.min}^{-1}$) males ($n=17$) participated in a 3 week

SIT intervention, comprising 9 exercise sessions performed in either a fasted or CHO-fed condition. Participants were randomised to FAST and FED groups at baseline. For each exercise session, participants arrived to the lab following an overnight fast (≥ 10 h) and ingested either 0.33 g.kg^{-1} body mass (BM) of non-caloric placebo (FAST), or 0.91 g.kg^{-1} BM maltodextrin (FED). SIT consisted of 4-6 X 30 s “all out” cycle sprints at a resistance of 7.5 % BM, interspersed with 4 min active recovery. Participants completed a 30 s Wingate test and a 20 min cycle performance test at baseline and 72-96 h following the final SIT session. (Non-)Responders were identified using twice the typical error (TE) of measurement for Wingate peak power (PP; $2 \times \text{TE} = 1.58 \text{ W.kg}^{-1}$), minimum power (MP; $2 \times \text{TE} = 0.81 \text{ W.kg}^{-1}$) and mean power (AP; $2 \times \text{TE} = 0.39 \text{ W.kg}^{-1}$), and for mean power during the cycle performance test (CP; $2 \times \text{TE} = 10.07 \text{ W}$). Independent t-tests determined between-group differences in change in exercise performance with data reported as mean $\pm$ SD. There was no significant difference between FAST and FED groups ΔPP (0.6 ± 1.2 vs. $1.0 \pm 1.0 \text{ W.kg}^{-1}$), ΔMP (0.5 ± 0.6 vs. $0.1 \pm 0.8 \text{ W.kg}^{-1}$), ΔAP (0.3 ± 0.5 vs. $0.3 \pm 0.4 \text{ W.kg}^{-1}$) or ΔCP (22.6 ± 11.3 vs. $19.3 \pm 20.3 \text{ W}$; all $p > 0.05$). Non-responders for PP (14/18, 77 %), MP (14/18, 77 %), AP (11/18, 61 %), and CP (7/18, 38 %) were identified across both groups. Global non-responders who did not improve any performance measure were also identified (3/18, 17 %). A greater number of responders to CP were identified in the FAST compared to FED (FAST: 88%; FED 44%). The number of responders between groups was similar for PP (FAST: 25%; FED 22%), AP (FAST: 50%; FED 33%) and MP (FAST: 25%; FED 22%). In conclusion, these findings indicate no significant difference in performance adaptations between FAST and FED SIT, however, heterogeneity is evident in the individual adaptations to SIT in measures of anaerobic and aerobic performance in both FAST and FED.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C072

Gravitational transitions induce systemic oxidative-nitrosative stress and cerebral hyperperfusion in humans

D.M. Bailey¹, D. Laneelle², J. Trihan³, B.S. Stacey¹, K. Tamiya⁴, T. Washio⁴, S. Ogoh^{1,4} and H. Normand⁵

¹Faculty of Faculty of Life Sciences and Education, University of South Wales, South Wales, UK, ²Centre Hospitalo-Universitaire, Caen, France, ³Centre Hospitalo-Universitaire, Poitiers, France, ⁴Toyo University, Saitama, Japan and ⁵Normandie University, Normandie, France

Background: While spaceflight-associated neuro-ocular syndrome (SANS) related to cephalad fluid shift-induced intracranial hypertension is considered NASA's top health risk for long-duration spaceflight including a manned mission to Mars, the underlying mechanisms remain unclear (Roberts *et al.*, 2017). In the present study,

we examined if repeated bouts of micro- and hypergravity during parabolic flight (PF) would result in exaggerated systemic oxidative-nitrosative stress reflected by a free radical-mediated reduction in nitric oxide (NO) bioavailability and regional cerebral hyper-perfusion.

Methods: Six participants (5♂, 1♀) aged 29 ± 11 years were examined before, during and after PF. Six gender and age-matched (27 ± 6 years) controls were subject to the same procedures/experimental timeline with the exception of PF. Duplex ultrasound was employed to simultaneously measure blood flow in the anterior (internal carotid artery, ICA) and posterior (vertebral artery, VA) circulation. Plasma and red blood cells were obtained from venous blood and assayed for the ascorbate radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy), nitrite (NO_2^-), S-nitrosothiols (RSNO) and S-nitrosohaemoglobin (SNO-Hb, ozone-based chemiluminescence) according to established methods (Bailey *et al.*, 2017). Changes in central blood volume (CBV) were measured using a tetrapolar high-resolution impedance monitor, four-channel digital impedance meter (Ogoh *et al.* (2018)).

Results: Transitioning from hyper-micro-hypergravity induced marked differential changes in CBV ($P < 0.05$). A selective increase in VA flow was observed in-flight during the most marked gravitational transition from micro- to hypergravity ($P < 0.05$). This was associated with an elevation in $A^{\bullet-}$ and corresponding reduction in (plasma + RBC) NO_2^- , RSNO and SNO-Hb, taken to reflect exaggerated systemic oxidative-nitrosative stress ($P < 0.05$).

Conclusions: Collectively, these data are the first to demonstrate that acute gravitational transitions result in selective hyperperfusion of the posterior cerebral circulation that appears to be subject to redox-regulation. This may have a teleological basis given that the territories it feeds, notably the medulla oblongata, cerebellum, hypothalamus, thalamus and brainstem, are phylogenetically older with priority over other (younger, more anterior) regions for oxygen and glucose supply given their arguably more critical roles in maintaining homeostasis. From a practical perspective, these findings may provide complementary insight into the pathophysiology of SANS and help inform the design of potential countermeasures. Roberts DR *et al.* (2017). *N Engl J Med* 377, 1746-1753

Bailey DM *et al.* (2017). *Circulation* 135: 166-176

Ogoh S *et al.* (2018). *J Appl Physiol* 125: 634-641

This work was supported by grants from Centre National d'Etudes Spatiales (CNES) and the European Space Agency (ESA). DMB is supported by a Royal Society Wolfson Research Fellowship (#WM170007).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C073

Treatment with the synthetic retinoid Fenretinide protects against fatty liver disease in LDLR^{-/-} mouse model of atherosclerosis

D. Thompson, S. Mahmood, P. Hoffman, R. Dekeryte, S. Kamli-Salino and N. Mody

Institute of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK

Obesity has reached epidemic proportions worldwide contributing to the pathophysiology of many diseases including type 2 diabetes, cardiovascular disease, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). As such, there is an urgent need for new therapeutics to halt the exponential rise in individuals presenting with these conditions. Increased lipotoxicity and ceramide accumulation has been attributed to be a major player in the progression of obesity-associated diseases. Therefore, targeting ceramide accumulation is an attractive target for manipulation.

Fenretinide (FEN) is a synthetic retinoid that blocks ceramide accumulation in adipose and liver tissue in both high-fat diet-fed mice and genetically-obese diabetic mice and prevents obesity and insulin resistance. However, the potential beneficial effects of FEN in either NAFLD or atherosclerosis is unknown. We sought to answer these questions using the LDLR^{-/-} mouse model of atherosclerosis. All animal procedures were performed under a project license approved by the U.K. Home Office under the Animals (Scientific Procedures) Act 1986. Mice were fed either control (n=14) or high cholesterol/high fat diet (HFD, n=18) for 14 weeks to induce atherogenesis and steatosis with or without 0.04% FEN (HFD-FEN, n=18). Data are expressed as mean $\pm$ S.E.M, and analysed by either one- or two-way ANOVA, followed by Bonferroni multiple comparison tests.

FEN treatment prevented weight gain in LDLR^{-/-} mice, specifically due to fat mass accumulation, and exhibited increased sensitivity to insulin when compared to mice fed HFD alone ($p<0.001$ and $p<0.01$ respectively, $n=10$ per group). Hepatic genetic analysis revealed that HFD increased expression of the inflammatory cytokines TGF1 β (3-fold, $p<0.05$) and TNF α (10-fold, $p<0.05$), and the fibrosis genes collagen (3-fold; *Col1a1*, *Col4a1*, $p<0.01$ and $p<0.05$ respectively) and matrix metalloproteinase protein 9 (10-fold *MMP9*, $p<0.001$). These were restored to control levels following FEN exposure. Fibrotic gene changes were reciprocated at the protein level as HFD-FEN fed mice had two-fold less MMP2 ($p<0.05$) than those on HFD alone. Finally, increased liver triglycerides are a hallmark of NAFLD. When compared to control mice, HFD feeding resulted in significantly increased liver triglycerides (14.24 ± 1.47 versus 35.28 ± 6.39 $\mu\text{g}/\text{mg}$ protein, $p<0.01$) which was attenuated following the addition of FEN to the diet (13.82 ± 0.97 $\mu\text{g}/\text{mg}$ protein). Taken together these data indicate, in addition to weight loss and restoring insulin sensitivity, FEN has beneficial effects on liver steatosis. Hence, this compound could also be used to curb NAFLD progression. Further analyses of tissues, including aortic atherosclerotic plaque formation, may reveal further beneficial phenotypes of FEN treatment in LDLR^{-/-} mice.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C074

Adiponectin inhibits contractile signalling via inhibition of Rho-associated protein kinase in human vascular smooth muscle cells

O. Katwan^{1,2}, S. Kennedy¹ and I. Salt¹

¹*Institute of Cardiovascular & Medical Sciences, University of Glasgow, Glasgow, UK* and ²*University of Diyala, Baqubah, Iraq*

Secretion of the adipocytokine adiponectin inversely correlates with adiposity. Adiponectin has been proposed to have insulin-sensitising and anti-inflammatory actions in metabolic tissues and anti-atherogenic actions in blood vessels. We have recently demonstrated that adiponectin secretion is reduced in perivascular adipose tissue (PVAT) from mice lacking the $\alpha 1$ catalytic subunit isoform of AMP-activated protein kinase (AMPK), a key regulator of cellular and whole body metabolism. Furthermore, adiponectin attenuated contraction of endothelium-denuded aortic rings in response to the thromboxane A2 receptor agonist U46619, suggesting adiponectin has direct anti-contractile effects on vascular smooth muscle. We therefore investigated whether adiponectin influenced contractile signalling pathways in cultured human aortic vascular smooth muscle cells (HAVSMCs).

The effect of globular adiponectin (gAd) on contractile signalling in HAVSMCs stimulated with U46619 was assessed by immunoblotting with specific antibodies to contractile signalling proteins. Intracellular HAVSMC Ca^{2+} concentrations were assessed using the ratiometric fluorescent dye Fura2. Rho-associated protein kinase (ROCK) activity and actin filament formation were assessed by ELISA and immunofluorescence microscopy of HAVSMCs stained with phalloidin respectively. Data are reported as mean $\pm$ SEM. Statistical significance was assessed by 2-way ANOVA. U46619 stimulated phosphorylation of myosin light chain (MLC) T18/S19 and the MLC phosphatase subunit MYPT1 at T696 2.8 $\pm$ 0.3-fold (n=4, p<0.0001) and 1.25 $\pm$ 0.02-fold (n=3, p<0.001) respectively. Preincubation of HAVSMCs with gAd completely abrogated U46619-stimulated phosphorylation of either protein (p<0.001). MYPT1 has been previously demonstrated to be inhibited by ROCK-mediated phosphorylation at T696, thereby increasing MLC phosphorylation and subsequent contraction. U46619 stimulated ROCK activity 2.3 $\pm$ 0.3-fold (n=3, p<0.05), which was inhibited by preincubation with gAd (p<0.05). In contrast, preincubation with gAd had no effect on U46619-stimulated increases in intracellular Ca^{2+} or protein kinase C (PKC) activation. Furthermore U46619-stimulated actin filament formation 1.9 $\pm$ 0.3-fold (n=3, p<0.01), an effect that was inhibited by preincubation with gAd or the ROCK inhibitor Y27632 (p<0.05 in each case). Intriguingly, although gAd has been previously reported to activate AMPK in many cell types, it had no effect on AMPK activity in HAVSMCs.

These data indicate that gAd inhibits U46619-stimulated contractile signalling in HAVSMCs by reducing ROCK activity, leading to decreased MYPT1 phosphorylation, increased dephosphorylation of MLC and reduced actin filament formation. The effects of gAd were independent of Ca²⁺, PKC and AMPK. These data identify a hitherto unappreciated mechanism by which adipose tissue may regulate vascular cell function.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C075

A novel *ex vivo* and *in silico* approach to determine placental tissue oxygenation in normal and fetal growth restricted pregnancies

G.A. Nye^{1,2}, A. Erlich³, E. Johnstone², R. Lewis⁴, I. chernyavsky^{3,2} and P. Brownbill²

¹Chester Medical School, University of Chester, Chester, UK, ²Maternal and fetal health, university of manchester, Manchester, UK, ³School of Mathematics, University of Manchester, Manchester, UK and ⁴Faculty of Medicine, University of Southampton, Southampton, UK

Introduction: Fetal growth restriction (FGR) is a global problem affecting 3-7% of pregnancies worldwide(1). Fetal growth depends on adequate oxygen supply(2). Understanding oxygenation at significant depths within organs is an unmet challenge, constrained by tissue accessibility and spatial resolution, preventing an understanding of metabolic dysregulation in the healthy and diseased state. This is especially challenging in the placenta with its complex anatomy and dual blood flows(3). **Methods:** Here we overcome this problem using novel methodology based on *ex vivo* perfusion of the human placenta employing high-resolution 3D oxygen-mapping of the intervillous space(IVS). Tissues were acquired following informed written consent from women attending St Mary's Hospital, Manchester, UK. Normal singleton pregnancies above the 20th centile for fetal weight(N=7) and diagnosed cases of FGR in pregnancy and a live birth weight below the 5th centile (FGR group, N=5) were recruited. *Ex vivo* perfusion was performed as previously described(4) adapted through using only one maternal side cannula, replicating a maternal single spiral artery and perfusing the IVS (Figure 1A). An oxygen-sensing optode and oxygen monitor measured oxygen readings in a Latin hypercube arrangement, predicted to optimise data collection for *in silico* modelling, capturing a 1.2x1.2x0.6 cm³ volume of the placental lobule (Figure 1B).

Results: There was a significant and consistent loss of oxygen with increased distance from the cannula in the normal group, equating to 90±34 mmHg at 8.9mm away from the cannula tip, or a loss of 10.1± 3.8 mmHg/mm (ANOVA P<0.05; *Dunnett's multiple comparisons test: P<0.05; Figure D). Further interrogation showed 47±13% of the oxygen provided was lost in the first 3mm from the source. Average IVS PO₂s revealed high variance within both groups (63.99, 58.48-79.41 mmHg and 64.76, 26.83-82.17 mmHg, median and interquartile

rages, normal and FGR, respectively; Figure 1E) but in the FGR group there was a clear stratification at the extremes of normality although this has not yet reached significance ($P=0.38$, F test) (Figure 1E).

Discussion: The stratification of oxygen gradients within the FGR group may be linked to the severity of the disease. The results gained from these methods have the potential to transform our understanding of spatial oxygen metabolism in the human placenta, providing a ground-breaking insight into the way oxygen diffusion, blood flow and microanatomy combine, stimulating further *in vivo* diagnostic development to assist in the management of pregnancy problems. The technology we report can be tailored to gain insight into spatial aerobic metabolism in a range of perfused tissue types.

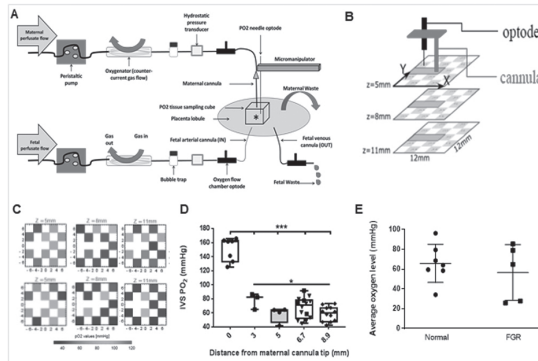


Figure 1. A novel ex vivo approach to determining placental tissue oxygenation in the intervillous space of a human placental lobule. A) Schematic representation of equipment employed in ex vivo dual perfusion of the human placenta with configuration for spatial PO₂ mapping in the intervillous space (IVS). All equipment, except for the pump was enclosed within a perfusion cabinet regulated at placental height to 37°C. B) Modified Latin hypercube sampling pattern used for PO₂ mapping in the IVS. C) 2 representative examples of spatial maps of oxygen values. D) IVS oxygen levels following spatial mapping at distances from the IVS inflow cannula in placentas from normal pregnancy (box and whisker plots; N=7; ANOVA $P<0.05$; *Dunnett's multiple comparisons test: * $P<0.05$, *** $P<0.001$). E) Average oxygen level in the measured tissue volume of the placental IVS in normal (N=7) and FGR pregnancies (N=5) demonstrating a stratification of oxygen consumption rates within the FGR group).

1. Romo A, Carceller R, Tobajas J. Intrauterine growth retardation (IUGR): epidemiology and etiology. *Pediatr Endocrinol Rev.* 2009;6 Suppl 3:332-6.
2. Carter AM. Placental Gas Exchange and the Oxygen Supply to the Fetus. *Compr Physiol.* 2015;5(3):1381-403.
3. Nye GA, Ingram E, Johnstone ED, Jensen OE, Schneider H, Lewis RM, et al. Human placental oxygenation in late gestation: experimental and theoretical approaches. *J Physiol.* 2018.
4. Brownbill P, Sebire N, McGillick EV, Ellery S, Murthi P. Ex Vivo Dual Perfusion of the Human Placenta: Disease Simulation, Therapeutic Pharmacokinetics and Analysis of Off-Target Effects. *Methods Mol Biol.* 2018;1710:173-89.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C076

Classification model for the detection of women at risk of preterm birth based on exosomal miRNAs and proteins in cervico-vaginal fluid at early gestation

H.J. Jenkins¹, C. Palma², A. Lai², D. Guanzon², N. Hezelgrave¹, A. Shennan¹, C. Salomon² and R.M. Tribe¹

¹*Women and Children's Health, Kings College London, London, UK and* ²*UQ Centre for Clinical Research, Exosome Biology Laboratory, Centre for Clinical Diagnostics, University of Queensland, Brisbane, QLD, Australia*

Every year, one in 13 babies are born prematurely in the UK. During pregnancy, the reproductive tract and cervico-vaginal fluid (CVF) is proposed to reflect inflammatory events which later contribute to early parturition. Exosomes, a specific subtype of secreted vesicles that have the capacity to transport bioactive molecules like miRNAs and proteins, are released from a wide range of cells including placental cells (Adam et al., 2017; Salomon et al., 2018). As the content of exosomes is cell type specific, we hypothesize that there will be distinct exosomal "fingerprints" associated with the risk of spontaneous preterm birth (sPTB). The aim of this study was to profile exosomes isolated from CVF obtained from women in the first and second trimesters of pregnancy who later had sPTB or term deliveries.

Thirty-seven pregnant women (n=10 PTB <37 weeks; n=27 >37 weeks' gestation) recruited as part of a larger prospective cohort study (INSIGHT REC No. 13/LO/0393; written informed consent) provided CVF between 10-23⁺⁶ weeks of pregnancy and clinical metadata. Exosomes were isolated from CVF by differential centrifugation and characterised by nanoparticle tracking analysis using the NanoSight NS500. An Illumina TrueSeq Small RNA kit was used to construct a small RNA library from CVF exosomal RNA and sequenced. Real time PCR was used to validate miRNA candidates in all samples. The exosomal proteomic profiles were identified in all samples by liquid chromatography mass spectrometry and quantified by Sequential Window Acquisition of All Theoretical (SWATH) analysis. LogitBoost regression was used to develop a classification model for early detection of sPTB cases using STATA software. Exosomes were identified as small vesicles (50-150 nm) in 30 CVF samples. Exosomal miRNA analysis revealed seven downregulated and nine upregulated miRNAs in sPTB cases compared with term. With a false discovery rate of 1 %, a total of 206 proteins within CVF exosomes were identified and quantified using SWATH. We identified three proteins that were significantly lower and one protein higher (p<0.05) in exosomes from sPTB compared with term. Using the miRNA and proteomic profiles of exosomes, a binomial classification algorithm was generated using a boosted logistic regression analysis (WEKA machine learning software). The algorithm was built using four miRNAs- and four proteins-associated with exosomes. The model delivered discrimination between women who had sPTB and term pregnancies, with an area under the curve of 0.822.

We provide evidence that first and second trimester CVF exosomes contain measurable miRNA and protein signals. In this small pilot study, the CVF proteomic signal

alone or in combination with miRNA shows potential in relation to the prediction of preterm birth. Validation of this novel exosomal biomarker panel is needed in a larger cohort of term and preterm pregnancies.

Adam et al. (2017). *Placenta* 54, 83-88.

Salomon et al. (2018). *CurrPharm Des* 9, 974-982.

We thank the CRN funded research midwives and research assistants involved with the study, Action Medical Research, RosetreesTrust, NIHR and the Tommy's Charity.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C077

Preeclampsia Link to Hypoxic Pregnancy

W. Tong^{1,2}, K.L. Brain¹, B.J. Allison¹, K.J. Botting^{1,2}, Y. Niu^{1,2}, S.G. Ford¹, P.B. Wooding¹, Q. Lyu¹, T.A. Garrud¹, L. Zhang¹, A. Sowton¹, K. O'Brien¹, T. Cindrova-Davies^{1,2}, B. Yung^{1,2}, G.J. Burton^{1,2}, A.J. Murray¹ and D.A. Giussani^{1,2}

¹*Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK* and ²*Centre for Trophoblast Research, University of Cambridge, Cambridge, UK*

Introduction: Preeclampsia remains a significant killer, affecting the mother and her offspring. However, underlying mechanisms remain uncertain, precluding plausible intervention. We investigated whether hypoxic pregnancy leading to fetal growth restriction (FGR) in sheep results in similar maternal cardiovascular and placental dysfunction, as in preeclampsia. We tested the hypothesis that preeclampsia is linked to hypoxic pregnancy.

Methods: Pregnant ewes were exposed to normoxia (N; n=10) or 10% hypoxia (H; n=7) for over 1 month from 105 to 138 days gestational age (dGA; term 145 dGA) in bespoke isobaric chambers. At 138 dGA, *in vivo* uterine resistance was assessed by Doppler while maintaining N or H, as appropriate. *Post mortem*, maternal, fetal and placental biometry was determined and placentomes collected for mitochondrial respirometry following permeabilization using saponin and molecular analysis. A separate cohort of pregnant ewes was instrumented at 120 dGA with a uterine Transonic flow probe and vascular catheters to record continuous maternal cardiovascular function via a wireless data acquisition system (CamDAS). Five days later, ewes were exposed to N (n=5) or H (n=5) for 10 days. Data were compared statistically via the Student's *t* test, two-way RM ANOVA and the Pearson correlation.

Results: Hypoxic pregnancy led to FGR and an increase in uterine artery pulsatility index (PI; Fig 1 A and B). It also prevented the fall in uterine vascular resistance and maternal blood pressure with advancing gestation measured in normoxic controls (Fig 1 C and D). Hypoxic pregnancy was associated with markers of increased placental oxidative stress and of unfolded protein response (UPR) activation in

the cytoplasm (UPR^{Cyt}), endoplasmic reticulum (UPR^{ER}) and mitochondria (UPR^{mt}; Fig 2 A-I). Hypoxic pregnancy did not affect mitochondrial density, but it led to a fall in mitochondrial citrate synthase activity and reduced mitochondrial oxygen consumption dependent on β -oxidation and Complex I (Figure 3). Conclusion: Hypoxic pregnancy leads to placental oxidative and mitochondrial stress associated with alterations in mitochondrial oxygen consumption and energy metabolism. These molecular mechanisms may provide a link between an increase in placental vascular resistance, FGR and maternal cardiovascular dysfunction in adverse pregnancy, as seen in preeclampsia.

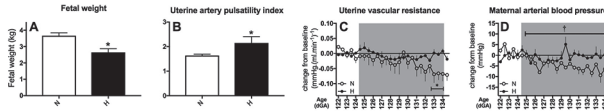


Figure 1. Fetal weight and changes in maternal cardiovascular function. Values are mean $\pm$ SEM for fetal weight (A), maternal uterine artery pulsatility index (B) and the change from baseline in uterine vascular resistance and maternal arterial blood pressure with advancing gestation (C and D). Significant differences ($P < 0.05$) are *N vs. H, Student's *t* test for unpaired data or N vs. H; † vs. baseline, two-way RM ANOVA where appropriate.

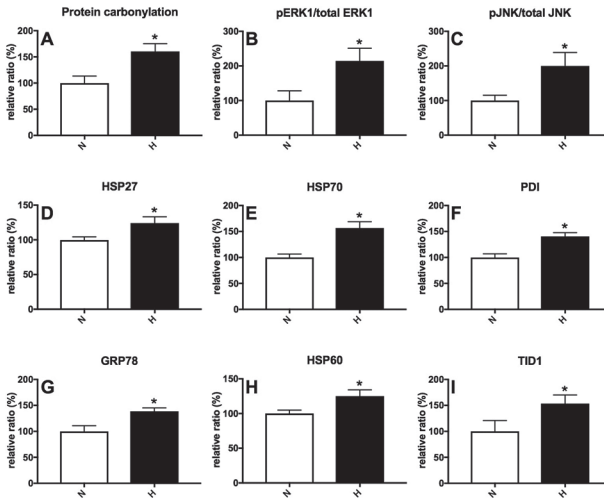


Figure 2. Placental stress response during hypoxic pregnancy correlates with asymmetric fetal growth restriction. Values are mean $\pm$ SEM for the relative ratio compared to normoxic controls of posttranslational protein carbonylation (A), of the ratio of phosphorylated stress kinases to total stress kinases ERK1 and SAPK/JNK (B and C), of the UPR^{Cyt} markers HSP27 and HSP70 (D and E), of the UPR^{ER} markers PDI and GRP78 (F and G) and of the UPR^{mt} markers HSP60 and TID1 (H and I). Significant differences ($P < 0.05$) are: *N vs. H, Student's *t* test for unpaired data.

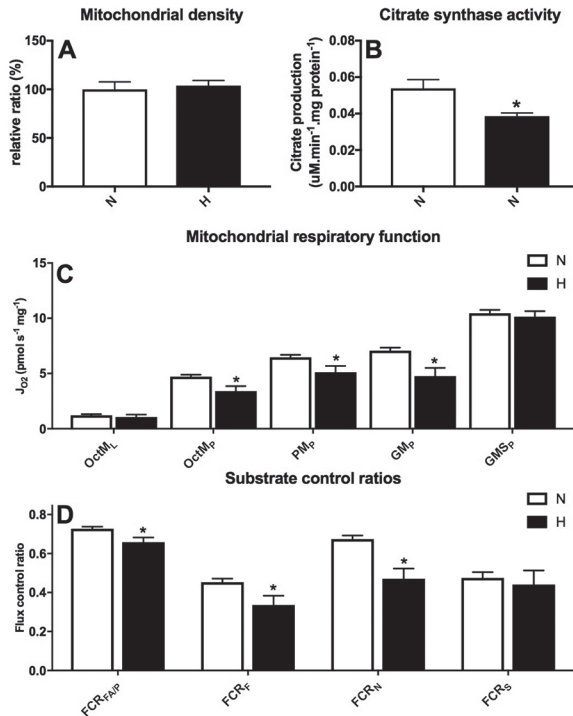


Figure 3. Chronic hypoxia alters placental mitochondrial respiration and substrate preference. Upper panel: Values are mean $\pm$ SEM for the relative ratio compared to normoxic controls of mitochondrial density (A) and citrate synthase enzyme activity (B). Middle panel: Values are mean $\pm$ SEM for mitochondrial oxygen consumption (J_{O_2}) normalized to mass during LEAK respiration (OctM_L) and OXPHOS stimulated by the F-pathway via β -oxidation (OctM_P), by the N-pathway via complex I (PM_P and GM_P) and by the NS-pathway via complex I and II (GMS_P). Lower panel: Flux control ratios (FCRs) for OXPHOS stimulated by octanoyl carnitine and malate relative to pyruvate and malate (FCR_{FAP}) and the contributions of the F-, N- and S-pathways to maximal OXPHOS capacity (FCR_F, FCR_N and FCR_S). Significant differences ($P < 0.05$) are: *N vs. H, Student's *t* test for unpaired data.

The British Heart Foundation and the Centre for Trophoblast Research.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C078

ATP reduces functional cell-to-cell tethering between renal tubular epithelial cells

P.E. Squires, E. Siamantouras, G.W. Price, J.A. Potter and C.E. Hills

Life Sciences, University of Lincoln, Lincoln, UK

Background: Loss of epithelial (E)-cadherin mediated cell-cell adhesion impairs gap junction formation and facilitates hemichannel-mediated ATP release in the diabetic kidney. Linked to inflammation and fibrosis, we hypothesize that local increases in inter-cellular ATP activate P2X7 receptors on neighbouring epithelial cells of the proximal tubule, to further impair cell-cell adhesion and exacerbate tubular injury.

Methods: Atomic force microscopy (AFM)-single cell force spectroscopy (SCFS) was used to quantify the unbinding forces needed to separate clonal tubular epithelial cells of the proximal kidney (HK2) following treatment with non-hydrolysable ATP γ S (100 μ M) $\pm$ the P2X7 purinergic receptor antagonist A438079 (50 μ M) over 48hr. Densitometry was used to semi-quantify Western blot protein expression. Biophysical measurements quantified maximum unbinding force (F_{max}), tether (or ligation cluster) rupture events ($TREs$) and work of detachment (W_d). Three force-displacement curves were acquired per cell. Data from SCFS are expressed as mean $\pm$ SEM. Sample numbers refer to separate cell passages (n) from multiple individual cells. Data was evaluated using univariate anova followed by Tukey's multiple comparisons post-test. $P < 0.05$ was taken to indicate statistical significance.

Results: The P2-purinergic receptor agonist ATP γ S (100 μ M), decreased E-cadherin expression by $54.4 \pm 4\%$ compared to control ($P < 0.01$, $n = 3$), an effect negated by the P2X7 antagonist A438079 (50mM; $113.6 \pm 9.7\%$ of control). The agonist (100mM) weakened intercellular ligation forces (F_{max}) by 26.8% ($P < 0.001$; $n = 3$) and affected the formation of ligation clusters by reducing tether rupture events ($TREs$) by 22.3% ($P < 0.001$; $n = 3$), effects largely reversed by A438079 (50mM). Total energy consumed during the pulling process prior to complete cell separation, was calculated by the integration of the retraction *Force-Displacement* curve and is referred as the work of adhesion W_d . Our results showed that control cells exhibited a W_d of $21.85fJ \pm 1.1$, whilst the total energy consumed in separating ATP γ S (50mM) treated cells was reduced to $13.32fJ \pm 1.13$, indicating a reduction of 39% ($P < 0.001$; $n = 3$). Co-incubation with A438079 (50mM) increased W_d back to control ($23.7fJ \pm 1.22$, $n = 3$) and negated the response to ATP.

Conclusion: Determining strength of adhesion in disease is challenging due to the underlying molecular assembly that regulates formation of the adherens junction. Force-displacement measurements during cell-cell separation can provide valuable information about the response to ligands and their antagonists. Nanoscale force-displacement measurements capture the molecular activity underlying ATP γ S evoked changes in cell adhesion, loss of which appears to be mediated by

downstream P2X7 receptor activation and supports a role for P2X7 as a potential therapeutic target in managing progression of diabetic nephropathy.

Hills, C. E. and Price, G. W. and Wall, M. J. and Kauffman, T. J. and Tang, S. C. and Yiu, W. H. and Squires, P. E. (2018) Transforming Growth Factor Beta 1 drives a switch in connexin mediated cell-to-cell communication in tubular cells of the diabetic kidney. *Cell Physiology and Biochemistry*, 45 (6). pp. 2369-2388. ISSN 1015-8987

This work is supported by Diabetes UK (12/0004546 and 16/0005427), and the European Foundation for the Study of Diabetes (EFSO) and Boehringer Ingelheim/European Research Programme in Microvascular Complications of Diabetes

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C079

Altered inner-mitochondrial membrane dynamics disrupt skeletal muscle core-clock gene expression in type 2 diabetes

B. Gabriel¹, J.A. Smith¹, L. Sardon-Puig¹, A. Altintas², R. Tjörnhjörðsson¹, A. Zorzano³, R. Barres², N. Pillon¹, A. Krook¹ and J. Zierath¹

¹Karolinska Institutet, Stockholm, Sweden, ²NNF Center for Basic Metabolic Research, Copenhagen, Denmark and ³University of Barcelona, Barcelona, Spain

Type 2 Diabetes Mellitus (T2D) is a growing global problem. Mutations in core-clock genes increase the risk of developing T2D¹, and a disrupted core-clock leads to obesity and insulin resistance in rodent models. Defective mitochondrial metabolism within skeletal muscle appears to play a major role in insulin resistance², and mitochondrial membrane dynamics may be an important dysregulated pathway in T2D^{3,4}. We sought to test the hypothesis that there are intrinsic rhythmic differences in skeletal muscle from people with T2D, and that this is linked to dysfunctional mitochondrial metabolism.

Synchronised human skeletal muscle cells were harvested every 6 hours over 42 hours and underwent RNA-seq. The core-clock genes *CLOCK*, *CRY1*, *NPAS2*, *NR1D1* and *PER3* displayed different cycling patterns in myotubes from normal glucose tolerant controls (NGT) compared to T2D ($p < 0.05$, determined via 'DODR' analysis). Myotubes from NGT had circadian rhythm in oxygen consumption rate oscillation ($p < 0.05$, RAIN), whereas T2D did not (both $n = 5$). Myotubes from NGT's displayed more cycling genes in the mitochondrial inner membrane than T2D (Gene ratio T2D: > 0.11 ; NGT: > 0.26 , adjusted p -value for compartment enrichment T2D: < 0.01 ; NGT: < 0.05). *Vastus lateralis* muscle from NGT ($n = 25$) and T2D ($n = 25$) volunteers was obtained using a needle biopsy technique. Microarrays were performed on all samples. These subjects also underwent a euglycemic-hyperinsulinemic clamp to determine an M-value for insulin sensitivity. Cycling genes specific to NGT from the inner-mitochondrial membrane (analysed as a pathway) positively correlated with M-value (correlation: $q < 0.05$), including inner-mitochondrial

membrane regulator OPA1. Skeletal muscle specific OPA1^{-/-} mice displayed altered gene expression of core-clock genes: *Clock* (p<0.001:t-test, OPA1^{-/-} 0.81±0.05:SEM vs Wild-type 1.21±0.11:SEM), *Bmal1* (p<0.05:t-test, OPA1^{-/-} 0.68±0.28:SEM vs Wild-type 1.37±0.11:SEM) and *Nr1d1* (p<0.001:t-test, OPA1^{-/-} 0.63±0.07:SEM vs Wild-type 1.41±0.09:SEM). siRNA targeting *OPA1* in synchronised primary human skeletal myotubes altered expression of *NPAS2* (a paralogue of *Clock*) and *PER2* (both genes: 2-way ANOVA: p<0.05 time-interaction; siOPA1 vs scramble). OPA1 depletion also resulted in an altered rhythmic oxygen consumption rate (12hrs siOPA1 vs 4hrs Scramble: p<0.01).

These are the first data to demonstrate intrinsically dysregulated core-clock rhythmicity in primary cells from patients with T2D. Mitochondrial dynamics have previously been implicated in circadian regulation of the core-clock, in this study we also show that OPA1 expression in skeletal muscle is positively correlated with insulin sensitivity. OPA1 expression oscillates in a circadian manner in primate skeletal muscle⁵, and these data demonstrate disruption of OPA1 in skeletal muscle *in vivo*. and *in vitro* alters retrograde signalling from the inner-mitochondrial membrane that affects the core-clock in skeletal muscle.

Corella, D.*et al.* in *Cardiovasc Diabetol* Vol. 15 (2016).

Phielix, E. & Mensink, M. Type 2 diabetes mellitus and skeletal muscle metabolic function. *Physiol Behav* 94, 252-258, doi:10.1016/j.physbeh.2008.01.020 (2008).

Yoon, Y., Galloway, C. A., Jhun, B. S. & Yu, T. Mitochondrial dynamics in diabetes. *Antioxid Redox Signal* 14, 439-457, doi:10.1089/ars.2010.3286 (2011).

Wang, Y.*et al.* Translocase of inner mitochondrial membrane 44 alters the mitochondrial fusion and fission dynamics and protects from type 2 diabetes. *Metabolism* 64, 677-688, doi:10.1016/j.metabol.2015.02.004 (2015).

Mure, L. S.*et al.* Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. *Science* 359, doi:10.1126/science.aao0318 (2018).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C080

Effects of two-weeks of time restricted feeding on basal and postprandial metabolism in healthy men

R. Jones, P. Pabla, J. Mallinson, T. Taylor and K. Tsintzas

MRC/ARUK Centre for Musculoskeletal Ageing, School of Life Sciences, University of Nottingham, Nottingham, UK

INTRODUCTION

Nutritional health research has traditionally focused on nutrient type and quantity. More recently, studies have investigated the concept of chrononutrition, the influence of nutrient timing. Mice fed in a daily time-restricted feeding (TRF) window gain less weight and display improved glucose tolerance (1). Results in humans are

mixed, with daily TRF improving indices of whole-body insulin sensitivity of overweight men with pre-diabetes (2) and reducing fasting concentrations of glucose, insulin and triglycerides in healthy men (3).

AIMS

Primary: Assess whether TRF improves whole-body and skeletal muscle insulin sensitivity in healthy young men.

Secondary: Assess changes in body composition and energy balance in response to TRF.

METHODS

The energy intake (EI) and expenditure (EE) of eight healthy males [Age 22 (2) years, BMI 24.1 (2.0) kg.m⁻²; mean (SD)] was assessed for one week of habitual diet (baseline) and two weeks of TRF. During TRF, participants fasted daily between 4pm-8am and subcutaneous glucose monitoring was performed throughout. Participants consumed a standardised evening meal ~12h before visiting the laboratory prior to and immediately after TRF, to assess their metabolic response to a liquid test meal (1g/kg/BW dextrose and 0.4g/kg/BW protein) using the arterio-venous forearm balance model. DEXA scans, indirect calorimetry measurements and fasted and postprandial vastus lateralis biopsies were also obtained.

RESULTS

Daily feeding periods decreased by an average of 5.5h during TRF and EI was reduced by ~400 kCal (t-test; $p<0.01$). Total EE did not change, resulting in a mean body mass loss of 1kg (t-test; $p<0.01$). TRF had no effect on fasting blood glucose concentrations but 24h continuous glucose monitoring revealed consistently lower blood glucose levels during the fasting phase of TRF (throughout the night) compared with the baseline period. There was an increase (t-test; $p<0.05$) in the Matsuda index (a composite of hepatic and peripheral insulin sensitivity), primarily driven by a reduction in postprandial insulin responses (ANOVA; $p<0.05$), indicating an improvement in whole-body insulin sensitivity post-TRF. Forearm (muscle) glucose uptake was higher (ANOVA; $p<0.05$) in the first 90 min after ingestion of the test meal, indicating an improvement in local muscle insulin sensitivity. Forearm branched chain amino acid (BCAA) uptake was also higher post-TRF (ANOVA; $p<0.01$) but no changes in lean body mass (LBM) were found.

CONCLUSION

TRF leads to favourable metabolic adaptations in healthy individuals including enhanced whole-body and skeletal muscle insulin sensitivity. Persistent elevations in BCAA uptake over longer periods of TRF may lead to better maintenance of LBM under conditions of significant weight loss.

Hatori et al., 2012, Cell Metabolism, 15, 848.

Sutton et al., 2018, Cell Metabolism, 27,1212.

Moro et al., 2016, Journal of Translational Medicine, 14, 290.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Can acute exercise alleviate the impairment in glucose regulation after sleep restriction in healthy humans? A randomised crossover trial.

E. Sweeney¹, D. Peart¹, J. Ellis², I. Kyza¹, T. Harkes¹ and I. Walshe¹

¹*Department of Sport, Exercise and Rehabilitation, Northumbria University, Newcastle Upon Tyne, UK and* ²*Northumbria Sleep Research Laboratory, Northumbria University, Newcastle Upon Tyne, UK*

Sleep restriction leads to impairments in glucose regulation in healthy individuals. Exercise can improve glucose regulation and hence may be beneficial for attenuating the impairment in glucose regulation after sleep restriction. This study aimed to investigate whether an acute bout of exercise performed the morning after a single night of sleep restriction would improve glucose regulation compared to no exercise.

Twelve healthy males were recruited for this randomised crossover study which consisted of four conditions – control (CON), sleep restriction (SR), control plus exercise (CON+Ex), and sleep restriction plus exercise (SR+Ex). Time in bed was 8 hr in the control conditions (approximately 23:00 - 07:00) and 4 hr in the sleep restriction conditions (approximately 03:00 - 07:00). Participants underwent a 1 week entraining period prior to each condition, during which they kept a consistent bed and wake time. Compliance was assessed by wrist actigraphy throughout the study. After consuming a 24 hr controlled diet participants arrived at the laboratory at 08:00 in a fasted state and conducted either a high intensity exercise bout or rested for the equivalent duration. The exercise protocol consisted of 4 all-out 30-s sprints against 7.5% of bodyweight, separated by 4.5 min of active recovery. Following a 30 min rest period an oral glucose tolerance test was conducted. Seven blood samples were obtained over a 2 hour period. Serum glucose and insulin were analysed from the blood samples. Total (0-120 min), early phase (0-60 min) and late phase (60-120 min) area under the curve (AUC) for glucose and insulin were calculated using the trapezoidal rule. Matsuda index and HOMA-IR were used to estimate whole-body insulin sensitivity. Repeated measures ANOVAs were used to determine differences between conditions.

Total AUC was not significantly different between conditions for glucose ($P=0.261$) or insulin ($P=0.678$). Similarly, early and late phase glucose AUC and early phase insulin AUC did not differ between conditions ($P>0.05$ for all). However, late phase insulin AUC showed a significant main effect ($P=0.010$). Post-hoc analysis revealed a significantly lower late phase AUC in SR+Ex than SR ($P=0.025$). Matsuda index and HOMA-IR showed a main effect of condition ($P=0.029$ and $P=0.009$, respectively), with a trend towards increased HOMA-IR in SR compared to CON ($P=0.053$).

These findings suggest that exercise improves the late post-prandial phase following a single night of sleep restriction. Whilst no effect of exercise on the Matsuda index or HOMA were found, the lower late-phase insulin AUC following exercise suggests that there may be some benefit of performing a single bout of

high intensity exercise following a night of sleep restriction. This may have implications for individuals who face unplanned sleep curtailment.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C082

HES1 expression is positively regulated by glycaemic challenges and exercise, and is perturbed in the skeletal muscle of type 2 diabetic individuals

J.A. Smith¹, T. de Castro Barbosa², P.S. Alm¹, S. Platt³, A. Krook¹, J. Zierath^{1,4,5} and B. Gabriel¹

¹Department of Physiology and Pharmacology (FyFa), Karolinska Institutet, Stockholm, Sweden, ²Department of Medicine (H7), Karolinska Institutet, Stockholm, Sweden, ³School of Life Sciences, University of Nottingham, Nottingham, UK, ⁴Department of Molecular Medicine and Surgery (MMK), Karolinska Institutet, Stockholm, Sweden and ⁵The Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

Transcriptional repressor hairy and enhancer of split 1 (HES1) is a basic helix-loop-helix DNA binding protein, and a downstream effector of Notch signalling. Hes1 has been metabolically implicated in adipose tissue, whereby overexpression inhibits the transcription of adipocyte-browning genes, *Ppargc1a* and *Prdm16*, through direct binding of their promoters (1). In skeletal muscle, Hes1 negatively regulates myogenic differentiation by suppressing *MyoD* (2). Intriguingly, HES1 has been identified as an insulin-responsive gene in human *vastus lateralis* (3), and is less abundant in the proteome of obese, type 2 diabetic individuals (4). We aimed to elucidate the metabolic role of HES1 in human skeletal muscle.

High-throughput analysis of the skeletal muscle transcriptome identified differential expression of *HES1* between men with normal glucose tolerance (NGT; n=25, age=58±9 years) and men with type 2 diabetes (T2D; n=25, age=62±6 years): after a 2-hour oral glucose challenge, *HES1* increased in males with NGT and T2D (P<0.0001, Wilcoxon sign test), however the extent of this increase was attenuated in those with T2D (3-fold, 95%CI [2.4, 3.8] vs 1.7-fold, 95%CI [1.4, 2.1]; P<0.0001, Mann-Whitney U test). Primary human skeletal muscle myotubes (pHskMM) were used to further characterise HES1. Insulin treatment (120 nM; 30 minutes) elevated *HES1* levels in pHskMM (1.6-fold, IQR [1.2, 4.9]; P=0.06, Wilcoxon sign test; n=5). *HES1* siRNA silencing (30%) altered the expression of glucose and fatty acid metabolism-regulating genes: *GLUT1* (0.7±0.2-fold), *HK2* (0.8±0.1-fold) and *CPT1B* (1.3±0.1-fold) (P<0.05, paired samples t-test vs scramble control; n=5). Furthermore, insulin-stimulated (10 and 120 nM) D-[¹⁴C (U)]-glucose incorporation into glycogen was attenuated in *HES1*-silenced myotubes, compared to scramble control (1.5±0.2-fold vs 0.9±0.1-fold and 1.9±0.2-fold vs 1.7±0.2-fold, respectively; P<0.05, RM 2-Way ANOVA with Šidák correction; n=5). Electrical pulse stimulation (3 hours) increased

HES1 abundance in pHskMM (1.8±0.3-fold; P=0.03, paired samples t-test; n=7). Moreover, cycling exercise (30 minutes at 85% of maximum heart rate) increased *HES1* expression in skeletal muscle of men with NGT (n=24, age 61±6 years; VO₂ max 35.5±7.6 mL.Kg.min⁻¹) and T2D (n=26, age 60±5 years; VO₂ max 31.4±7.6 mL.Kg.min⁻¹) (P<0.0001; paired samples t-test).

In summary, *HES1* is blunted in the skeletal muscle of men with T2D after an oral glucose tolerance test. *HES1* silencing modulates the expression of key metabolic genes and recapitulates an insulin-resistant phenotype in skeletal muscle, as indicated by reduced incorporation of glucose into glycogen with insulin stimulation. *HES1* is increased by skeletal muscle contraction and responds similarly in NGT and T2D individuals after exercise.

Our results implicate *HES1* in the development of skeletal muscle insulin resistance and tentatively suggest that physical activity could restore *HES1* levels in T2D.

Bi P, Shan T, Liu W, Yue F, Yang X, Liang XR, *et al.* Notch signalling regulates adipose browning and energy metabolism. *Nat Med.* 2014 Aug; 20(8): 911-18

Kuroda K, Tani S, Tamura K, Minoguchi S, Kurooka H, Honjo T. Delta-induced Notch signaling mediated by RBP-J inhibits MyoD expression and myogenesis. *J Biol Chem.* 1999 Mar; 274(11): 7238-44

Parikh H, Carlsson E, Chutkow WA, Johansson LE, Storgaard H, Poulsen P, *et al.* TXNIP regulates peripheral glucose metabolism in humans. *PLoS Med.* 2007 May; 4(5): e158

Giebelstein J, Poschmann G, Højlund K, Schechinger W, Dietrich JW, Levin K, *et al.* The proteomic signature of insulin-resistant human skeletal muscle reveals increased glycolytic and decreased mitochondrial enzymes. *Diabetologia.* 2012 Apr; 55(4): 1114-27

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C083

Targeted metabolomics in human skeletal muscle and plasma reveals distinct differences in key biomarkers of Type 2 diabetes.

P. Pabla^{1,2}, D. Kim², D. Barrett², F. Stephens³ and K. Tsintzas¹

¹MRC/ARUK Centre for Musculoskeletal Ageing, School of Life Sciences, University of Nottingham, Nottingham, UK, ²Centre for Analytical Bioscience, Advanced Materials and Healthcare Technologies Division, School of Pharmacy, University of Nottingham, Nottingham, UK and ³Department of Sport and Health Sciences, University of Exeter, Exeter, UK

Increased lipid delivery and disturbances in skeletal muscle lipid metabolism play a central role in the development of insulin resistance. In a previous cross-sectional study, we observed a reduced suppression of plasma fatty acids in response to an oral glucose tolerance test and increased dietary fat oxidation in middle-aged, but not older, patients with Type 2 diabetes (T2D). Elevated concentrations of several

circulating lipid and branched chain amino acid (BCAA) derived metabolites, such as acylcarnitines (AC), have been consistently associated with T2D and ageing. However, their role in the development of skeletal muscle insulin resistance and T2D remains unclear.

The aim of this study was to use a novel quantitative methodology to (a) investigate the association between T2D and a range of AC, BCAA and their respective keto acids (KA) in both plasma and human skeletal muscle from the volunteers in our previous study, and (b) examine to what extent ageing alters their profiles in T2D.

Seven middle-aged (MA; 46.0 ± 1.1 years, BMI $29.6.1 \pm 1.1$) and 7 older-aged patients (OA; 63.4 ± 0.6 , BMI 29.6 ± 1.3) with T2D, matched for disease duration, lean mass and metformin use, and 7 middle-aged normoglycemic controls (CON; 46.1 ± 2.9 years, BMI 31.1 ± 1.5) were recruited. Fasting vastus lateralis muscle biopsies and plasma samples were analysed for a range of individual AC, BCAA (leucine, isoleucine and valine) and their KA by liquid chromatography coupled to high resolution tandem mass spectrometry (LC-MS/MS) in a targeted approach. AC were grouped as short (C_3 - C_5), medium (C_6 - C_{10}) and long-chain (C_{12} - C_{20}). The muscle and plasma concentrations were quantified using an isotopically labelled internal standard method. Data are mean $\pm$ SEM and were compared by one-way ANOVA and Fisher's LSD post hoc test where appropriate.

Plasma short-chain AC (45 %, $P < 0.01$) and BCAA (17 %, $P < 0.05$) were higher in MA, but not OA vs. CON. Medium-chain AC were elevated (46 %, $P < 0.05$) in OA only. Inversely, the long-chain AC were lower in both MA (23 %, $P < 0.05$) and OA (22 %, $P < 0.05$) vs. CON. Skeletal muscle AC did not differ between groups. Muscle BCAA were elevated in MA (49%, $P < 0.01$) and OA (23 %, $P < 0.05$) vs. CON, with higher values in MA vs. OA ($P < 0.05$). There was no difference in plasma or muscle KA between groups.

These findings reveal a lack of association between plasma and intramuscular concentrations of AC, suggesting that these species may not accurately reflect skeletal muscle lipid metabolism in both middle- and older-aged T2D patients. However, higher levels of plasma and muscle BCAA in MA than OA may better reflect perturbations in energy metabolism as indicated by higher dietary fat oxidation in the former group.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Endocrine regulation of fetal mitochondrial density in skeletal muscle and liver in fetal sheep

K. Davies¹, R.M. Hess¹, E.J. Camm¹, A.J. Forhead^{1,2}, A.J. Murray¹ and A.L. Fowden¹

¹*Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, UK and* ²*Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK*

Prenatally, skeletal muscle and liver must prepare for new postnatal roles demanding extra energy, like locomotion and gluconeogenesis (1). Mitochondria are the primary source of this energy via oxidative ATP production. Fetal cortisol and tri-iodothyronine (T₃) levels rise naturally towards term and stimulate maturation of tissues essential for neonatal survival (1). Both hormones also regulate adult metabolism (2,3). This study investigated the effects of these hormones on mitochondrial density in fetal ovine liver and skeletal muscle.

All procedures were carried out under the Animals (Scientific Procedures) Act 1986. Under general anaesthesia, 12 single Welsh Mountain fetuses were catheterised at 118 days of gestation (d; term ~145d) and were infused with either cortisol (n=6; 2-3mg/kg/day) or saline (n=6) between 125-130d before euthanasia at 130d (200mg/kg sodium pentobarbitone, ewe and fetus). Another 12 twin-bearing ewes underwent thyroidectomy (TX) of one fetus and sham operation of its control twin under anaesthesia at 102d before euthanasia at 127d (n=6 ewes) or 143d (n=6 ewes). Samples of *biceps femoris* muscle (BF) and liver were collected from all fetuses and snap frozen. Fetal plasma cortisol was measured by ELISA and T₃ by radioimmunoassay. Citrate synthase (CS) activity, a marker of mitochondrial density (4), was measured spectrophotometrically. Gene expression of PGC1 α involved in mitochondrial biogenesis was measured by qRT-PCR. Data were analysed by *t*-test.

Cortisol infusion significantly increased fetal cortisol levels at 130d (8.4 ± 1.7 vs 49.2 ± 2.4 ng/ml, $P < 0.0001$). Liver CS activity was significantly higher in cortisol than saline infused fetuses (Fig A), with a trend for higher PGC1 α gene expression ($P = 0.07$). However, at 130d, CS activity of the BF was unaffected by cortisol-infusion (Fig B). TX significantly reduced T₃ levels at both ages (127d: 0.33 ± 0.01 vs 0.12 ± 0.03 ng/ml; 143d: 0.60 ± 0.14 vs 0.24 ± 0.01 ng/ml, $P < 0.05$). At 127d, CS activity of the BF was significantly lower in TX than control fetuses, whereas TX had no significant effect on liver CS activity (Fig C & D). At 143d, CS activity was significantly lower in TX than control fetuses in both liver ($P < 0.05$) and BF ($P < 0.001$). Liver and skeletal muscle PGC1 α expression was unaffected by TX at both ages. The results show cortisol and thyroid hormones have tissue specific effects on mitochondrial density in fetal ovine tissues at 127-130d, with cortisol more effective in liver than skeletal muscle. The reverse was seen with the thyroid hormones, although, by term, TX affected mitochondrial density in both tissues. Overall, the

study suggests that both hormones are involved in prepartum preparation of mitochondria for the increased neonatal energy demands.

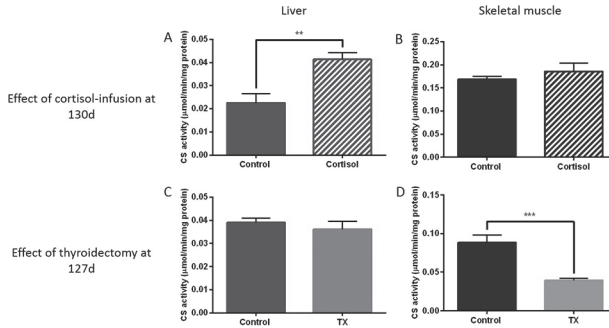


Figure. Mean±SEM citrate synthase (CS) activity in skeletal muscle and liver of A&B) control vs cortisol-infused fetuses at 130 days of gestation (d) and C&D) control vs thyroidectomised (TX) fetuses at 127d. ** P<0.01; *** P<0.001 by t-test.

Fowden et al. (1998) *Proc Nutr Soc* 57:113

Cioffi et al. (2013) *Mol Cell Endocrinol* 379:51

Weber et al. (2002) *Endocrinology* 143:177

Larsen et al. (2012) *J Physiol* 590:3349

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C085

A novel protein regulator of adipogenesis

P.J. Verschoor¹, J. Rochford^{1,2}, M. Delibegovic¹ and G. Nixon¹

¹Aberdeen Cardiovascular & Diabetes Centre, University of Aberdeen, Aberdeen, UK and

²University of Aberdeen, Rowett Institute, Aberdeen, UK

Adipogenesis relies on a tightly controlled cascade in which various factors respond in a sequential manner. It has been hypothesized that adipocyte differentiation is directed by a carefully timed activation of extracellular signal-regulated kinases (ERK1/2). Previous studies have demonstrated that ERK1/2 gene expression is controlled by the cytoplasmic 'anchor' called phosphoprotein enriched in astrocytes (PEA-15), regulating nuclear translocation of ERK1/2¹. Here, we reveal for the first time that PEA-15 regulates adipogenesis. Values are mean±S.E.M. and compared by ANOVA and unpaired t test. PEA-15 knockout (KO) mice and C57BL/6 (WT) mice were fed high fat diet (HFD, 42% kcal from fat) for 8 weeks (ethical approval by Ethics Review Board of University of Aberdeen). There was an increased body weight gain in PEA-15 KO compared to WT mice (PEA-15 KO–13.3±0.8g vs.

WT- 8.0 ± 0.7 g weight gain, $p < 0.001$, $n = 8$) and 50% greater mass of epididymal adipose tissue (eWAT) (PEA-15 KO- 2.7 ± 0.2 g vs. WT- 1.8 ± 0.2 g, $p < 0.05$, $n = 4$). Remarkably, serum triglyceride levels were reduced in PEA-15 KO mice (0.9 ± 0.1 mg/mL vs. 1.6 ± 0.3 mg/mL in WT, $p < 0.05$, $n = 4$). Despite the increased body weight, there was no additional hepatic lipid accumulation (TG assay) nor worsening of glucose intolerance (GTT assay). These changes in lipid handling could be explained by an increased efficiency in lipid storage in PEA-15-deficient adipose tissue, since adipocytes in eWAT sections were larger in PEA-15 KO mice ($5.9 \pm 0.3 \mu\text{m}^2$ vs. WT- $4.4 \pm 0.5 \mu\text{m}^2$, $p < 0.05$, $n = 6$). Interestingly, PEA-15 protein levels were increased in the adipose tissue of WT mice after HFD compared to WT mice on chow. Isolated pre-adipocytes from the eWAT of PEA-15 KO mice showed an increased initiation of adipogenesis followed by impaired maturation, as measured by protein levels of markers of adipocyte development. This suggests a previously uncharacterized and significant role for PEA-15 during adipocyte development. Confocal images of ERK1/2 immunostaining of adipocytes in PEA-15 KO eWAT showed that ERK1/2 is predominantly located in the nuclei compared to the cytoplasmic localization in WT eWAT. The concept of ERK1/2 translocation was confirmed in 3T3L1, in which differentiation led to ERK1/2 translocation. To unravel the regulatory mechanism of PEA-15, 3T3L1 adipocytes were transfected to knockdown (KD) PEA-15 gene expression. To determine whether PEA-15 KD led to increased downstream components of the ERK1/2 cascade, the activation of Elk1 was measured, being the transcription factor directly activated by ERK1/2. Indeed, Elk1 was constantly active in PEA-15 KD cells, as detected by EMSA (3.6 ± 0.9 vs. control cells- 0.4 ± 0.2 , $p < 0.05$, $n = 3$). This study demonstrates that PEA-15 protein deletion attenuates the HFD-associated lipid burden by increasing lipid storage capacity in adipocytes and preventing lipid overflow. Therefore, decreasing PEA-15 levels may initiate such favourable adiposity.

Greig et al. (2017), J Am Heart Assoc 6 (9)

Research funded by the British Heart Foundation and University of Aberdeen

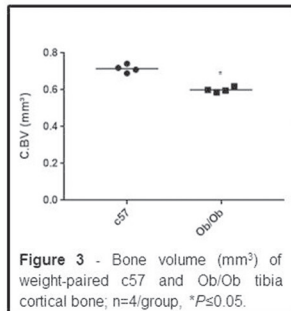
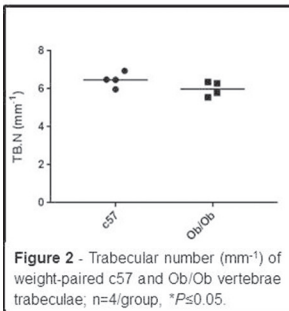
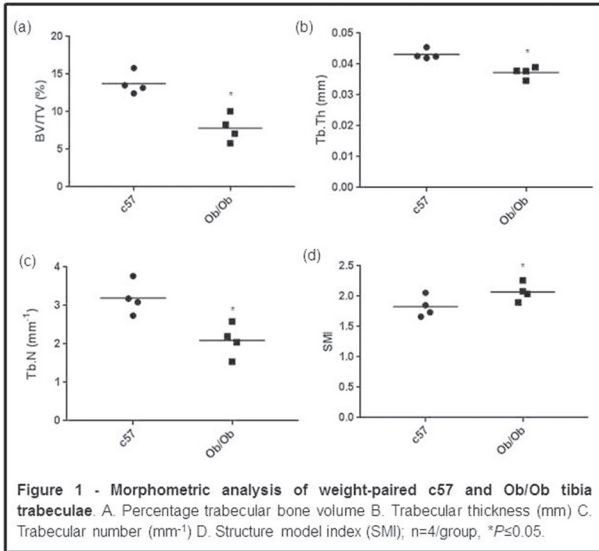
Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Leptin differentially remodels vertebrae and tibia trabecular bone independent of load *in vivo*

S. Shafiq¹, H. Evans², C. Le Maitre¹, J. E Hall³ and N. Aberdein¹

¹Biomolecular Science Research Centre, Department of Bioscience and Chemistry, Sheffield Hallam University, Sheffield, UK, ²Sheffield Myeloma Research Team, Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK and ³Mississippi Centre for Obesity Research, Department of Physiology and Biophysics, University of Mississippi Medical Centre, Jackson, MS, USA

Leptin is a 166AA adipokine predominantly secreted by adipocytes. Circulating leptin concentrations are determined chronically by the volume of adipose tissue and acutely by fluctuations in food intake. Leptin contributes to energy metabolism, acting to reduce appetite and body weight as well as increased heart rate and blood pressure, however the effects of leptin on bone metabolism remain contradictory. In this study high resolution μ CT (SKYSCAN 1272) was used to scan and analyse vertebrae and tibia samples collected from leptin deficient Ob/Ob mice (n=4), which were weight matched with c57 controls (n=4), from 4-22 weeks of age. Bone samples were scanned at a resolution of 4.3 μ m, 0.7° rotation step and reconstructed using NRecon. Post-reconstruction, CTAn was used to calculate percentage trabecular bone volume (BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.N) structural model index (SMI), bone mineral density (BMD) and cortical bone volume (C.BV). BV/TV of the Ob/Ob tibias was significantly decreased compared to c57 controls (12.73 ± 0.73 vs 7.81 ± 0.91 , respectively) (Figure 1a). Compared to c57, Ob/Ob mice tibia had a significant reduction in trabecular thickness: 0.043 ± 0.001 mm vs. 0.037 ± 0.001 mm (Figure 1c), and number: 3.19 ± 0.21 mm⁻¹ vs. 2.09 ± 0.22 mm⁻¹ (Figure 1d), respectively. Furthermore, Ob/Ob mice tibia trabeculae showed a significant increase in SMI (2.07 ± 0.07) compared to c57 (1.83 ± 0.09), demonstrating tibia trabeculae were becoming more rod like (Figure 1b), indicative of increased osteoclast activity. Similar trends were observed in the trabeculae of c57 and Ob/Ob vertebrae samples for BV/TV, T.Th., however vertebrae T.N. was not significantly reduced in Ob/Ob mice compared to c57 controls (6.47 ± 0.20 mm⁻¹ to 5.99 ± 0.20 mm⁻¹, respectively) (Figure 2). Cortical BV of the tibia was reduced in Ob/Ob compared to c57 controls (0.72 ± 0.01 mm³ to 0.60 ± 0.02 mm³, respectively) (Figure 3). In all samples analysed, BMD did not significantly differ between weight-paired Ob/Ob and c57 controls, demonstrating leptin deficiency, independent of load, did not affect density of trabecular or cortical bone. However, findings on thickness, percentage bone volume and SMI suggest leptin plays a significant role in promoting bone resorption and/or inhibiting bone formation in both trabecular and cortical areas, and that this occurs differentially between the spine and long bones independent of weight.



Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Effect of maternal resveratrol administration on offspring sugar preference and glucose tolerance in a rat model of obese pregnancy

N. Itani¹, P. Sethupathi¹, T. Perez¹, P.W. Nathanielsz², L.G. Gonzalez-Rodriguez³, E. Zambrano³ and P.D. Taylor¹

¹Women and Children's Health, King's College London, London, UK, ²Wyoming Center for Pregnancy and Life Course Health Research, University of Wyoming, Wyoming, WY, USA and ³Reproductive Biology Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico

Background/Aims: Increasing evidence from epidemiological and animal studies supports maternal obesity during pregnancy as a major risk factor for childhood obesity and cardio-metabolic disease in adulthood. Altered neuroendocrine response to rewarding stimuli may contribute to increased energy intake, culminating to impaired glucose tolerance and/or obesity in later life. Employing an established rodent model of maternal obesity, we hypothesised that resveratrol, a polyphenol with antioxidant properties, would improve offspring metabolic phenotype. The aim of the current study was to determine the effects of resveratrol supplementation pre-pregnancy through to lactation on offspring sucrose preference and glucose tolerance.

Method: Female rats (n=10/group) were fed control (C) or obesogenic (O) diet *ad libitum* from weaning and then supplemented with resveratrol (R, 20mg/kg/d) or vehicle from 90d of age, through mating at 120d, to the end of lactation. Offspring were weaned on to normal chow. One male and one female from each litter (four groups; maternal (M)C, MO, MCR and MOR) was subjected to a sucrose preference test at 40d of age: singly housed rats received two identical bottles each filled with water or sucrose solution (4%) with normal chow *ad libitum*. The position of the bottles was switched after 24h. Change in body weight and food, water and sucrose consumption were measured after 48h. At 8 months, offspring were subjected to glucose tolerance test (GTT): rats were given a glucose load (2g/kg intraperitoneal) and their blood glucose level was measured at -10, 10, 20, 30, 45, 60, 90 and 120min. Data were calculated as mean±SEM and analysed by Two-way ANOVA with Tukey's posthoc test. **Results:** Maternal obesity significantly enhanced offspring intake of sucrose solution (4%) in males and females at 40 days, which were prevented by maternal R (Figure1A and B). No difference was found in water intake. At 8 months, both male (MC:667.3±13; MO:768.2±33* g) and female (MC:341.3±12; MO:397.6±16* g) MO offspring had significantly higher body weight, and MO females displayed a significantly impaired glucose tolerance which was prevented by maternal R (Figure1C). Similar effects were not observed in male offspring.

Conclusions: Maternal obesity in pregnancy programmes appetitive behaviour in offspring which promotes the development of obesity in later life. Maternal supplementation with resveratrol throughout pregnancy and lactation prevented the programming of an enhanced sucrose preference in the offspring of obese rat dams and impaired glucose tolerance in the female but not male offspring. The extent to which maternal obesity and resveratrol alters neurodevelopment of

the reward pathways of the nucleus accumbens and mechanisms underlying the sexually divergent effect of maternal obesity is the focus of future studies.

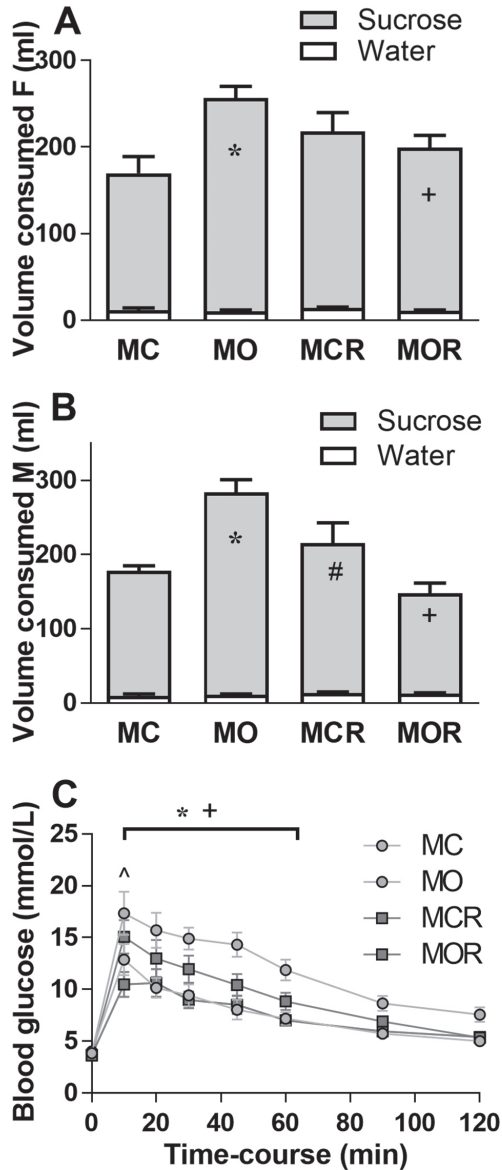


Figure 1. Volume of sucrose solution (4%) and water during a 48h-challenge in (A) males and (B) females at 40 days of age. Blood glucose level following a single dose (2g/kg) of glucose in females at 8 months of age (C). Significant difference * MC vs. MO; + MO vs. MOR; ^ MC vs. MCR; and # MCR vs. MOR ($P < 0.05$, Two-way ANOVA with Tukey post hoc test).

Supported by the Newton Fund.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C088

5 β -reductase (AKR1D1) deletion drives hepatic inflammation, fibrosis and tumour development *in vitro* and *in vivo*.

S. Harris¹, N. Nikolaou¹, A. Arvaniti^{1,2}, L. Gathercole² and J. Tomlinson¹

¹Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK and ²Biological and Medical Sciences, Oxford Brookes University, Oxford, UK

The enzyme 5 β -reductase (AKR1D1) catalyses an essential step in bile acid synthesis, but in addition, controls intra-cellular steroid hormone availability by generating inactive 5 β -reduced dihydrosteroid metabolites. As disturbances in steroid hormone and bile acid metabolism have potent effects on metabolic health and lipid homeostasis, we hypothesize that AKR1D1 may play a role hepatic lipid accumulation and contribute to the development of metabolic disease.

We generated global AKR1D1 knockout mice (KO, n=18) alongside littermate wildtype controls (WT, n=18). Mice were fed either normal chow (NC, n=9) or the American lifestyle induced obesity syndrome diet (ALIOS; 45% fat, 55% fructose:45% glucose in H₂O, n=9), which replicates the clinical features of non-alcoholic fatty liver disease (NAFLD), for 52 weeks. Data (mean $\pm$ SEM) were assessed using Students t-test.

Whilst weight gain was similar in WT normal chow and ALIOS treated animals, in WT mice, the ALIOS diet induced liver inflammation and fibrosis in comparison with NC. AKR1D1 KO mice fed the ALIOS diet had increased hepatic steatosis in comparison with WT mice (WT: 16.7 $\pm$ 3.3, KO: 21.7 $\pm$ 3.6mg/g, P<0.005). In addition, there was evidence of increased hepatic inflammation on H&E stained sections in male AKR1D1 KO mice on a NC, but not ALIOS diet (1.6 vs. 1.1, P<0.01). However, at 37 weeks of age, liver biochemistry was significantly elevated in AKR1D1 KO mice fed the ALIOS diet in comparison with WT mice (ALT; WT: 140.7 $\pm$ 51.9, KO: 404.7 $\pm$ 171.4U/l, P<0.05. AST; WT: 136.7 $\pm$ 39.0, KO: 360.7 $\pm$ 121.7U/l, P<0.05). Endorsing observations in our rodent model, AKR1D1 knockdown experiments in human hepatoma cells (Huh7 and HepG2) increased mRNA expression and secretion of the pro-inflammatory cytokines IL1 β , IL-6 and IL-8 as well as inducible nitric oxide synthase (iNOS).

Hepatic inflammation is a key driver of fibrosis. AKR1D1 KO mice fed the ALIOS diet for 52 weeks had increased hepatic fibrosis as quantified by sirius red staining (WT: 5.4 $\pm$ 2.6%, KO: 10.0 $\pm$ 4.9%, P<0.01). Furthermore, it is well-established that advanced fibrotic metabolic liver disease increases the risk for the development of hepatocellular carcinoma (HCC) and AKR1D1 mice were more prone to tumour development in comparison with WT mice (WT: 11.5%, KO: 42.1%, P<0.05).

Deletion of AKR1D1 in combination with a dietary stress evokes increased hepatic triacylglycerol content and fibrosis, which could exacerbate the progression of NAFLD to NASH and potentially fuel the development HCC. These data suggest that AKR1D1 may have a pivotal role in the regulation of the NAFLD progression.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C089

Carotid sinus nerve denervation improves hepatic function in young and old animals with metabolic dysfunctions exacerbated by long-term hypercaloric diet consumption

C.C. Batista, J.F. Sacramento, B.F. Melo and S. Conde

CEDOC, NOVA Medical School | Faculdade Ciências Médicas, Universidade Nova de Lisboa, Lisbon, Portugal

The carotid body (CB) has been described as a metabolic sensor implicated in the genesis of insulin resistance and glucose intolerance (1-2). Hence, modulation of the activity of its sensitive nerve, the carotid sinus nerve (CSN) could be a therapeutic strategy for metabolic diseases (MD) (3). Knowing that non-alcoholic fatty liver disease is regarded as the hepatic manifestation of MD and since the liver has a fundamental role in dysmetabolism (4), herein we investigated the effect of CSN resection on liver function in young and aged animals with metabolic dysfunction exacerbated by long-term hypercaloric diet consumption.

Male Wistar rats (8-10 weeks) were used: a control (CTL) group fed a standard diet and a high-fat high-sucrose (HFHSu) group fed with a 60% lipid-rich diet-35% sucrose, during 14 and 44 weeks. After this period, animals were anesthetized (medetomidine 0.5 mg/kg; ketamine 75 mg/kg) and submitted to CSN resection or to a sham surgery, with 9 weeks follow-up. At a terminal experiment, the liver was collected for hematoxylin/eosin (H&E) and Massons trichrome staining. Liver triglycerides (TGL) and lipid content were measured, as western blots of key proteins involved in tissue inflammation and fibrosis. Serum total cholesterol and TGL were evaluated. One- and two-way Anova tests were used. Laboratory care was in accordance with the European Union Directive for Protection of Vertebrates Used for experimental and Other Scientific Ends (2010/63/EU).

Ageing and long-term HFHSu diet consumption increased total cholesterol levels (CTL_{Young} = 82.22±4.25mg/dl; CTL_{Old} = 97.74±25.77mg/dl; HFHSu_{Old} = 111.30±13.12mg/dl, *p*<0.01) as well as serum TGL (CTL_{Young} = 83.18±13.72mg/dl; CTL_{Old} = 142.65±32.35mg/dl; HFHSu_{Young} = 134.40 ±32.35mg/dl, *p*<0.05; HFHSu_{Old} = 118.87±38.66mg/dl), an effect that was restored with the CSN resection in HFHSu animals. Liver TGL levels increased with age and diet (CTL_{Young} = 8.15±0.83µmoles/mg liver; CTL_{Old} = 13.48±2.45µmoles/mg liver; HFHSu_{Young} = 10.58±1.73µmoles/mg liver; HFHSu_{Old} = 42.34±3.01µmoles/mg liver,

$p < 0.0001$), an effect that decreased with CSN resection (HFHSu_{Old with CSN resection} = 17.55 ± 1.09 $\mu\text{moles/mg liver}$, $p < 0.001$). Ageing increased liver lipid deposition (CTL_{Young} = $1.48 \pm 0.10 \text{gr}^{-1}$ liver; CTL_{Old} = $2.98 \pm 0.25 \text{gr}^{-1}$ liver) an effect that was exacerbated by HFHSu diet (HFHSu_{Young} = $2.19 \pm 0.30 \text{gr}^{-1}$ liver, $p < 0.01$; HFHSu_{Old} = $5.47 \pm 0.56 \text{gr}^{-1}$ liver, $p < 0.0001$). CSN resection decreased liver lipid deposition by 21.46 ($p < 0.01$) and 35.28% ($p < 0.001$) in young and old HFHSu animals, respectively, results that were supported by H&E staining.

In conclusion, ageing induced an increase in the levels of total cholesterol, TGL and lipid deposition in the liver, an effect that was exacerbated by long-term hypercaloric diet consumption. Moreover, CSN resection ameliorated these effects. Our results suggest that modulation of CB activity might be important in liver-associated metabolic dysfunctions in young and old animals.

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

Sacramento JF et al. Diabetologia. 2017; 60(1):158-68.

Sacramento JF et al. Diabetologia. 2018; 61(Suppl).

Takahashi Y et al. World Journal of Gastroenterology. 2012; 18(19): 2300-2308.

J.F. Sacramento and B.F.Melo were supported by the Portuguese Foundation for Science and Technology grants PD/BD/105890/2014 and PD/BD/128336/2017 respectively.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C090

Grazing induces phasic preference for high-fat diet in male rats

J.L. Dovey and T. Wells

School of Biosciences, Cardiff University, Cardiff, UK

Despite epidemiological evidence^{1,2}, the physiological impact of temporal feeding patterns remains a major unanswered question^{3,4}. To address this, we have used a CLAMS-based feeding station to demonstrate that the alignment of acute food preference with base diet in meal-fed rats is abolished in grazing animals⁵. We have now modified our feeding station to test the hypothesis that grazing influences long-term food choice.

Bespoke food hoppers were used to supply low- (LFD; Special Diet Services (SDS) 10% AFE fat) and high-fat diet (HFD; SDS 45% AFE fat) to male Sprague-Dawley rats (4-weeks old; Charles River, UK) in either *ad libitum* (AL; $n=9$), grazing (GR; provided 1/24th of the total daily intake of AL-fed rats every 30 mins during the dark phase; $n=5$) or meal-fed (MF; three 1h periods of AL feeding at 18:00h, 23:30h and 05:00h; $n=6$) patterns for 6 weeks, with water available AL in a 12h light:12h dark cycle (lights on 06:00h). Body weight and food intake was monitored daily (at 09:00h) and a range of metabolically active tissues dissected and weighed *post-mortem*. Data presented are

mean $\pm$ SEM with statistical comparisons performed by 1-way ANOVA and Bonferroni post hoc test (between groups) and paired Student's t-test (within groups).

As shown with single diets, total cumulative food intake in GR and MF rats was 23% lower than in AL-fed rats ($P < 0.0001$). While mean cumulative LFD intake (cLFDi) in GR and MF rats was 67% and 70% of that in AL rats, neither cLFDi nor cumulative HFD consumption were significantly altered ($P > 0.1$). Although neither GR nor MF affected cumulative LFD/HFD preference, GR rats favoured HFD ($P < 0.05$) on 15 out of 42 days in a clear phasic pattern. In contrast, MF rats showed significant preference for LFD for either HFD or LFD for one day, while AL rats showed a higher preference for LFD for the first 2 days. Despite GR and MF rats showing a 22% reduction in total cumulative caloric intake ($P < 0.001$), there were no concomitant reductions in body weight gain or any of the measures of skeletal growth (nose-anus length, tibial and femoral lengths) in GR and MF rats. Although epididymal white adipose tissue (WAT) weight was not significantly increased, fat storage efficiency in GR and MF rats was elevated by 47% and 62% respectively ($P < 0.01$). Broadly similar results were obtained for inguinal and retroperitoneal WAT and interscapular brown AT. Thus, grazing not only abolishes the alignment between base diet and acute food preference seen in MF rats⁵, but induces periods of phasic preference for HFD when continuously exposed to a choice between LFD and HFD. The mechanisms linking these feeding patterns with food preference and growth outcomes remain to be determined. Our study suggests that when combined with dietary choice, contemporary changes in feeding pattern² may contribute to consumption of energy-dense diets.

Laska MN et al. (2014). *Public Health Nutr* 18, 2135-2145.

Duffey KJ & Popkin BM. (2011). *PLoS Med* 8, e1001050.

Abbasi J. (2017). *JAMA* 317: 243-244.

Allison DB, et al. (2015). *Front Nutr* 2, 26.

Dovey J, et al. (2018). *Chrononutrition: from epidemiology to molecular mechanism*, The Physiological Society, Abstract C02.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C091

G α_q -coupled Receptors Primarily Opts Direct G α_q Gating for Inhibiting TRPM8 Ion Channels

X. Zhang

School of Life & Health Sciences, Aston University, Birmingham, UK

Cold inhibits pain through activating the cold sensing TRPM8 ion channel, and has been widely used as an approach to pain relief. However, TRPM8 is inhibited during inflammation leading to disinhibition of the analgesic effect of cold, contributing to aggravated pain. Inflammatory mediators such as bradykinin and histamine inhibit

TRPM8 through acting on $G\alpha_q$ -protein coupled receptors (GPCRs). Both activated $G\alpha_q$ and the downstream hydrolysis of the membrane lipid phosphatidylinositol 4, 5-bisphosphate (PIP_2) inhibit TRPM8. However, how $G\alpha_q$ directly gates TRPM8 and the relative role of $G\alpha_q$ gating and PIP_2 signaling in TRPM8 mediated cold signaling remain elusive. Here, we took advantage of $G\alpha_q$ knockout mice in which phospholipase C β (PLC β)- PIP_2 signaling is intact carried by $G\alpha_{11}$, thus allowing for discrimination between $G\alpha_q$ gating and PIP_2 signaling in TRPM8 modulation. Combined with cell-attached electrophysiological recordings, we show that direct $G\alpha_q$ gating is essential for the basal cold sensitivity of TRPM8, and is also the sole mechanism for TRPM8 inhibition caused by bradykinin in native sensory neurons without the involvement of PLC β - PIP_2 signaling or $G\alpha_{11}$ in this process, because deleting $G\alpha_q$ markedly enhanced TRPM8-mediated firing evoked by cold, and abolished the inhibitory effect of bradykinin on TRPM8-mediated firing responses in sensory neurons, while sparing PLC β - PIP_2 signaling. We then identified three key arginine residues in the N-terminal melastatin homology regions (MHRs) of TRPM8 as $G\alpha_q$ gating sites using protein binding assays and mutagenesis. Neutralization of these positively charged residues dramatically enhanced the sensitivity and voltage-dependent gating of TRPM8, and completely abolished TRPM8 inhibition by both $G\alpha_q$ and bradykinin without affecting the channel sensitivity to PIP_2 . Interestingly, TRPM8 binding to $G\alpha_q$ also reciprocally competes with $G\alpha_q$ binding and coupling to PLC β - PIP_2 , leading to reduced PIP_2 hydrolysis and signaling based on live cell imaging and functional assays. Furthermore, the bradykinin receptor B2R also binds to TRPM8 rendering TRPM8 insensitive to PIP_2 depletion. Thus, TRPM8 and B2R/ $G\alpha_q$ form a bidirectional signaling complex that act together to opt $G\alpha_q$ gating rather than PIP_2 signaling as a primary means for TRPM8 inhibition during inflammation. These findings implicate a critical role for the N-terminal MHRs in TRPM8 gating and cold signaling. The revealed $G\alpha_q$ gating sites represent interesting targets for TRPM8 drug discovery.

Zhang, X., Mak, S., Li, L., Parra, A., Denlinger, B., Belmonte, C. & McNaughton, P.A. Direct inhibition of the cold-activated TRPM8 ion channel by Galpha(q). *Nat. Cell Biol* (2012) 14, 851-858.

Li, L. & Zhang, X. Differential inhibition of the TRPM8 ion channel by Galphaq and Galpha 11. *Channels (Austin.)* (2013) 7, 115-118.

Rohacs, T., Lopes, C.M., Michailidis, I. & Logothetis, D.E. PI(4,5)P₂ regulates the activation and desensitization of TRPM8 channels through the TRP domain. *Nat. Neurosci* (2005) 8, 626-634

Proudfoot, C.J., Garry, E.M., Cottrell, D.F., Rosie, R., Anderson, H., Robertson, D.C., Fleetwood-Walker, S.M. & Mitchell, R. Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. *Curr. Biol* (2006) 16, 1591-1605.

De Caro, C., Russo, R., Avagliano, C., Cristiano, C., Calignano, A., Aramini, A., Bianchini, G., Allegretti, M. & Brandolini, L. Antinociceptive effect of two novel transient receptor potential melastatin 8 antagonists in acute and chronic pain models in rat. *British journal of pharmacology* (2018) 175, 1691-1706.

The research was funded by the MRC and Aston University.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Modified hippocampal long-term potentiation in *mdx* mice can be recapitulated in wild type comparators by acute exposure to the pro-inflammatory cytokine, interleukin-6.

K.A. Stephenson, M.G. Rae and D. O'Malley

Physiology, University College Cork, Cork, Ireland

Patients with Duchenne Muscular Dystrophy (DMD) have an increased incidence of mild to moderate cognitive impairment with deficits in verbal, short-term and working memory (1). In addition to similar cognitive deficits being observed in the dystrophin-deficient *mdx* mouse model of DMD, hippocampal long-term potentiation (LTP), the cellular correlate of memory formation, is also altered in *mdx* mice relative to controls (2). Although typically found at low levels in healthy brains, interleukin (IL)-6 can be elevated in diseased brains where cognitive function is also impaired (3). The aim of the present study was to assess hippocampal LTP in wild type (WT) and *mdx* mice to determine if exposure to IL-6 modifies LTP.

In accordance with the European Directive 2010/63/EU, *mdx* and WT mice (8-12 weeks old) were anaesthetised with isoflurane, decapitated and their whole brains removed. Hemispheres were sectioned into 300µm slices in ice-cold aCSF. Slices were maintained in either aCSF (control) or recombinant IL-6 (1nM) in aCSF for 1 hour before experimentation. LTP was induced in both WT and *mdx* slices by high frequency stimulation of Schaffer collateral fibres innervating neurons of the CA1 hippocampal region. Comparisons were subsequently made between the slope function of field excitatory postsynaptic potentials (fEPSP) in WT versus *mdx*, and untreated versus treated slices. All fEPSP slope speeds (mV/ms) are provided as a normalised percentage of averaged 20 minute fEPSP slope pre-LTP baselines. Statistical analyses were performed using unpaired *t* tests with data expressed as mean normalised post-LTP slope speed $\pm$ standard deviation.

We found that the magnitude of LTP was significantly reduced in *mdx* slices relative to WT ($187.3 \pm 34\%$ vs $209.9 \pm 48\%$, $n=5$, $p=0.005$). Further, chronic exposure to IL-6 (1nM) significantly reduced LTP slope in WT slices relative to untreated controls ($181.2 \pm 18\%$ vs $218.7 \pm 49\%$, $n=4$, $p<0.0001$). Chronic treatment of *mdx* slices with IL-6 (1nM) did not significantly affect the mean post-LTP EPSP slope relative to untreated controls. Interestingly, WT slices treated with recombinant IL-6 mirrored the reduced LTP pattern observed in *mdx* control experiments (WT= $181.2 \pm 18\%$, $n=4$ vs *mdx*= $187.3 \pm 34\%$, $n=5$).

In contrast to previous findings, we have shown that LTP in *mdx* hippocampal slices was decreased relative to WT comparators. This may be a functional manifestation of the memory deficits described in *mdx* mice and DMD patients. Exposure to the pro-inflammatory cytokine IL-6 reduced post-LTP EPSP slope in WT hippocampal neurons to the reduced mean post-LTP EPSP slope observed in dystrophin-deficient *mdx* slices. IL-6 did not impact upon LTP generation in *mdx* mice. Thus, in *mdx* mice, chronic exposure to elevated IL-6 may contribute to impaired memory formation.

1. Snow WM, Anderson JE & Jakobson LS. (2013). Neuropsychological and neurobehavioral functioning in Duchenne muscular dystrophy: a review. *Neuroscience and biobehavioral reviews* 37, 743-752.
2. Vaillend C, Billard JM & Laroche S. (2004). Impaired long-term spatial and recognition memory and enhanced CA1 hippocampal LTP in the dystrophin-deficient Dmd(mdx) mouse. *Neurobiol Dis* 17, 10-20.
3. Gruol DL. (2015). IL-6 regulation of synaptic function in the CNS. *Neuropharmacology* 96, 42-54.

K.A.S is supported by an Investigator Lead Project from the Health Research Board, Ireland

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C093

The parkinsonian neurotoxin MPTP induces cerebrovascular leakage that can be mitigated by photobiomodulation

M. San Miguel, K. Martin and D. Johnstone

University of Sydney, University Of Sydney, NSW, Australia

The neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which crosses the blood-brain barrier (BBB) and destroys dopaminergic neurons, is commonly administered to animals to model Parkinson's disease (PD). While the destructive effects of MPTP on dopaminergic cells are well established, recent evidence suggests that MPTP may also compromise the integrity of the cerebral vasculature, although the timeline of vascular damage remains unclear. Our team has demonstrated that photobiomodulation (PBM) – the irradiation of tissue with low-intensity red to near-infrared light – mitigates MPTP-induced damage to the nigrostriatal pathway, but the effects of PBM on the brain vasculature have not been investigated. In this study, we aimed to delineate the time course of cerebrovascular disruption as a result of MPTP intoxication, and to determine whether PBM could mitigate this disruption.

Male C57BL/6 mice were injected with 80 mg/kg MPTP, in 4 injections over 6 hours (n=20), or a corresponding volume of saline vehicle (n=8). At 1, 2, 3, 7 or 14 days after injection, mice were euthanased and perfused transcardially with FITC-albumin tracer (5 mg/ml). Fluorescence microscopy of coronal sections of the substantia nigra pars compacta (SNc) and caudate-putamen complex (CPu), followed by analysis of the proportion of pixels positive for FITC, revealed maximum vascular leakage at 7 days post-injection for both the SNc (3.2 ± 0.2 vs 15.7 ± 0.4 , $p < 0.0001$) and CPu (2.9 ± 0.2 vs 26.2 ± 1.4 , $p < 0.0001$).

In a separate cohort of mice injected with 80 mg/kg MPTP (n=12) or saline (n=8), a subset of MPTP mice (n=6) received daily transcranial PBM, commencing 24 hours after the final MPTP injection. PBM was directed at the cranium using a

handheld LED panel (WARP10; 670 nm, 50 mW/cm², 180 s daily), and tissue was collected 7 days post-injection. Transcranial PBM significantly mitigated MPTP-induced vascular leakage in both the SNc (19.9±1.7 for MPTP-sham vs 7.9±1.4 for MPTP-PBM, $p<0.0001$) and the CPu (30.2±1.7 vs 13.4±3.9, $p=0.0005$). Vascular leakage in PBM-treated MPTP mice was not significantly greater than saline-injected control mice (SNc: 5.6±0.6, $p=0.38$; CPu: 5.9±0.4, $p=0.07$).

This study has demonstrated that (i) the neurotoxin MPTP, which is used to model PD, induces transient cerebrovascular permeability that peaks at ~7 days post-injection, and (ii) that post-conditioning with daily transcranial PBM can mitigate cerebrovascular damage in this model. The observed disruption of blood-brain barrier integrity following MPTP exposure brings into question the validity of using the MPTP model for pre-clinical trials of pharmacological PD treatments. While further research is required to understand the mechanism of action, the discovery that PBM can mitigate MPTP-induced BBB disruption provides the rationale for more widespread deployment of this neuroprotective intervention for conditions that involve vascular damage, such as stroke and traumatic brain injury.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C094

The effects of acute hypoxia on cognitive and cardiovascular parameters in healthy subjects

M. Subhan, R. Ravi, R. Lang, L. Shute and N. Carter

School of Biomedical Sciences, University of Plymouth, Plymouth, UK

High altitude, lung disease and carbon monoxide poisoning are all known to cause hypoxia. High altitude continues to be a threat to the safety and operations of pilots and mountaineers. Hypoxic exposure can affect various functions in the human body, particularly cognition and the cardiovascular system. However, there is an uncertainty surrounding the effect hypoxia may have on both cognition and the cardiovascular system.

The objectives of the study were to examine cognition using the Wechsler Abbreviated Scale of Intelligence – First Edition (WASI-I), evaluate brain tissue oxygenation levels using near-infrared spectroscopy (NIRS) and investigate cardiovascular parameters using heart rate variability (HRV) under different O₂ concentrations of 12%, 16.5% and 21%.

Seventeen healthy volunteers (nine males and eight females) with a mean ($\pm$ SD) age of 21.11 $\pm$ 1.21 years and BMI of 22.51 $\pm$ 2.58 kg m⁻² participated in two tasks of the WASI-I; block design (BD) and matrix reasoning (MR). BD, MR and HRV were measured during the three different gas interventions; which were randomised. HRV data was also normalised by considering the heart rate. A subset of ten subjects had their cerebral tissue oxygenation levels (TOI) evaluated using NIRS.

Cognitive testing under different O₂ concentrations for BD (p=0.133) and MR (p=0.237) were not significantly different. HRV data showed a decrease in the non-normalised high frequency (HFnu) for MR subset (p=0.001) with decreasing O₂ concentration. Mean heart rate for BD (p=0.016) and MR (p=0.007) also significantly declined with decreasing O₂ concentration. NIRS data showed the mean TOI did not significantly change (p=0.611) during BD, however during MR, TOI (p=0.003) significantly decreased with lowering O₂ concentration.

The cognitive tests did show decreasing trends, albeit non-significant, with increasing severity of hypoxia. However, parasympathetic activity and cerebral tissue oxygenation both fell during MR with increasing hypoxia. HR also decreased during hypoxia for both BD and MR. Data from this study can benefit patients who suffer from hypoxia, athletes, and persons at high altitude or in industry.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C095

Ion channels involved in pain pathways: An automated patch clamp study

A.R. Obergrussberger, N. Becker, M. Rapedius, T.A. Goetze, M.G. Rotordam, N. Brinkwirth, I. Rinke-Weiß, S. Stölzle-Feix, C. Haarmann, M. George, A. Brüggemann and N. Fertig

Nanion Technologies GmbH, Munich, Germany

Chronic and neuropathic pain is a significant problem affecting millions of people worldwide each year, with 1 in 5 individuals experiencing moderate to severe chronic or persistent pain. A number of different ion channels have been identified which play roles in pain transmission including Na_v, TRP, P2X, NMDA and, more recently, HCN1 and HCN2 channels. We have used automated patch clamp instruments recording from 8 or 384 cells simultaneously to record Na_v1.7, Na_v1.8 and Na_v1.9 in CHO cells. Current-voltage plots and pharmacology using protoxin II will be shown, as well as state-dependence of tetracaine.

TRP channels are a family of cation channels with roles in many biological functions including pain perception and thermosensation. A number of selective TRPV1 antagonists were developed for the treatment of pain, however clinical studies discovered that there were undesirable side effects on core body temperature which has led to drug development targeting this ion channel to be largely halted. We recorded TRPV1 and TRPV3 activated by either ligand or heat. In this way, antagonists for ligand application of TRP channels could be identified which do not block the heat activated response.

Ligand-gated ion channels involved in pain pathways include NMDA and P2X receptors. NMDA receptors have been implicated chronic and neuropathic pain. We have recorded NMDA receptor combinations NR1/NR2A and NR1/NR2B to investigate both positive modulation and negative modulation. P2X receptors,

in particular P2X₃ homomers or P2X_{2/3} heteromers, are thought to be involved in pain conditions such as allodynia and hyperalgesia. P2X_{2/3} and P2X₃ receptors expressed in CHO or 1321N1 cells were activated by ATP and αβ-methylene ATP in a concentration-dependent manner and blocked by suramin.

HCN1 and 2 are primarily expressed in dorsal root ganglion neurons. The Na⁺/K⁺ inward current which flows due to activation of HCN channels, I_h, appears to have a role in mediating neuropathic pain. Indeed, in animal models some gain-of-function mutations or over-expression of HCN support this theory. We have measured HCN2 which was blocked by ivabradine and ZD-7288 in a concentration-dependent manner with an IC₅₀ of 5.8 μM (n = 88) and 17.3 μM (n = 93), respectively.

Human induced pluripotent stem cell-derived neurons (hiPSC-neurons) are an excellent model to study neuronal ion channels in a more native environment. Indeed, hiPSC-neurons may also be important models to study neurodegenerative diseases such as motor neuron disease, Alzheimer's and Parkinson's disease. We have used human glutamatergic-enriched cortical neurons derived from hiPSCs and Na_v currents and ligand-gated currents mediated by GABA_A, AMPA and nAChα7 receptors.

Our results demonstrate that pain pathways can be successfully studied on automated patch clamp systems, facilitating the discovery of novel pain therapeutics.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C096

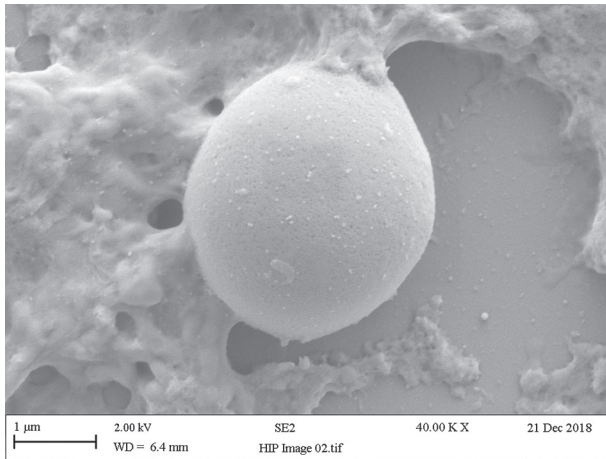
Estimation of synaptosome mitochondrial function in the TgF344-AD rat brain

S.D. Webb and L.D. Orton

School of Healthcare Science, Manchester Metropolitan University, Manchester, UK

Alzheimer's disease (AD) has been associated with changes in metabolic function and synaptic dysfunction. How synaptic metabolism is modified in AD is under-explored. To address this gap, we are investigating synaptic mitochondrial function in the TgF344-AD rat model of AD. This model expresses mutant human amyloid precursor protein and human presenilin 1 with Δexon9 mutation; both driven by a mouse prion promoter. A three factor design was employed, with age (7- or 15-months), genotype (wild-type or mutant) and brain region (frontal cortex, auditory cortex, hippocampus, superior colliculus, inferior colliculus and cerebellum) as factors. Fresh brains were extracted and the above brain regions and dissected. Each brain region was homogenised in 0.32M sucrose and 10mM Tris-Cl buffer and then centrifuged multiple times at 4°C to extract synaptosomes. Protein content was estimated with a BCA assay. 5μg of protein per brain region was subject to analyses. A Seahorse XF Cell Mito Stress Test Kit was used in conjunction with a Seahorse XFP flux analyser (both Agilent) to measure i) basal oxidative

consumption rate, ii) proton leak, iii) maximum respiration, iv) spare respiratory capacity, v) non-mitochondrial oxygen consumption, vi) ATP production, and vii) coupling efficiency (all $n=6$ except wild-type 7-months, for which $n=3$). Three-way ANOVAs found a possible real effect of greater coupling efficiency in wild-type (7-months mean=24.3%, standard deviation ± 15.0 ; 15-months mean=20.2, ± 17.8) than mutant (7-months mean=15.4, ± 17.4 ; 15-months mean=5.2, ± 12.0) (genotype factor $P<0.05$) and trends toward more non-mitochondrial oxygen consumption, spare respiratory capacity and proton leak in mutant rats. We also prepared a separate sample of synaptosomes for scanning electron microscopy, following glutaraldehyde fixation and gold sputter coating. Micrographs were collected from all samples on a Zeiss Supra 40VP microscope. Synaptosome perimeter measures were performed in ImageJ. Preliminary findings lacked evidence of morphological differences between groups. Synaptosomes ranged in perimeter from $2.7\mu\text{m}$ in inferior colliculus to $10.1\mu\text{m}$ in hippocampus. Taken together, these data suggest potential differences in aspects of synaptic mitochondrial function relating to genotype in the TgF344-AD rat. Future work will expand on these data to provide greater statistical power and will furthermore employ correlative PET imaging and molecular biological approaches.



Scanning electron micrograph showing a putative hippocampal synapse from the synaptosome homogenate used in this study.

NB: the bleached rectangle in the centre is due to a prior higher magnification image being collected from this sample.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Kv3.1 and Kv3.3 differentially contribute to action potential repolarization in principal neurons of the auditory brainstem

N. Choudhury¹, A. Richardson¹, D. Linley², S. Robinson³, M. Anderson¹, V. Marra¹, C. Kopp-Scheinflug⁴, J.R. Steinert¹ and I.D. Forsythe¹

¹Neuroscience, Psychology and Behaviour, University of Leicester, Leicester, UK, ²University of Leeds, Leeds, UK, ³MRC Toxicology Unit, Leicester, UK and ⁴Ludwig-Maximilians-University, Munich, Germany

The classical 'delayed rectifier' potassium current is mediated by Kv3 channels which open at voltages during action potential (AP)-mediated depolarisation and thus provide the repolarization drive to support high-frequency firing in neurons. Multiple Kv3 genes (KCNC1-4) are co-expressed in several parts of the brain, either as hetero- or homo-tetramers (1-3) but subunit specific roles are largely unknown. Neurons of the medial nucleoid trapezoid body (MNTB) and the lateral superior olive (LSO) in the superior olivary complex (SOC) of the auditory brainstem compute sound source localisation through integration of binaural stimuli. Both MNTB and LSO neurons express Kv3.1 and Kv3.3 subunits, but lack Kv3.2 and Kv3.4 subunits (4). We have used their neuronal network to investigate the necessity or redundancy of Kv3.1 and Kv3.3 subunits in generating functional Kv3 channels and during repolarization of the postsynaptic AP. The study was conducted on wildtype (WT), Kv3.1 knockout (KO), and Kv3.3 KO mice in a CBA/Ca background strain in accordance with the Animals (Scientific Procedures) Act UK 1986 and as revised by European Directive 2010/63/EU. Expression of Kv3 channels in the MNTB and LSO principal neurons was investigated by qRT-PCR, western blot and immunohistochemistry. For *ex vivo* whole cell patch-clamp electrophysiology, transverse sections of SOC were obtained from brainstem and used for recording currents and voltages in the neurons under different pharmacological and biophysical conditions. Intracellular Ca²⁺ transients were studied by fura-2 ratiometric fluorescence measurement. Statistical significance was determined using Student's t-test and one-/two-way ANOVA and expressed as mean $\pm$ SD.

The results obtained reveal that MNTB neurons effectively employed either Kv3.1 or Kv3.3 subunits in Kv3 channels, with similar whole-cell current amplitude, however, the fastest APs were achieved when both subunits were expressed together (WT: 0.29 ± 0.07 ms, n=14; Kv3.1 KO: 0.41 ± 0.11 ms, n=11, p=0.0207; Kv3.3 KO: 0.48 ± 0.06 ms, n=11, p<0.0001). In the LSO, Kv3.3 subunits were essential; Kv3.1 mRNA was present, but somatic Kv3 channels must contain at least one Kv3.3 subunit, since Kv3.3KO mice had little or no TEA-sensitive Kv3 current (Ctrl: 6.0 ± 2.5 nA, n=11; TEA: 5.0 ± 1.8 nA, n=7), and AP halfwidths (0.69 ± 0.15 ms, n=7, p<0.0001) were increased compared to WT (0.28 ± 0.04 ms, n=10). Measurement of whole-cell voltage-gated Ba²⁺ currents showed no change in the MNTB or LSO, but the longer AP duration in the KOs increased activity-dependent [Ca²⁺]_i in an activity-dependent manner. We conclude that Kv3 channels make major contributions

to AP repolarization in both the MNTB and LSO. Kv3.1 and Kv3.3 subunits each contribute to Kv3 currents in the MNTB, while Kv3.3 is crucially dominant for Kv3 channels in the LSO, both affecting Ca^{2+} influx in these neurons.

Weiser M, Vega-Saenz de Miera E, Kentros C, Moreno H, Franzen L, Hillman D, et al. Differential expression of Shaw-related K⁺ channels in the rat central nervous system. *J Neurosci*. 1994;14(3 Pt 1):949-72.

Baranauskas G, Tkatch T, Nagata K, Yeh JZ, Surmeier DJ. Kv3.4 subunits enhance the repolarizing efficiency of Kv3.1 channels in fast-spiking neurons. *Nat Neurosci*. 2003;6(3):258-66.

Puente N, Mendizabal-Zubiaga J, Elezgarai I, Reguero L, Buceta I, Grandes P. Precise localization of the voltage-gated potassium channel subunits Kv3.1b and Kv3.3 revealed in the molecular layer of the rat cerebellar cortex by a pre-embedding immunogold method. *Histochem Cell Biol*. 2010;134(4):403-9.

Li W, Kaczmarek LK, Perney TM. Localization of two high-threshold potassium channel subunits in the rat central auditory system. *J Comp Neurol*. 2001;437(2):196-218.

Medical Research Council, United Kingdom

Preclinical Research Facility, Biomedical Services, University of Leicester, UK

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C098

Systemic LPS challenge induced cognitive dysfunction accompanied by decreased hippocampal brain-derived neurotrophic factor.

F.O. IWHIWHU, J. Wolugbom, M. Oyeleke and C. Ajonijebu

1Department of Physiology, College of Medicine and Medical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria., Afe Babalola University Ado-Ekiti, Ekiti State, ADO-EKITI, Nigeria

Correlational studies have linked pathogenic infections, including those induced by lipopolysaccharide (LPS), with learning and/or memory impairments, as opposed to beneficial roles of IL-4 on hippocampal-mediated cognitive performance. However, there is still paucity of information regarding the exact domain of cognition affected by systemic LPS exposure with reference to endogenous secretions and signalling of bioactive immune modulators (TNF- α versus IL-4). This study was aimed at investigating the neurotoxic effects of LPS on hippocampal-mediated cognitive functions, assessed through critical evaluation of spatial learning and reference memory tasks in the Morris water maze (MWM), with reference to synaptic plasticity and perturbed neuro-immune responses. Thirty-six BALB/c mice (18-20g) were randomly divided into six groups (n=6/group) which received i.p injections of sterile saline or 5mg/kg E.coli lipopolysaccharide (LPS), aspirin (100mg/kg) or cyanocobalamin (1000mcg/kg) by gavage, while others received combined treatments of either LPS/aspirin or LPS/cyanocobalamin. 48h

post LPS challenge, mice were pre-treated with either aspirin or cyanocobalamin one hour before the swimming sessions in MWM. Thereafter, mice were anaesthetized, and blood was collected via cardiac puncture. The brains were removed using appropriate forceps, while the hippocampi were dissected out on ice cold surface and immediately processed for molecular assessment of TNF- α and IL-4 by ELISA. Whole brains were also preserved in neutral buffer formalin for subsequent morphological analysis by H&E staining and expression of hippocampal Nissl bodies using cresyl violet stain. Our data showed that systemic LPS injection significantly increased the latency to platform crossings during acquisition phase of MWM task without any significant change during the probe test. These changes were accompanied by elevated TNF- α and decreased BDNF levels in the hippocampi of LPS treated mice compared to control. This suggest a decrease in memory consolidation due to neurotoxic effect of LPS. Surprisingly, cyanocobalamin treatment appears to have neurotoxic effects when given at a moderate dose of 1000mcg/kg. Conversely, hippocampal IL-4 was not significantly different among all the treated groups. Also, LPS-induced morphological changes was partly reversed while moderate expression of nissil bodies was associated with LPS treatment. This study underlines the impact of systemic LPS challenge on modulation of endogenous hippocampal molecules with severe consequences on learning components of cognition.

Czerniawski, J. (2015). "Systemic lipopolysaccharide administration impairs retrieval of context-object discrimination, but not spatial, memory: evidence for selective disruption of specific hippocampus-dependent memory functions during acute neuroinflammation." Brain, behavior, and immunity **44**: 159-166.

Lewicki, S., Lewicka, A., Kalicki, B., Klos, A., Bertrandt, J. and Zdanowski, R. (2014). The influence of vitamin B12 supplementation on the level of white blood cells and lymphocytes phenotype in rats fed a low-protein diet. Central-European journal of immunology, **39**(4), 419.

Murray, L. K., Otterstetter, R., Muller, M. D., & Glickman, E. L. (2011). The effects of high and low-dose aspirin on thermoregulation during and after acute cold exposure. Wilderness & environmental medicine, **22**(4), 321-325.

Prakash, R. (2017). "Neuroprotective activity of ethanolic extract of *Tinospora cordifolia* on LPS induced neuroinflammation." Translational Biomedicine **8**(4): 1-8.

Yi, B., Wu, C., Shi, R., Han, K., Sheng, H., Li, B. and Wu, H. (2018). Long-term administration of salicylate-induced changes in BDNF expression and CREB phosphorylation in the auditory cortex of rats. Otology & Neurotology, **39**(3), e173.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Presynaptic modulation of synaptic release and plasticity via NMDA receptor activation at the dorsal cochlear nucleus multisensory synapses

M. Svent, N. Hartell and M. Hamann

Neuroscience, Psychology and Behaviour, University of Leicester, Leicester, UK

Recent studies have shown that acoustic over-exposure, which leads to gap detection deficits in a rodent model of tinnitus, also increases synaptic release of glutamate at dorsal cochlear nucleus (DCN) multisensory synapses (1). This activates NMDA receptors (NMDARs) and leads to saturation of long-term potentiation (LTP) (1). The aim of this study is to investigate the presynaptic role of NMDARs in modulating synaptic release and plasticity at DCN multisensory synapses. Whole cell recordings were used to record excitatory postsynaptic currents (EPSCs) from fusiform cells in CBA mouse DCN slices. Inhibitory transmission was blocked by 10 μ M strychnine and 10 μ M gabazine. Values are reported as mean $\pm$ SD. Fusiform cell LTP was induced by high frequency stimulation of parallel fibres (2) (increase of EPSC slope from 153 $\pm$ 81 pA/ms to 196 $\pm$ 96 pA/ms, ** p <0.01, one-tailed ratio paired t-test, n =4). To study the effect of NMDARs on presynaptic glutamate release, miniature EPSCs (mEPSCs) were isolated with 1 μ M tetrodotoxin and recorded in presence of NMDAR agonist (500nM NMDA) with and without antagonist (50 μ M D-AP5). Friedman test with Dunn's correction was used for statistical comparisons. In 6/12 fusiform cells, NMDA increased mEPSC frequency (decreased inter-event interval from 2257 $\pm$ 1467ms to 1170 $\pm$ 805ms) which was reversed by the addition of D-AP5 (inter-event interval 3340 $\pm$ 2417ms) (control vs NMDA * p <0.05, NMDA vs NMDA+D-AP5 ** p <0.01, control vs NMDA+D-AP5 p >0.99, F =11.14, n =7). Activation of NMDARs had no effect on mEPSC amplitude or decay τ (p =0.62, F =1.14 and p =0.49, F =2 respectively). Selective effect on mEPSC frequency suggests increased presynaptic release. This was further assessed in transgenic mice expressing SyGCaMP2-mCherry, a ratiometric Ca²⁺ sensor which allows the visualisation of Ca²⁺ level changes in presynaptic boutons (3). Epifluorescence imaging of DCN molecular layer showed that activating NMDARs (500nM NMDA) increases the fluorescence associated to baseline presynaptic Ca²⁺ levels (from 0.99 $\pm$ 0.005 to 1.01 $\pm$ 0.006 normalised F_0 , * p <0.05, Mann-Whitney test, U =0, n_{control} =4, n_{NMDA} =5). Blocking NMDARs (50 μ M D-AP5) decreased the peak fluorescence response (from 1.09 $\pm$ 0.06 to 1.06 $\pm$ 0.05 F/F_0 , *** p <0.001, two-tailed paired t-test, t =5.5, n =8) evoked by parallel fibre stimulation (50pulses, 50Hz, 0.5-1mA). Multiphoton imaging of individual boutons showed that 50 μ M D-AP5 decreases both the number of responding presynaptic boutons (from 32% (n =130) to 21% (n =117)) and the amplitude of Ca²⁺ response per bouton (from 2.30 $\pm$ 0.73 to 1.91 $\pm$ 0.36 F/F_0 , n_{control} =42, $n_{\text{D-AP5}}$ =25, N =1). In conclusion, blocking NMDARs at DCN multisensory synapses could be used to restore basal levels of presynaptic release and in this way reverse the effect on LTP saturation and gap detection deficits observed after acoustic over-exposure (1).

Tagoe T, Deeping D, Hamann M. Saturation of long-term potentiation in the dorsal cochlear nucleus and its pharmacological reversal in an experimental model of tinnitus. *Experimental Neurology*. 2017;292:1-10.

Fujino K, Oertel D. Bidirectional synaptic plasticity in the cerebellum-like mammalian dorsal cochlear nucleus. *Proceedings of the National Academy of Sciences*. 2003;100(1):265-270.

Al-Osta I, Mucha M, Pereda D, Piqué-Gili M, Okorochoa A, Thomas R et al. Imaging Calcium in Hippocampal Presynaptic Terminals With a Ratiometric Calcium Sensor in a Novel Transgenic Mouse. *Frontiers in Cellular Neuroscience*. 2018;12(209).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C100

The effects of daily transcutaneous vagus nerve stimulation on autonomic tone

B. Bretherton^{1,2}, L. Atkinson¹, S. Deuchars¹ and J. Deuchars¹

¹*School of Biomedical Sciences, University of Leeds, Leeds, UK and* ²*Leeds Teaching Hospitals NHS Trust, Leeds, UK*

Aging is associated with increases in sympathetic and decreases in parasympathetic nervous activity, potentially contributing to age related disorders¹. In young, healthy adults, non-invasive, transcutaneous electrical stimulation of the vagus nerve (tVNS) promoted parasympathetic and reduced sympathetic activity². Since the magnitude of change was linked to the starting levels of autonomic activity², here we tested if daily tVNS for 14 days promoted increases in parasympathetic and decreases in sympathetic activity in volunteers aged ≥ 55 years.

Twenty-nine healthy participants (11 males) attended on two occasions. During both visits, participants underwent a 10 minute baseline period, 15 minutes of tVNS (administered via a TENS machine bilaterally to the tragus) and a 10 minute recovery period. Heart rate variability (HRV) and baroreflex sensitivity (BRS, responsiveness of changes in RR interval following changes in blood pressure) were derived. Participants self-administered tVNS at home once daily for 15 minutes over 14 days following visit 1. Data were analysed by repeated measure ANOVA, paired t-test and linear regression, values are the mean $\pm$ 1 S.E.M.

In the first lab-visit, tVNS was associated with significantly higher values for measures reflecting parasympathetic activity (nSD2: 59 ± 5 , $p = 0.001$) and overall variability in heart rate (SDRR: 47 ± 5 ms, $p = 0.001$) compared to baseline (nSD2: 46 ± 3 ; SDRR: 36 ± 3 ms). A measure of baseline parasympathetic outflow tended to be improved after two weeks of daily tVNS (pRR50: visit 1: $10 \pm 3\%$, visit 2: $14 \pm 4\%$, $p = 0.053$). Resting BRS in visit 1 significantly predicted change at visit 2 ($R^2 = 0.330$, $p = 0.002$): low visit 1 values were linked with greater increases after 14 days of tVNS. Baseline autonomic balance (LF/HF) significantly predicted change to tVNS both before and after two weeks of daily tVNS (visit 1: $R^2 = 0.352$, $p = 0.001$; visit 2: $R^2 = 0.697$, $p < 0.001$): imbalance during visit 1 was related to greater improvements following 14 days of daily tVNS. For those who responded to tVNS in

visit 1 (n = 9), most encountered improvements in autonomic balance at visit 2 (n= 6). This was even the case for seven participants who did not respond to tVNS in visit 1. Findings show that daily tVNS for 14 days confers autonomic benefits in individuals aged ≥ 55 years. Importantly, baseline autonomic tone seems to influence the direction and magnitude of change after 14 days of daily tVNS: low initial autonomic function is associated with greater improvements following daily tVNS for 14 days. Therefore, daily tVNS appears to help attenuate autonomic changes that occur with ageing, having the most pronounced effects in individuals with impaired autonomic balance. This means that tVNS is a simple and effective tool that can temporarily halt age-related changes in autonomic function and boost the period of healthy aging.

Kuo TBJ, Lin T, Yang CCH, Li CL, Chen CF, Chou P. Effect of aging on gender differences in neural control of heart rate. *American Journal of Physiology-Heart and Circulatory Physiology*. 1999;277:H2233-H2239.

Clancy JA, Mary DA, Witte KK, Greenwood JP, Deuchars SA, Deuchars J. Non-invasive Vagus Nerve Stimulation in Healthy Humans Reduces Sympathetic Nerve Activity. *Brain Stimulation*. 2014;7:871-877.

We would like to acknowledge the Dunhill Medical Trust.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C101

Amyloid- β inhibits mitochondrial large-conductance calcium-activated potassium channel

Y. Kravenska^{1,2}, H. Nieznanska¹, K. Nieznanski¹, A. Szewczyk¹ and P. Koprowski¹

¹Nencki Institute of Experimental Biology of the Polish Academy of Sciences, Warsaw, Poland and ²Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

Alzheimer's disease is the most common form of dementia. This neurodegenerative disorder is characterized by the overproduction and extracellular deposition of the pathological amyloid- β (A β) peptides. It was shown that one of the hallmarks of Alzheimer's disease are defects of mitochondria. We hypothesize that the mitochondrial large conductance calcium activated potassium (mitoBK(Ca)) channel could be one of the molecular targets for A β . It was previously found that mitoBK(Ca) is present in mitochondria of various tissues including the brain, and proved to play cytoprotective function during neuropathology. Therefore, the aim of our research was to investigate the effects of A β on the activity of mitoBK(Ca) channels. For our studies, mitochondria were isolated from the human astrocytoma U-87 MG cell line by the differential centrifugation. The activity of single mitoBK(Ca) channels was recorded from mitoplasts by the patch-clamp technique in the «inside-out» mode. The β_{1-42} -amyloid (A β_{1-42}) and its D-enantiomer (D-A β_{1-42}) in the form of either monomers, oligomers or amyloid fibrils were added directly to the medium with the recording pipette. The probability of channel opening was determined with

the Clampfit 10.3 software (Molecular Devices, USA). Detection of A β ₁₋₄₂ structural forms was performed using Thioflavin-T fluorescence assay and electron microscopy. For the first time, we showed that all three forms of A β ₁₋₄₂ – monomers, oligomers and fibrils – significantly inhibit mitoBK(Ca) channels. We used 0.1 – 0.5 μ M of A β ₁₋₄₂ and described its concentration-dependent block with a partial wash-out. The strongest inhibition of mitoBK(Ca) channels was caused by oligomers, which are known to be inserted deep into lipid bilayers. Interestingly, A β ₁₋₄₂ blocked mitoBK(Ca) channels from the both sides of the membrane indicating the lack of a specific binding site in the channel molecule. Similarly, D-A β ₁₋₄₂ inhibited channel showing the lack of stereoisomer specificity. We concluded that the action of A β ₁₋₄₂ can be explained by its interaction with the mitochondrial membranes. Thus, we demonstrated that the impairment of mitoBK(Ca) channel functioning is one of the probable mechanisms of A β -induced cytotoxicity. These results may help to develop potential methods of the prevention and treatment of Alzheimer's disease in the future.

This work was supported by Visegrad Fund Scholarship to Kravenska Y. and the Polish National Science Center grant no. 2017/26/M/NZ3/00727 to Koprowski P.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C102

Mapping atrial receptor distribution in the right atrial subendocardial neural plexus of the rat

T. Campbell¹, F. Shenton², S. Pyner² and J.F. Jones¹

¹*School of Medicine, University College Dublin, Dublin, Ireland and* ²*Department of Biosciences, Durham University, Durham, UK*

Introduction:

Cardiac reflexes originating from sensory receptors in the heart ensure blood supply to all tissue and organs in the face of constantly changing demands. Atrial receptors are mechanically sensitive vagal afferents which relay to the medulla and hypothalamus, affecting vasopressin release and renal sympathetic activity. Although the mechanism of volumetric mechanotransduction has not been studied before, vesicle recycling is an important component in other mechanotransduction systems (1). Two distinct sensory endings for atrial receptors have been localised to the subendocardial space using methylene blue staining or anterograde tracing (2) and these are referred to as end-nets and flower spray endings. To map the distribution of atrial receptors in the subendocardial space, we have double-labelled rat right atrial whole mounts [neurofilament (NF) & synaptic-vesicle protein 2 (SV2)] and generated high resolution maps of the rat subendocardial neural plexus.

Methods:

In accordance with EU directive 2010/63 and local ethical committee approval, 5 female wistar rats (150-350g) were euthanised by cervical dislocation following

isoflurane anaesthesia (5%). The heart was rapidly removed and the right atrium was isolated, pinned flat on silicone with the endocardial surface exposed and fixed & permeabilised in precooled methanol (-20 °C, 30 mins). Subsequently the tissue was blocked and incubated overnight in a humidity chamber at 4°C with primary antibody [1:500 chicken anti-neurofilament (Abcam, ab72996), 1:25 mouse anti-synaptic-vesicle protein 2 (Hybridoma Bank)]. Primary antibodies were rinsed off with blocking solution prior to secondary incubation in a light-protected humidity chamber overnight at 4°C [1:250 goat anti-chicken IgY Alexa Fluor®568 (Abcam, ab175477), 1:100 goat anti-mouse IgG-FITC (Sigma F8771)]. Samples were imaged using an apochromatic objective lens. Images were stitched using the *Pairwise stitching* function included in Fiji (ImageJ) (3).

Results:

The subendocardial space of the right atrium was richly innervated in all specimens and a recurrent hourglass-like arrangement of axons was present in the posterior atrial wall. Extensive end-net formations were visualised at the cavo-atrial junctions and interatrial septum. NF and SV2 antibodies differentially stained axons with NF labelling larger axons while SV2 labelled smaller axons. Nets of SV2 labelled endings were coincident with NF labelled nets particularly at the cavo-atrial and mid-atrial regions

Conclusion:

A smaller set of vesicle filled terminals extends from the larger and previously described end-nets. The study has provided an anatomical basis by which atrial deformation (atrial filling and contraction) can be communicated to the brain.

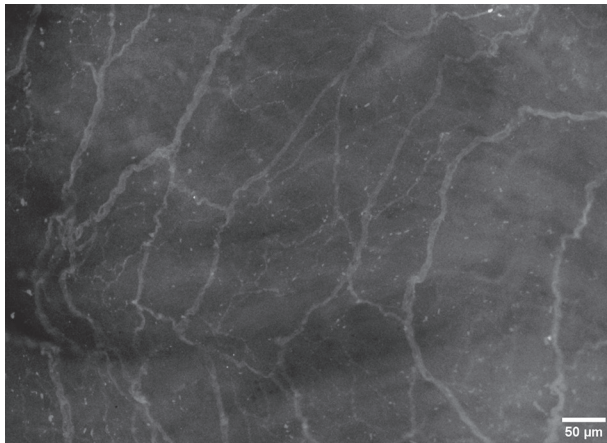


Figure 1: Subendocardial end-net of the inferior venous-atrial junction. Epifluorescent imaging of rat right atrial whole mount showing large red end-nets (neurofilament heavy chain) and smaller green nets (synaptic vesicle protein 2). The presence of synaptic vesicle protein 2 indicates the presence of vesicles. No varicosities are observed in the synaptic vesicle protein 2 net which suggests that these are sensory in nature. No flower spray endings are present. Images were captured using an apochromatic plan objective lens. Images were contrast enhanced and overlaid using Fiji (ImageJ).

Bewick GS, Reid B, Richardson C, Banks RW. Autogenic modulation of mechanoreceptor excitability by glutamate release from synaptic like vesicles: evidence from the rat muscle spindle primary sensory ending. *The Journal of physiology*. 2005;562(2):381-94.

Cheng Z, Powley TL, Schwaber JS, Doyle FJ. Projections of the dorsal motor nucleus of the vagus to cardiac ganglia of rat atria: An anterograde tracing study. *The Journal of Comparative Neurology*. 1999;410(2):320-41.

Preibisch S, Saalfeld S, Tomancak P. Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics*. 2009;25(11):1463-5.

This work was supported by the British Heart Foundation (PG14/53/309000) and the UCD School of Medicine. We would also like to thank Dr Mark Pickering (University College Dublin) for the provision of antibodies.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C103

ASICs in mechanosensation: ASIC1b deletion in mice reduces blood pressure, perturbs fine motor control *in vivo*, and reverses amiloride sensitivity of muscle spindle afferent firing.

G.S. Bewick¹, C. Lee⁴, A.T. Leeson-Payne², J. Rochford², C. Chen⁴ and R.W. Banks³

¹*Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK,* ²*The Rowett Institute, University of Aberdeen, Aberdeen, UK,* ³*Department of Biosciences, University of Durham, Durham, UK and* ⁴*Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan*

Amiloride-sensitive ion channels (ASICs) are highly expressed in primary mechanosensory nerve endings, including muscle spindle primary endings and baroreceptors monitoring blood pressure. However, their role there is unclear. Indeed, their possible role in neurosensory mechanotransduction is disputed^{1,2,3}. Nevertheless, they are one of the few putative mechanosensory channel families that display amiloride sensitivity and a selective conductance for Na⁺, characteristics of the mechanosensory receptor potential in muscle spindles^{4,5}. We have shown that ASIC1b is selectively expressed in primary mechanosensory terminals³. We therefore examined the effect of ASIC1b deletion on blood pressure and fine motor control *in vivo*, plus muscle spindle mechanosensory responses *ex vivo*.

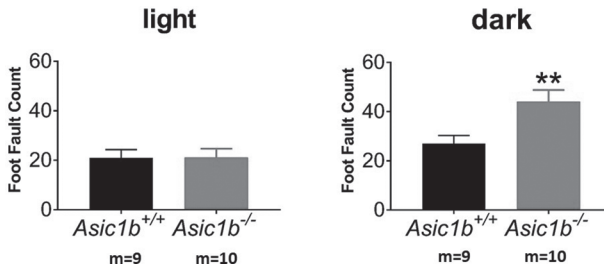
Experiments were covered by relevant national and local animal experimentation ethical approvals. Characterization of ASIC1b-expressing neurones was performed by immunofluorescence staining. Blood pressure was measured using a tail cuff, after 5 days habituation, and motor skills by hind foot placement faults during grid walking. Soleus nerve-muscle preparations were removed after humane killing (Schedule 1, Animals (Scientific Procedures) Act, 1986). The number of action potentials (APs) in the muscle nerve during hold phase of stretch-hold-release (ramp) profiles was quantified, as was the firing in amiloride, a broad-spectrum

blocker of ASICs. Data are expressed as mean $\pm$ SEM, from 'n' muscles and 'm' mice, and WT vs KO. The significance of differences was ascertained by ANOVA or *t*-test as appropriate, with a *P*=0.05 threshold.

ASIC1b-expressing neurones represented ~40% of NF200 positive (large myelinated), ~35% of peripherin positive (non-/poorly-myelinated) and ~47% of IB4 positive (nonpeptidergic), but only ~3.5% CGRP positive (peptidergic) neurones. *ASIC1b*^{-/-} mice had significantly lower systolic (105.5 $\pm$ 6.0 vs 88.5 $\pm$ 4.4 mmHg; *P*<0.02, *m*=5/group) but not diastolic (84.7 $\pm$ 4.1 vs 76.8 $\pm$ 4.3 mmHg; *P*=0.18, *m*=5/group) blood pressure. They also displayed higher foot faults during grid walking without visual or whisker cues (hind foot faults per 5 min: 27.0 $\pm$ 3.3, *m*=9 vs 44.1 $\pm$ 4.7, *m*=10; *P*<0.01, *t*-test). Mean hold phase spindle afferent firing rate was greater in WT than *ASIC1b*^{-/-} mice (124.3 $\pm$ 16.7 vs 90.8 $\pm$ 15.8 APs/sec), but the difference was not significant (*P*=0.3, *n*=26, *m*=21). Interestingly, while amiloride inhibited hold-phase firing in WT (0.1 mM: 92.3 $\pm$ 5.3% initial, *n*=5, *m*=4; 1 mM: 61.9 $\pm$ 8.0% initial, *n*=5, *m*=4) amiloride increased firing in *ASIC1b*^{-/-} spindles (0.1 mM: 131.7 $\pm$ 13.4% initial *P*<0.01; 1 mM: 125.8 $\pm$ 13.9% initial, *P*<0.001, both *n*=5, *m*=5; data normalised to final control value before amiloride addition).

We conclude that ASIC1b is a molecular determinant contributing to fine-tuning mechanosensitivity firing rates in proprioceptors.

Grid walking behaviour



ASIC1b KO greatly increases foot placement errors in grid walking task. Mean $\pm$ SEM, *m* (no. of mice). ** *P*<0.01, *t*-test.

Drew LJ *et al.* (2004) *J Physiol* 556, 691–710.

Chen CC & Wong CW (2013) *J Cell Mol Med* 17, 337–349.

Simon A *et al.* (2010) *J Physiol* 588, 171–185.

Hunt CC *et al.* (1978) *J Gen Physiol* 71, 683–698.

Bewick GS & Banks RW (2015) *Pflügers Arch* 467, 175–190.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Modelling Temperature Sensing by Parvocellular Neurones of the Paraventricular Nucleus of the Hypothalamus

F. O'Brien, C.H. Feetham, C. Staunton and R. Barrett-Jolley

University of Liverpool, Liverpool, UK

The paraventricular nucleus (PVN) of the hypothalamus is important in maintaining homeostasis and can alter cardiovascular function via autonomic control, however, the mechanisms underlying this control are not fully understood. PVN neurones are home to a wide array of ion channels and we have previously reported coupling between TRPV4 and small-conductance calcium-activated potassium channels (SK) to facilitate osmosensing in the PVN [1]. The PVN is also known to have thermoregulatory roles, therefore, we investigate whether TRPV4 may play a role in thermosensing.

To characterise single-channel gating of TRPV4 and spontaneous action current firing from parvocellular PVN neurones, we used patch-clamp electrophysiology on mouse hypothalamic brain slices. Heart rate, blood pressure and tail blood volume were recorded from CD1 mice using non-invasive tail plethysmography. Results are given as mean $\pm$ SEM, statistical significance was assessed by ANOVA or Student's t-tests as appropriate.

We identified a TRPV4-like channel on parvocellular PVN neurones with a conductance of 59.7 ± 1 pS and V_{rev} of -5.9 ± 3 mV ($n=9$) that was sensitive to the TRPV4 inhibitor GSK2193874. When temperature was increased, we observed a dramatic increase in open probability (P_o), from 0.19 ± 0.07 at 22°C to 0.54 ± 0.07 at 32°C and 0.82 ± 0.07 at 37°C , which was mediated by profound increases in the mean open durations ($n=9$, $**p < 0.01$, $***p < 0.001$). A NEURON model, adapted from Feetham *et al.*, (2015) [1] but with temperature dependent stochastic gating of TRPV4, and secondary Ca^{2+} -dependent activation of SK channels, predicted that action potential frequency of these neurones would decrease with temperature. This was confirmed with action current frequency (Apf) measurements from parvocellular PVN neurones. Apf decreased when temperature was increased from 22°C to 37°C ($**p < 0.001$, $n=6$). This effect was inhibited at temperatures up to 32°C by the broad-spectrum channel blocker gadolinium ($100 \mu\text{M}$) but not at physiological temperatures (37°C). In the presence of the TRPM2 blocker Econazole ($10 \mu\text{M}$), the effect was inhibited at all temperatures, indicating a role also for TRPM2 in thermosensing in the PVN. In whole animal experiments, inhibition of TRPV4 (GSK2193874, $300 \mu\text{g/kg}$, *i.p.*) resulted in an increased tail blood volume response to ambient heat application ($>33^\circ\text{C}$), which was not observed in control ($n=4$, $**p < 0.01$).

Our data shows that TRPV4-like channels on mouse parvocellular PVN neurones are sensitive to temperature change; increasing temperature has profound effects on channel gating. We postulate that TRPV4 channels on the PVN may be involved in thermosensing and show that pharmacological inhibition of TRPV4 results in an

increased temperature evoked vasodilation response in mice. Our spontaneous action current data, however, suggests that the temperature sensing in the PVN is complex, and is likely mediated by an interplay of multiple channel subtypes, including TRPV4 and TRPM2.

1. Feetham, C.H., et al., *TRPV4 and KCa ion channels functionally couple as osmosensors in the paraventricular nucleus*. British Journal of Pharmacology, 2015. 172(7): p. 1753-1768.

This work was funded by the BBSRC

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C105

The paralamina nucleus of the amygdala: a potential nexus in the regulation of stress in major depressive disorder

A. Nasa^{1,3}, A. Shah^{1,4}, L. Tobin-Schnittger^{2,1}, C.B. Kennedy^{2,1}, E. Roman^{1,4}, K. Levins⁵, E. O'Hanlon¹, V. O'Keane¹ and D.W. Roddy^{1,2}

¹Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland, ²Department of Physiology, University College Dublin, Dublin, Ireland, ³School of Medicine, Trinity College Dublin, Dublin, Ireland, ⁴School of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland and ⁵Department of Anaesthetics, Intensive Care and Pain Medicine, St Vincents University Hospital, Dublin, Ireland

Embedded beneath the lateral, basal and cortico-amygdaloid regions of the amygdala is the paralamina nucleus (PL), a poorly understood structure that has evolved to be relatively larger in humans and non-human primates. Studies show that it has dense mood regulatory serotonergic connections and indicate it to be a site for amygdalar neuroplasticity (1). Corticotropin-releasing factor receptors and glucocorticoid receptors are abundantly expressed in the PL facilitating interactions between emotions, the amygdala and stress (2). As such, this amygdalar nucleus may be involved in the pathogenesis of mood disorders. Until recently, neuroimaging was unable to visualise this enigmatic nucleus in vivo. The purpose of this study was to investigate the involvement of the PL in major depressive disorder (MDD) and evaluate whether the cortisol awakening response (CAR), a measure of HPA (hypothalamic-pituitary-adrenal) axis function, was related to its volumetrics. 20 healthy human controls and 15 depressed subjects were scanned using high-resolution T1 (1mm isotropic) magnetic resonance imaging at Trinity College Institute of Neuroscience (3T, Phillips Itega). Whole amygdala and paralamina nuclear volumes were generated using state of the art automated amygdalar segmentation in FreeSurfer 6.0 (3). Participants also provided three morning salivary cortisol samples (0, 30, 60 mins after waking). The CAR was calculating using two well-defined calculations; area under the curve with respect to ground and increase (AUCg and AUCi respectively) (4). Ethics for this study was granted under the Tallaght hospital REC.

Post-hoc ANCOVAs highlighted that the left PL was smaller in patients with MDD [$p = 0.018$] with no change in whole amygdalar volume between groups. Partial correlations correcting for age, sex and total intracranial volume (eTIV) revealed AUCi to be inversely correlated only with the left PL in the control group [$p = 0.006$], whereas the AUCg was inversely correlated only with the right PL in the depressed group [$p = 0.006$].

A smaller left PL implicates a potential role for this amygdalar nucleus in MDD. The relationship between the left PL and the AUCi in controls is lost in the MDD group. This suggests that the smaller left PL in MDD may impact the normal cortisol response to waking, a known neuroendocrine disturbance in the disorder (5). The relationship between the AUCg and the right PL in MDD suggests that total cortisol output may be associated in right PL volumes in MDD. This is consistent with the hypothesis of cortisol oversecretion in MDD and previous findings of larger right amygdalae in MDD and right-sided amygdalar affective dominance for negative emotions. In summary, this study suggests a role for the PL in MDD and in particular a role for this nucleus at the interface between HPA stress axis dysfunction and MDD.

Fudge JL. Where and what is the paralaminar nucleus? A review on a unique and frequently overlooked area of the primate amygdala. *Neuroscience & Biobehavioral Reviews*. 2012 Jan 1;36(1):520-35.

Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: implications for major psychiatric disorders. *Biological psychiatry*. 2003 Sep 1;54(5):515-28.

Fischl B. FreeSurfer. *Neuroimage*. 2012 Aug 15;62(2):774-81.

Fekedulegn DB, Andrew ME, Burchfiel CM, Violanti JM, Hartley TA, Charles LE, Miller DB. Area under the curve and other summary indicators of repeated waking cortisol measurements. *Psychosomatic medicine*. 2007 Sep 1;69(7):651-9.

O'Keane V, Frodl T, Dinan TG. A review of atypical depression in relation to the course of depression and changes in HPA axis organization. *Psychoneuroendocrinology*. 2012 Oct 1;37(10):1589-99.

We would like to acknowledge all the patients and volunteers for their time and effort in taking part in the study. We'd also like to thank the staff at the Trinity College Institute of Neuroscience (TCIN) for their expertise and assistance.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Maternal obesity alters intermediate memory and anxiety-related behaviours in adult mouse offspring

E.J. Mort, S.R. Heritage, E.J. Camm and A.L. Fowden

Physiology Development & Neuroscience, University of Cambridge, Cambridge, UK

UK rates of adult obesity have doubled in the last 25 years, leading to more overweight pregnant women (1). Maternal obesity during pregnancy impairs cardiometabolic health of the adult offspring in experimental animals, and is associated with behavioural and cognitive impairments in humans (2,3). Identifying whether these neurological changes are due to the pre- or post-natal environment is difficult in humans but is possible in experimental animals like mice. This study examined the effects of maternal obesity on the behaviour of their adult offspring fed standard chow post-weaning.

Under the Animal (Scientific Procedures) Act, C57BL6/J female mice were fed either a control (Con, 11%kcal fat, 7%kcal simple sugars) or high fat-high sugar diet (HFHS, 35.9%kcal fat and 29.5%kcal simple sugars) from 6 weeks before pregnancy and throughout pregnancy and lactation. The HFHS diet significantly increased body fat percentage of the dams before pregnancy (Con $20.8 \pm 0.6\%$ vs. HFHS $32.5 \pm 1.7\%$, $p < 0.0001$). Pregnant dams delivered naturally and their litters were reduced to 6 at postpartum day 2 (PN2). All offspring were weaned onto the control diet. From 12 weeks of age male and female offspring were tested using an elevated plus maze (EPM for anxiety; Con $n=13$ ♀, $n=14$ ♂, HFHS $n=8$ ♀, $n=7$ ♂) followed by a novel object recognition task (NOR for intermediate memory; Con $n=14$ ♀, $n=14$ ♂, HFHS $n=8$ ♀, $n=7$ ♂). Behavioural data analysis used Two-way ANOVA with *post hoc* Sidak's multiple comparison test or Unpaired t-test. Fetal and PN2 weights used a Linear mixed model repeated-measures ANOVA with mother as subject followed by *post hoc* Bonferroni t-test. Data expressed as mean or estimated marginal mean $\pm$ SEM.

At embryonic day 19 HFHS pups were growth restricted (Con 1.01 ± 0.01 g; HFHS 1.04 ± 0.01 g, $p=0.0005$) relative to controls, but not at PN2 (Con 1.48 ± 0.04 g vs HFHS 1.47 ± 0.08 g, $p>0.05$). Both male and female HFHS offspring displayed a deficit in intermediate memory showing no preference for the novel over the familiar object (discrimination index (DI) $p<0.0001$, Figure 1A). In the EPM task, HFHS offspring displayed reduced anxiety with increased entries ($p=0.0053$, Figure 1B) and time spent in the open arm ($p=0.0066$, Figure 1C). By 14 weeks of postnatal age, there were no differences in body weight, body fat percentage and brain weight between the control and HFHS offspring ($p>0.05$).

This study has shown maternal obesity causes memory impairment and a reduction in anxiolytic behaviour in the offspring, despite eating a healthy balanced diet post-weaning and without any change in their adiposity, body or brain weight.

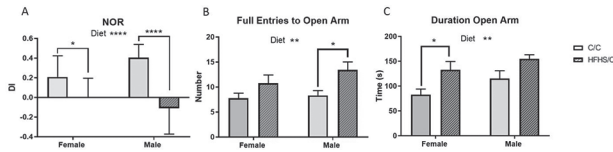


Figure 1: Behavioural results of 12 week old Control and HFHS offspring: (A) Discrimination index in the NOR task, (B) Entries into the open arm and (C) Duration in the open arm measures within the EPM task. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

1. Public Health England: Maternal Obesity <http://webarchive.nationalarchives.gov.uk/20170110171847>

2. Glastras SJ, Chen H, Pollock CA, Saad S. Maternal obesity increases the risk of metabolic disease and impacts renal health in offspring. Biosci Rep [Internet]. 2018;61(2):BSR20170050. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/29483369>0A<http://bioscirep.org/lookup/doi/10.1042/BSR20180050>

3. Contu L, Hawkes CA. A review of the impact of maternal obesity on the cognitive function and mental health of the offspring. Int J Mol Sci. 2017;18(5).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C107

Effect of spinal cord stimulation on autonomic function in patients with failed back surgery syndrome

S. Black^{2,1}, B. Bretherton^{1,2}, G. Baranidharan^{2,1}, S. Deuchars¹ and J. Deuchars¹

¹School of Biomedical Sciences, University of Leeds, Leeds, UK and ²Leeds Teaching Hospitals NHS Trust, Leeds, UK

Failed back surgery syndrome (FBSS), a chronic pain condition, is characterised by impaired autonomic tone with sympathetic prevalence and vagal withdrawal¹. Spinal cord stimulation (SCS) is an approved pain relief treatment. However, it is currently unknown whether SCS is associated with improvements in autonomic function in individuals with FBSS. The objectives of this study were to measure autonomic function by means of heart rate variability (HRV), baroreflex sensitivity (BRS) and muscle sympathetic nerve activity (MSNA) in individuals with FBSS prior to undergoing SCS, and to evaluate change in autonomic function 3-6 months following SCS implantation.

Autonomic function was measured in individuals with FBSS prior to, and 3-6 months following SCS. Measurements included: HRV, using statistical, Fourier and Poincaré analysis of the RR interval; BRS, using sequence method analysis of changes in heart rate in response to changes in systolic blood pressure; and the frequency (units/minute) and incidence (units/100 heart beats) of single MSNA units. Data were analysed by paired t-test and linear regression.

Baseline autonomic measures were obtained in 14 participants (7 males) prior to SCS implant. Autonomic tone varied between participants: some had high sympathetic prevalence (as indexed by high LF/HF and MSNA frequency and incidence) and low vagal tone (indexed by low BRS), whilst others had values considered in the 'normal' range². Ten participants (4 males) underwent further measurement 3-6 months following SCS implantation in which autonomic tone altered: there were trends towards reduced sympathetic prevalence (LF/HF and MSNA) and increases in vagal tone (BRS). Importantly, baseline measures of autonomic tone, particularly LF/HF ($R^2 = 0.669$, $p = 0.007$) and BRS ($R^2 = 0.483$, $p = 0.038$), significantly predicted change at 3-6 months post SCS implant. This means that patients with high LF/HF (reflecting sympathetic prevalence) and low BRS (reflecting low vagal tone) encountered greater improvements at 3-6 months follow-up. This is the first study combining HRV, BRS and MSNA to explore autonomic function in individuals with FBSS. Results revealed great heterogeneity in resting autonomic tone in this patient group, with some experiencing high sympathetic prevalence and low vagal tone. In addition, SCS tended to elicit a reduction in sympathetic prevalence, suggesting this may be an effective treatment for restoring autonomic balance in individuals with FBSS. Predicting autonomic response to SCS based on pre-implant BRS values is a key finding, suggesting that autonomic measurement might be a useful tool to identify those patients who have attenuated vagal control and heightened sympathetic outflow and who might benefit the most from SCS. With a greater sample, these findings could have important clinical impact, contributing to improving patient outcomes and safety.

Sahin N, Müslümanoğlu L, Karatas O, Cakmak A, Özcan E, Berker E. Evaluation of sympathetic response in cases with failed back surgery syndrome. *Agri*. 2009;21:10-15.

Camm AJ, Malik M, Bigger JT, Breithardt G, Cerutti S, Cohen RJ, et al. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *European Heart Journal*. 1996;17:354-381.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C108

Effects of indirect cocaine exposure and early postnatal fostering on epigenetic DNA modification and behavioral phenotypes of drug naïve mice.

D.C. Ajonijeju¹, M. Mabandla², W. Daniels³ and O. Abboussi⁴

¹Physiology, Nelson Mandela University, Port Elizabeth, South Africa, ²Physiology, University of KwaZulu-Natal, Durban, South Africa, ³Physiology, University of Witwatersrand, Johannesburg, South Africa and ⁴Dundee University, Scotland, UK

Abstract Body: Here, we explored the hypothesis that parental cocaine administration could alter epigenetic machinery in drug-naïve offspring while early postnatal

fostering may further modify the accompanied neurochemical and functional components. Variant drug naïve pups were produced from cocaine exposed or non-drug exposed C57BL/6 female mice that were matched with their male counterpart for mating. Within three days of birth, some of the pups were fostered and nurtured by non-biological lactating dams. The pups were initially examined for locomotor activity and memory performance and subsequently for changes in DNA methylation in promoter regions of cAMP response element modulator (*Crem*) and *Fosb* in the prefrontal cortex (PFC) at 48 days postnatum. The impact of postnatal fostering on these parameters was also investigated to further evaluate the influence of the environment on epigenetic expression. Our results showed that cocaine administration significantly decreased both *Crem* and *Fosb* methylation in the prefrontal cortex of the progenitor mice compared to their controls. We also found that similar patterns of methylation in the parents were replicated in the same brain region of all groups of drug naïve non-fostered offspring mice. Whereas, fostering the offspring at early life did not only prevent expression of epigenetic marks associated with parent's cocaine experience but also impaired recognition memory, especially in descendants lineally inclined with either paternal (ME) and/or both progenitors (MFE) that directly administered cocaine, as opposed to intact memory performance in their non-fostered counterparts. We also found that locomotor activity was unaltered in all groups of mice tested in the open field. Our data provide some evidence that indirect cocaine administration may cause marked epigenetic changes within the cortical networks of drug naïve descendants and that mediation by *Crem/Fosb* signalling in this brain region may be beneficial, while early postnatal fostering may further engineer molecular switching that may predispose the individual to future risky behaviours as well as accumulative potential to developing cognitive impairment later in life.

The authors would like to thank Mr. Simeon Eche for help with real-time qPCR and Dr. Kogi Moodley for procurement of consumables and excellent technical assistance. We also thank Dr. Linda Bester for assistance with mating and fostering protocols. This work was financially supported by the National Research Foundation of South Africa and the College of Health Sciences – UKZN.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Association between vascular stiffness and oscillatory flow pattern in patients with recent Myocardial Infarction

S. Badhwar¹, D.S. Chandran¹, A.K. Jaryal¹, R. Narang², C. Patel³ and K.K. Deepak¹

¹Physiology, All India Institute of Medical Sciences, New Delhi, New Delhi, India,

²Cardiology, All India Institute of Medical Sciences, New Delhi, New Delhi, India and

³Nuclear Medicine, All India Institute of Medical Sciences, New delhi, New Delhi, India

Induction of an oscillatory flow pattern or increasing retrograde flow has been associated with proatherogenic effects in endothelial cell culture models¹. Retrograde flow in peripheral arteries is also known to increase with ageing². The flow pattern at any point depends on pressure difference across that point³. One of the factors which can influence the pressure waveform, is the velocity of propagation of the reflected waves, which are directly affected by vascular stiffness. The aim of the study was to investigate the association between vascular stiffness and brachial artery flow profiles in patients of myocardial infarction. Patients of recent (<6months) myocardial infarction (n= 25) were recruited from the Department of Cardiology, AIIMS, New Delhi. Flow profile was assessed at the brachial artery using Pulsed wave doppler (M7, MindRay) with simultaneous acquisition of the diameter in duplex mode for estimation of shear rate ($\text{Shear Rate} = 4 \times \text{Velocity} / \text{Diameter}$). Oscillatory shear index was calculated using the formula: $\text{Retrograde shear rate} / (\text{Anterograde shear rate} + \text{Retrograde shear rate})$. Regional vascular stiffness was assessed by measuring the Pulse Wave Velocity (PWV) of the carotid-radial (cr) arterial segment using applanation tonometry (Sphygmocor⁰). Carotid-Intima-Media Thickness (CIMT) was measured using B-mode ultrasound (M7, MindRay) for estimation of subclinical atherosclerotic burden. Carotid artery wall properties were derived from carotid artery diameter changes during the cardiac cycle for calculation of compliance and Incremental Elastic modulus (Medical Imaging applications Carotid Analyser). A significant positive correlation was observed between cr-PWV and oscillatory shear index (OSI) ($r = 0.44$, $p = 0.01$) and between far-wall CIMT and OSI ($r = 0.45$, $p = 0.01$). A significant negative correlation was seen between Carotid compliance and OSI ($r = -0.35$, $p = 0.03$) and a significant positive correlation was observed between Incremental Elastic modulus and OSI ($r = 0.38$, $p = 0.02$). Both OSI and retrograde flow correlated positively with Age ($r = 0.49$, $p = 0.004$ and $r = 0.36$, $p = 0.03$). The current study highlights the factors associated with the atherogenic-oscillatory flow pattern. Preliminary results suggest a potential role of increase in vascular stiffness of the corresponding regional arterial segment and a decrease in carotid artery compliance. Additionally, the study shows an association between oscillatory shear pattern and macroscopic sub clinical atherosclerosis. A decrease in carotid artery compliance would alter the characteristic plateau pattern of the central pressure waveform to a sharper wave with a steep decline in pressure. This would be further augmented by increase in regional arterial stiffness leading

to an amplification of the reflected waves. The net effect of the altered pressure waves would result in development of an oscillatory shear pattern in patients of myocardial infarction.

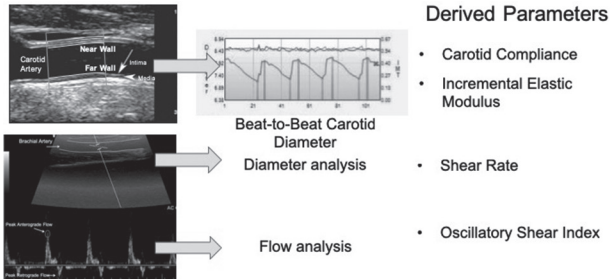


Figure 1. Representative Images of carotid and brachial artery for analysis

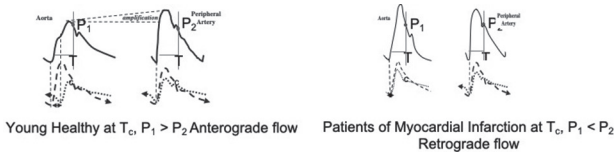


Figure 2. Proposed mechanism for alteration in waveforms leading to oscillatory flow in Myocardial Infarction

Jenkins NT, Padilla J, Boyle LJ, Credeur DP, Laughlin MH, Fadel PJ. Disturbed blood flow acutely induces activation and apoptosis of the human vascular endothelium. *Hypertension*. 2013 Mar;61(3):615–21

Credeur DP, Dobrosielski DA, Arce-Esquivel AA, Welsch MA. Brachial artery retrograde flow increases with age: relationship to physical function. *Eur J Appl Physiol*. 2009 Sep;107(2):219–25.

Nichols WW, Nichols WW, McDonald DA, editors. *McDonald's blood flow in arteries: theoretic, experimental, and clinical principles*. 6th ed. London: Hodder Arnold; 2011. 755 p.

We would like to acknowledge Indian Council of Medical Research (ICMR), New Delhi for funding the study.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

GPR81 activation causes endothelin-1 dependent renal vasoconstrictionN.K. Jones¹, K. Stewart¹, J.W. Wiseman², J.J. Mullins¹ and M.A. Bailey¹¹BHF Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, UK and²Discovery Sciences, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

G protein-coupled receptor 81 (GPR81) is abundant in adipocytes and activation by the endogenous ligand lactate or by the specific agonist AZ'5538 inhibits lipolysis^{1,2}. GPR81 is also found in non-adipose tissue¹ and AZ'5538 infusion has been shown to increase blood pressure (BP)². Here, we localised *Gpr81* mRNA in mouse arteries and kidney and examined the *in vivo* effects of AZ'5538 on renal haemodynamics. The expression of *Gpr81* in tissues from WT and *Gpr81*-KO mice was assessed by PCR and *in situ* hybridisation. Separately, WT and *Gpr81*-KO mice were anaesthetised (Inactin; 120mg/kg, IP), the carotid artery cannulated for BP measurement and renal blood flow (RBF) measured by a Doppler ultrasound probe on the right renal artery. Doppler probes were also inserted into the cortex and medulla for measurement of intrarenal perfusion during IV infusion of either AZ'5538 or 5% mannitol vehicle. Plasma endothelin-1 (ET-1) was assessed by ELISA at baseline and post AZ'5538 or vehicle infusion. In a final group of mice, we examined the effect of ET receptor antagonism on the cardiorenal actions of AZ'5538. All animal work was performed under UK Home Office licences after ethical review by The University. Data are mean±SD. *Gpr81* was expressed in the renal arteries, aorta and mesenteric arteries of wildtype mice (n=4). *In situ* hybridization showed *Gpr81* expression in the vascular smooth muscle, glomeruli and perivascular cells in the medullary rays. In wildtype mice, AZ'5538 increased systolic BP by 14.9±6.5mmHg; vehicle had no significant effect (0.7±3.1mmHg, n=10). AZ'5538 also decreased RBF (-0.33±0.23mL/min vs vehicle 0.04±0.02mL/min, P=0.027, n=5). AZ'5538 had no effect in *Gpr81*-KO mice. In separate studies, AZ'5538 decreased cortical flux in wildtype animals only (-312.6±113.7PU vs 12.0±42.3PU for WT and KO respectively, P<0.0001, n=6); the reduction in medullary flux did not reach statistical significance (P=0.07). In C57 mice, plasma ET-1 was increased by AZ'5538 from baseline compared to vehicle (0.44±0.28pg/mL vs 0.12±0.28pg/mL respectively, n=8, P=0.04) but there were no differences in kidney or aorta levels. Prior infusion of endothelin-A receptor (ETRA) inhibitor BQ-123 prevented the AZ'5538 mediated BP increase (n=4, P=0.04), whereas BQ-788, an endothelin-B receptor antagonist, did not (n=5, P=0.18).

We show *Gpr81* expression in vascular smooth muscle, glomerulus and perivascular cells of the mouse kidney. *In vivo*, GPR81 activation influences cardiorenal haemodynamics in an ETRA mediated manner by stimulating circulating ET-1. The sensitivity of the renal circulation to GPR81 activation may become physiologically significant when circulating lactate, the endogenous ligand of GPR81, concentration rises, as can occur in ischemic renal disease.

Liu C, Wu J, Zhu J, Kuei C, Yu J, Shelton J, et al. Lactate inhibits lipolysis in fat cells through activation of an orphan G-protein-coupled receptor, GPR81. *J Biol Chem*. 2009;284(5):2811–22.

Wallenius K, Thalén P, Björkman J, Johannesson P, Wiseman J, Böttcher G, et al. Involvement of the metabolic sensor GPR81 in cardiovascular control. *JCI Insight*. 2017;2(19):1–18.

Supported by The British Heart Foundation (FS/15/63/32033) and AstraZeneca (Research Agreement 10028531)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C111

The effect of the small conductance calcium-activated potassium channel (SK) inhibitor ICAGEN in intact atria and atrial cardiomyocytes

S. Dobi, A. Campbell, A.J. Workman and G.L. Smith

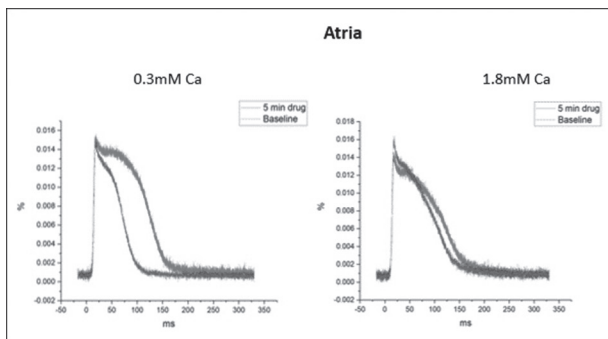
Institute of Cardiovascular and Medical Sciences, Glasgow, UK

Small-conductance calcium-activated potassium channels (*SK channels*) have been suggested to mediate abnormal atrial electrophysiology in heart failure and sustained atrial fibrillation [1, 2]. However, a mechanistic explanation of the function of SK channels in health and disease is lacking. In this study we examined the effects of a putatively specific SK channel blocker on the electrophysiology of atria and ventricle of rabbit hearts. Experiments were performed in isolated atrial cardiomyocytes and intact atria and ventricle of isolated rabbit hearts using fluorescence based methods. Isolated Langendorff perfused hearts were perfused with Di-4-ANEPPS to allow measurements of atrial and ventricular action potentials (APs) optically. Addition of the drug ($1\mu\text{M}$) to the perfusate caused significant prolongation of atrial AP duration (APD) at 5mins, (APD75 19.52% change $\pm$ 2.4 and APD90 23.90% change $\pm$ 6.63, $n=4$), but no change in ventricular APD (APD75 3.03% change $\pm$ 1.70, APD90 2.30% change $\pm$ 0.84, $n=4$). This effect was confirmed with corresponding, independent, experiments in isolated atrial and ventricular cells. To test the dependence of the effect on intracellular calcium, the effect of the drug in hearts perfused for 20mins in low (0.3mM) extracellular Ca^{2+} was examined. This manoeuvre which was designed to lower intracellular Ca^{2+} did not alter the drug-induced prolongation of APD. Similar results were observed in isolated atrial myocytes. This apparent lack of sensitivity of the ICAGEN effect to intracellular Ca^{2+} is counter to the supposed Ca^{2+} -dependence of SK channels. Further studies are required to understand the mechanism underlying the electrophysiological effects of the drug and the SK channel in rabbit atrial electrophysiology.

Calcium manipulations and their effect in APDs (Action Potential Duration)

5 min drug perfusion			
		1.8mM Ca^{2+}	0.3mM Ca^{2+}
	Atria	Ventricle	Atria
APD75	19.52 $\pm$ 2.4	3.03 $\pm$ 1.70	12.37 $\pm$ 6.85
APD90	23.90 $\pm$ 6.63	2.30 $\pm$ 0.84	34.61 $\pm$ 13.02

All values reported as the mean ($n=4$) APD value in % change in comparison to the baseline $\pm$ SEM (Standard Error of the Mean)



The effect of 5 min ICAGEN perfusion in the rabbit atria in physiological calcium concentration and low calcium

1. Gu, M., et al., *Small-conductance Ca(2+)-activated K(+) channels: insights into their roles in cardiovascular disease*. Exp Mol Med, 2018. 50(4): p. 23.
2. Diness, J.G., et al., *Role of Calcium-activated Potassium Channels in Atrial Fibrillation Pathophysiology and Therapy*. J Cardiovasc Pharmacol, 2015. 66(5): p. 441-8.

Professor Godfrey Smith and the Smith group

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C113

Involvement of M₂ muscarinic receptors and Kv7 channels in cholinergic-mediated contractions of murine bronchial rings.

T. Alkawadri¹, K. Thornbury¹, M. Hollywood¹, L. McGarvey², F. Lundy², G. Litherland³ and G. Sergeant¹

¹Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland,

²Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Science, Queen's University Belfast, Belfast, UK and ³Centre for Musculoskeletal Science, University of the West of Scotland, Glasgow, UK

Acetylcholine regulates bronchial tone in airway smooth muscle (ASM) by binding to both M₂ and M₃ muscarinic receptors (Fisher *et al.*, 2004), however, the mechanisms underlying M₂R-mediated contractions of ASM are still unclear. Recent studies have indicated that Kv7 channels are functionally expressed in ASM and that Kv7 currents are suppressed by cholinergic agonists (Brueggemann *et al.*, 2012). The purpose of the present study was to investigate if M₂R-mediated contractions of ASM were affected by Kv7 channel modulators. Adult male and female C57BL/6 mice were euthanized by intraperitoneal injection of pentobarbital (100 mg/kg) and the lungs were removed to obtain primary bronchial rings. The bronchial rings

were mounted into organ baths and isometric tension recordings were employed to examine the effect of M₂R antagonists and Kv7 channel modulators on contractions induced by application of the cholinergic agonist carbachol (CCh).

The M₂ muscarinic antagonist methoctramine (100 nM) reduced the mean contraction amplitude of responses induced by CCh (100 nM) by 68% from 5751 ± 768 to 1227 ± 624 mN.s (n=6, p<0.0001, two way ANOVA, Bonferroni's multiple comparisons test). The EC₅₀ for CCh was shifted from 100 to 278 nM in methoctramine (n=6, CI 95%: 75 – 160 nM and 234 – 333 nM, respectively). Similarly, the Kv7 channel activator retigabine (10 µM) reduced the amplitude of CCh-induced contractions, and increased the EC₅₀ from 90 to 240 nM (n=6, CI 95%: 60 – 127 nM and 190-304 nM, respectively). The M₃R antagonist 4-DAMP (3 nM) reduced the amplitude of CCh-induced contractions (300 nM) by 69% and the residual contractions were abolished by methoctramine (n=9, p<0.001, two tailed Student's *t*-test). 4-DAMP-resistant CCh responses were also abolished by retigabine (n=10, p<0.01, two tailed Student's *t*-test) and enhanced by the Kv7 channel blocker XE-991 (n=28, p<0.0001, ANOVA, Bonferroni's multiple comparisons test).

These data suggest that; 1) M₂Rs may have a greater functional role in ACh-induced contractions of ASM than previously realised and 2) that M₂R-dependent responses may involve Kv7 channels.

Brueggemann, L., Kakad, P., Love, R., Solway, J., Dowell, M., Cribbs, L. and Byron, K. (2012). Kv7 potassium channels in airway smooth muscle cells: signal transduction intermediates and pharmacological targets for bronchodilator therapy. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 302(1), pp.L120-L132.

Fisher, J., Vincent, S., Gomez, J., Yamada, M. and Wess, J. (2004). Loss of vagally mediated bradycardia and bronchoconstriction in mice lacking M2 or M3 muscarinic acetylcholine receptors. *The FASEB Journal*, 18(6), pp.711-713.

This study was funded by the EU under the Interreg VA and managed by the special EU programmes body (SEUPB).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C114

Descending vasa recta pericytes mediate medullary no-reflow after renal ischaemia

F. Freitas and D. Attwell

Neuroscience, Physiology & Pharmacology, University College London, London, UK

Motivation: Renal ischemia-reperfusion injury is one of the main causes of acute kidney injury. Medullary hypoxia occurs during reperfusion after ischemia, however, little is known of the mechanisms involved. The descending vasa recta mediate blood flow to the loop of Henle. These are capillary-sized microvessels, with contractile abluminal pericytes which could play a role in post-ischemic

medullary hypoxia. We investigated if pericyte-mediated constriction of medullary descending vasa recta contributes to renal ischaemia. Methods: Adult male Sprague-Dawley rats (200–250 g; n=6-8) were anaesthetized with pentobarbital sodium. Both kidneys were exposed, and the renal arteries and veins were carefully dissected. Left kidneys were subjected to 60 min ischaemia induced by a renal artery and vein cross clamp followed by 30 min reperfusion (ISCH). The right kidneys underwent the same procedures but the blood perfusion was not blocked (SHAM). Two laser-doppler probes were used to measure simultaneously the renal perfusion *in vivo* on both kidneys. At the end of the ischaemia/reperfusion protocol animals were overdosed with pentobarbital sodium and transcardially perfused with saline followed by 4% for fixation and then 20 ml of 5% gelatin solution containing FITC-albumin to visualize the perfused renal microvasculature. Pericytes were labelled with anti-NG2 and anti- α -SMA antibodies and the capillary basement membrane and pericytes were labelled with isolectin B4-Alexa Fluor. Data are mean $\pm$ s.e.m and were analyzed using an unpaired Student's t-test. Results: Ischaemia decreased renal perfusion by $\sim$ 86% ($p < 0.0001$), and this recovered 30 min following reperfusion. Perfusion was stable in the contralateral kidney (SHAM) throughout the experiment. Renal perfusion assessed *ex vivo*, as the mean intensity of FITC-albumin, demonstrated that renal ischaemia and reperfusion led to no-reflow in the medulla compared with the non-ischaemic kidneys (blood volume was reduced by $\sim$ 50%, $p < 0.0001$). Similar reductions were seen in the medulla for the total microvessel length ($p = 0.038$), the number of microvessel branches ($p = 0.053$) and the overall microvascular density ($p = 0.0003$). Ischaemia-induced medullary no-reflow was associated with blockages of the descending vasa recta. In ischaemic kidneys, the percentage of descending vasa recta blocked in the medulla of ischaemic kidneys was $78 \pm 9\%$ compared with $10 \pm 4\%$ in SHAM ($p < 0.0001$). The descending vasa recta diameter at pericyte somata after ischaemia was reduced ($4.4 \pm 0.4 \mu\text{m}$; $p = 0.0001$) compared with sham-operated kidneys ($7.6 \pm 0.2 \mu\text{m}$). The distance from blockage sites to the nearest pericyte soma was $4.9 \pm 0.4 \mu\text{m}$ ($p < 0.0001$), about 1/4 of the distance between pericytes, consistent with pericyte constriction generating the blockages. Conclusion: Medullary no-reflow reflects blockage by pericyte constriction of the descending vasa recta. Pericytes might be a therapeutic target for preventing renal medullary injury after ischaemia.

Regner KR & Roman RJ (2012). *Curr Opin Nephrol Hypertens* 21, 33-38.

Peppiatt-Wildman CM *et al.* (2013). *Acta Physiol (Oxf)* 207, 212-225.

Supported by a Rosetrees Trust and Stoneygate Trust award (M153-F2) to FF and DA and an ERC Advanced Investigator Award to DA.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

The H₂S-releasing NSAID, ATB-346, significantly attenuates human myometrial contraction

A. Mijuskovic, S. Wray and S. Arrowsmith

Cellular and Molecular Physiology, University of Liverpool, Liverpool, UK

Hydrogen sulphide (H₂S), similar to nitric oxide and carbon monoxide, is an endogenous gaseous signalling molecule, shown to play a prominent role in the regulation of contraction of many smooth muscles including myometrium^{1,2}. H₂S is predominantly produced from L-cysteine via the cystathionine γ -lyase and cystathionine β -synthase³. Both are expressed in uterus⁴. H₂S and H₂S-releasing compounds are potent inhibitors of rat and human myometrial contractions with evidence to suggest activities at the K_{ATP} channels, Cl⁻ channels and on Ca entry^{1,2}. H₂S also exerts anti-inflammatory, anti-oxidant and cytoprotective actions.

A better understanding of how uterine contractility is controlled is required in order to develop better tocolytics to reduce the morbidity and mortality associated with preterm delivery. Non-steroidal anti-inflammatory drugs (NSAIDs) e.g. indomethacin, which inhibit cyclo-oxygenase (COX) and production of prostaglandins, are effective in attenuating uterine contractions, but have limited use due to adverse fetal side-effects. ATB-346 is a NSAID, derived from naproxen but coupled to a H₂S-releasing moiety, 4-hydroxy-thiobenzamide (TBZ)⁵. This study aimed to compare the effects ATB-346 with naproxen and TBZ alone and the H₂S donor, Na₂S, on human myometrial contractility.

Isometric tension recordings were performed on strips of pregnant myometrium (1x5x2mm), from 15 women undergoing pre-labour Caesarean section (with informed consent and ethical approval). Firstly, the effect of increasing concentrations (0.3 μ M-100 μ M) of Na₂S on force of contraction (mN) and contraction integral (area under the curve, AUC) was investigated (n=5). Next, the effect of ATB-346 (10 μ M and 30 μ M) was compared to the unconjugated naproxen or TBZ alone (n=5-10). Significance was taken as P<0.05 by one-way ANOVA and Dunnett's multiple comparison test.

Acute application of Na₂S to contracting strips of human myometrium produced a concentration-dependent decrease in activity. The decrease in force amplitude of contraction was significant at 30 μ M Na₂S and for AUC at 100 μ M Na₂S (P<0.01 and P<0.001 respectively). ATB-346 also produced a significant decrease in force amplitude and AUC which was observed at 10 μ M: 74.5($\pm$ 8.4)% and 70.8($\pm$ 5.8)% of control activity respectively (P<0.05). This effect was further potentiated at 30 μ M; 41.2($\pm$ 14.0) % and 39.0($\pm$ 11.3)% of control activity respectively (P<0.01). Naproxen and TBZ alone had small but non-significant effects on contraction amplitude and AUC at both concentrations (P>0.05).

Data confirm the tocolytic effect of H₂S donors in human myometrium. The combination of COX inhibition together with the release of H₂S resulted in a significant reduction of human myometrial contractility compared to Na₂S alone. The more

profound effect of ATB-346 compared to naproxen alone is likely due to the presence of H₂S. Combination approaches involving H₂S donors should be further explored as potential tocolytic therapies in myometrium.

Robinson et al., 2012; PLoS One. 7(9):e46278

Mijuskovic et al., 2015; Br J Pharmacol.172(14):3671-86

Rose et al., 2017; Cell. Mol. Life Sci. 74(8):1391-1412

You et al., 2011; PLoS One. 6(8):e23788

Wallace et al., 2010; Br J Pharmacol. 159(6):1236–1246

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C116

Adaptive remodeling recovers impaired skeletal muscle function and microvascular perfusion after compensatory hypertrophy of the rat heart.

P. Tickle¹, P. Hendrickse², H. Degens² and S. Egginton¹

¹University of Leeds, Leeds, UK and ²Manchester Metropolitan University, Manchester, UK

Microvascular rarefaction (loss of functional capillaries) may contribute to a decline in skeletal muscle performance accompanying cardiac and vascular diseases. It is not clear to what extent loss of microvascular density occurs in the earliest stages of these conditions, if impaired arterial blood flow delivery to muscle is an aggravating factor, and whether muscle remodeling *via* angiogenic therapies can restore performance. Therefore, we have characterised muscle performance, arterial blood flow and microvascular perfusion and how these change when the muscle is functionally overloaded in a rat model of compensatory cardiac hypertrophy.

Effects of cardiac hypertrophy on peripheral blood flow and muscle function were investigated by surgical implantation (isoflurane anaesthesia) of a titanium band to effect abdominal aortic stenosis, with determination of acute (Aob-acute; n=6) and chronic (Aob; n=7) effects after 4 weeks. To assess adaptive remodeling, a third group (Aob+OV; n=7) underwent a 2-week overload (by removal of a synergist muscle) of the extensor digitorum longus (EDL), while another overload group (OV; n=6) was otherwise intact. Bilateral EDL twitch force and fatigue-resistance were determined by indirect stimulation at 10Hz to elicit maximal isometric contractions for 180s. Carotid pressure (mmHg) and bilateral femoral artery flow (ml·min⁻¹) were monitored. Histological assessment of cryosections enabled calculation of functional rarefaction by comparison of fluorescent Dextran (perfused) and lectin (total) labelled capillary density.

Aortic banding was associated with hypertension (mean±SE: Aob-acute:140±5; Aob:157±10; Aob+OV:147±4) compared to control (122±1 mmHg, *P*<0.05). Increased heart mass (% body mass) was confirmed post-mortem in Aob (0.36±0.03)

and Aob+OV (0.31 ± 0.01) ($P < 0.05$ vs. control 0.26 ± 0.004 , $n=7$). Fatigue index (FI: max. force at end/start of stimulation) in control was $49 \pm 6\%$, but was reduced in Aob-acute ($24 \pm 12\%$; $P < 0.001$) and Aob ($37 \pm 5\%$; $P = 0.048$). FI was restored to control levels in Aob+OV ($49 \pm 8\%$; $P = 0.99$), while a similar proportional improvement above control occurred in OV ($61 \pm 7\%$; $P = 0.03$). Reduced femoral blood flow occurred in Aob-acute ($P < 0.001$) but was similar to controls in other groups, indicating that arterial perfusion did not constrain muscle performance following intervention. Reduced ($P < 0.05$) perfused CD occurred after stenosis (control: 548 ± 37 ; Aob: 232 ± 14 ; Aob+OV: 349 ± 49 ; OV: 556 ± 84 mm²), indicating a potential microvascular limitation on muscle performance. Within banded animals, functional overload stimulated an improved ($P < 0.01$) perfused and total (Aob: 565 ± 30 ; Aob+OV: 722 ± 47) CD, indicating that angiogenic capacity remained despite the systemic effects of aortic stenosis. These data show that deleterious effects of cardiac hypertrophy can be countered by a non-pharmacological angiogenic intervention, providing a future therapeutic target for improving the symptoms of patients with chronic heart failure.

Supported by the BHF.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C117

Increased β -amyloid production is associated with diabetes-induced vascular dysfunction

P.J. Meakin^{1,2}, Z. Tuharska², B. Coull², C. McCaffery², F. Khan² and M. Ashford²

¹Discovery & Translational Medicine, University of Leeds, Leeds, UK and ²Division of Systems Medicine, University of Dundee, Dundee, UK

Introduction: β -amyloid ($A\beta$) is produced via the cleavage of amyloid precursor protein (APP) by β -secretase (BACE1), resulting in the formation of amyloid plaques, a hallmark Alzheimer's disease (AD) pathology. AD, type 2 diabetes, obesity and cardiovascular disease appear intimately linked with endothelial dysfunction, inflammation, insulin resistance and elevated $A\beta$ levels all common features. Impaired endothelial function is represented by impaired endothelium-dependent, nitric oxide (NO)-mediated relaxation. Tight regulation of vasoactive compounds such as endothelin-1 (ET-1) and NO are required to maintain vascular tone. An imbalance, resulting in increased ET-1 and reduced NO levels promotes vascular stiffening and consequently diseases such as diabetic retinopathy, nephropathy and stroke. Diseases caused by an increased atherosclerotic burden including coronary heart disease and stroke are major causes of death in obese and diabetic populations.

Aims: Determine (1) whether enhanced A β levels are sufficient to induce vascular dysfunction and (2) if reducing A β production can reverse diet-induced vascular dysfunction.

Methods: Measurements of vascular function was determined *in vivo* by the vascular response to Acetylcholine (endothelial dependent) or Sodium Nitroprusside (endothelial independent) using laser Doppler imaging in two studies; (i) Wild-type (C57bl/6) infused with murine A β_{42} or scrambled peptide (ScP) (3.36 μ g/kg) in aCSF for 6 weeks mice fed a regular chow diet or (ii) a BACE1 inhibitor (M-3; 10 mg/kg) or vehicle (DMSO/PBS) into diet induced obese (DIO) C57bl/6 mice. Western blotting and ELISAs were used to measure vascular NO signalling and A β production.

Results: Circulating levels of A β_{42} , not the more prevalent A β_{40} isoform, are increased both in high fat feed mice and obese/diabetic human patients ($P < 0.01$). Infusion of M-3 into DIO mice rescued endothelial dependent reactivity (M-3 27.1 ± 5.9 , vehicle 1.3 ± 2.9 ; $P < 0.01$). In contrast, infusion of A β_{42} promoted impaired vascular responses (A β_{42} 14.1 ± 3.7 , ScP 38.3 ± 3.2 ; $P < 0.001$) on regular chow with no change in body weight. In line with our hypothesis, infusion of A β_{42} increases the ET-1/NO ratio (ScP 1.12 ± 0.05 , A β_{1-42} 5.35 ± 0.89 ; $P < 0.001$), while DIO mice treated with a BACE1 inhibitor, thus with reduced plasma A β_{42} levels, have a low ET-1/NO ratio (M-3 1.29 ± 0.9 , DIO 4.62 ± 1.3 ; $P < 0.05$).

Conclusions: We suggest that amyloid processing has a role in normal vascular function with aberrant processing leading to endothelial dysfunction and hypertension. Here we show that pharmacological inhibition of BACE1 can reverse diet induced endothelial dysfunction, via modulation of A β_{42} levels, as infusion of A β_{42} can promote the dysfunction independent of a high fat diet.

Implications: Our data suggest that plasma A β_{42} could be a novel bio-marker for diabetes-induced vascular disease and thus BACE1 inhibitors, currently in clinical trials for AD, may be an innovative treatment.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C118

Phosphodiesterase 1: A Novel Drug Target for Pulmonary Arterial Hypertension

Z. Al Bakour¹, J. Dyson¹, J. Hislop¹, J. Breitenbucher² and F. Murray¹

¹Aberdeen Cardiovascular and Diabetes Centre, University of Aberdeen, Aberdeen, UK and ²Institute for Neurodegenerative Diseases, University of California, San Francisco, CA, USA

Pulmonary arterial hypertension (PAH) is characterized by increased proliferation of pulmonary arterial smooth muscle cells (PASMCs), which leads to remodelling of the pulmonary artery and increased pulmonary artery pressure. Multiple risk factors, such as female bias, circulating co-mitogens and hypoxia, lead to the

progression of PAH. Decreased levels of cyclic nucleotides, both cAMP and cGMP, in PASMC contribute to the abnormal tone and remodelling in the pulmonary vasculature seen with PAH. Phosphodiesterases (PDEs) catalyse the hydrolysis of cAMP and cGMP. We have shown that an increase in the expression of PDE1 isoforms (PDE1A and PDE1C), accounts, at least in part, for lower agonist-induced cAMP levels and increased proliferation of PASMC isolated from PAH patients. Recently, novel selective PDE1 inhibitors have been developed and tested in phase II clinical trials for central nervous system disorders. We aimed to investigate the regulation of PDE1 isoforms in human PASMC and provide evidence for the repurposing of such PDE1 inhibitors for PAH. Data are presented as means $\pm$ S.E.M., compared by ANOVA. Real-time PCR showed that PDE1A and PDE1C increase in response to serotonin (5-HT, 10 μ M, 24 hr, 4.2 ± 0.3 and 2.9 ± 0.2 fold-increase respectively, $n=3$, $p<0.01$) and hypoxia (72 hours 1% O₂, 7.6 ± 0.9 and 59 ± 11 respectively, $n=3$, $p<0.05$) and are higher expressed in female- PASMC compared to male-PASMC (3.5 ± 1.4 and 3.6 ± 0.6 fold-increase in female vs. male-PASMC respectively, $n=3$, $p<0.05$). Of interest, increased PDE1C protein expression (western blot) and translocation to the nucleus correlated with the proliferation of PASMC, whereas arresting PASMC in the G1 phase of the cell cycle inhibited PDE1C expression. Inhibiting PDE1 activity, with 16K (10 μ M), decreased the proliferation (MTS assay) of normoxic and hypoxic PASMC ($14\% \pm 2.9$ and $21\% \pm 2.4$ decrease respectively, $n=3$, $p<0.05$) and decreased the expression of PCNA ($n=3$). 16K increased cAMP levels (151 ± 8.6 pmol/mg protein for forskolin vs. 224 ± 16.8 pmol/mg protein for forskolin + 16K, $n=3$, $p<0.01$, cAMP ELISA assay) and phospho-VASP in PASMC ($n=3$). In rat pulmonary artery precontracted with phenylephrine, PDE1 inhibition (1nM-10 μ M) induced a concentration-dependent relaxation, which was independent of an intact endothelium (control $25.3 \pm 5.2\%$, $n=5$ vs. endothelium-denuded $20.1 \pm 9.2\%$, $n=6$, performed in accordance with the United Kingdom Home Office regulations and approved by Aberdeen University Ethics Committee). Taken together, these data show that the increased expression of PDE1 isoforms in PAH could be attributed to multiple risk factors and that selective PDE1 inhibitors could be novel drugs for the disease.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Soluble fms-like Tyrosine Kinase 1 (sFlt1) is Downregulated in Aortic Valve Stenosis, Promoting Intravalvular Neovascularisation

C.T. Lewis¹, H.M. Wilson¹, K.M. Kerr² and G. Nixon¹

¹Aberdeen Cardiovascular and Diabetes Centre, University of Aberdeen, Aberdeen, UK and ²Department of Pathology, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, University of Aberdeen, Aberdeen, UK

Aortic valve stenosis (AVS) is the progressive calcification and loss-of-function of the aortic valve, leading to heart failure, left-ventricular hypertrophy and ultimately death. Despite affecting 3% of the population over 65 years there exists no drug treatment for AVS, leaving invasive surgery as the only option. Critical to the progression of AVS is the neovascularisation of the aortic valve leaflets. This allows for the infiltration of immune cells, such as macrophage and monocytes, into the valve tissue resulting in an inflammatory and proliferative response. The resultant tissue hypoxia stimulates angiogenesis and AVS disease progression. Inhibiting angiogenesis is therefore an attractive potential target for the treatment of AVS. The aim of this study was to determine the role of angiogenesis mediators during aortic valve neovascularisation and the progression of AVS

Human aortic valve tissue was obtained by informed consent from patients undergoing aortic valve replacement surgery at Aberdeen Royal Infirmary. *In vitro* techniques were performed using isolated and cultured primary human aortic valve interstitial cells (VICs). Immunohistochemistry of CD31 positive endothelial cells within human aortic valves showed that diseased aortic valves had significantly increased intravalvular vessel number in comparison to healthy aortic valves (results expressed as mean $\pm$ SEM; control - 3.93 ± 1.25 vessels/mm² versus diseased - 14.93 ± 2.69 vessels/mm², $p < 0.01$, $n=10$).

Soluble fms-like tyrosine kinase 1 (sFlt1) is a soluble isoform of the VEGF Receptor 1 which acts to inhibit angiogenesis by sequestering VEGF and thereby preventing signalling via the membrane-bound VEGF receptors. Hypoxia (induced by incubation in a hypoxic chamber at 1% O₂, 5% CO₂), which is observed within valve tissue after the onset of AVS progression, reduced the protein expression of sFlt1 (results expressed mean $\pm$ SEM; 0.54 ± 0.11 fold decrease in sFlt1 protein expression vs normoxic control after 24hr hypoxia incubation, $p < 0.05$, $n=6$)

siRNA knockdown of sFlt1 on cultured VICs indicate that loss of sFlt1 promotes angiogenesis *in vitro* using a HUVEC based assay measuring endothelial tubule formation (results expressed mean $\pm$ SEM; $31.3\% \pm 13.6\%$ increase in tubule formation in sFlt1 siRNA knockdown compared to control siRNA, $p < 0.05$, $n=6$). Together this demonstrates that hypoxia-induced modulation of pro- and anti-angiogenic factors is able to promote angiogenesis in aortic valves.

In summary, this data suggests that sFlt1 is an important factor in maintaining aortic valve avascularity. We have also shown that sFlt-1 reduction is caused by the pathologically relevant hypoxic changes which occur during the progression

of AVS. Together this work highlights the potential of sFlt1 as a therapeutic target in the treatment of AVS via the prevention of intravalvular neovascularisation.

The authors would like to acknowledge Mrs Joan Wilson and Miss Kristine Nellany at the NHS Grampian Biorepository for their expertise and assistance.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C120

Sex differences in basal cerebral perfusion: contribution of nitric oxide

A. Ward¹, K.J. Carter¹, O. Wieben^{2,3}, M.W. Eldridge⁴, S.A. Hagen⁴, B.J. Walker⁵, J.W. Lee⁵, A.M. Al-Subu⁴ and W.G. Schrage¹

¹Kinesiology, University of Wisconsin-Madison, Madison, WI, USA, ²Medical Physics, University of Wisconsin-Madison, Madison, WI, USA, ³Radiology, University of Wisconsin-Madison, Madison, WI, USA, ⁴Pediatrics, University of Wisconsin-Madison, Madison, WI, USA and ⁵Anesthesiology, University of Wisconsin-Madison, Madison, WI, USA

Females demonstrate higher cerebral perfusion compared to males; however, the mechanisms for this difference remain unknown. The role of estrogen has been implicated in the upregulation endothelial nitric oxide synthase (NOS) in animal models and speculated in humans. Therefore, it was hypothesized that females exhibit greater NO contribution to basal cerebral perfusion compared to males. Healthy females (n=6) and males (n=6) underwent quantitative MRI cerebrovascular assessment during intravenous infusion of saline and the NOS inhibitor L-NMMA (5mg/kg/min) in a randomized, single-blind design. Pseudo-continuous arterial spin labeling magnetic resonance imaging (MRI) was used to quantify microvascular perfusion which was normalized to gray matter volume. Heart rate, mean arterial pressure, and end-tidal CO₂ were measured during study procedures. Data were analyzed via two-way repeated measures ANOVA (Sex x Condition) and by unpaired *t*-test as appropriate. Data are mean ± SD and significance was set at *p*<0.05. Females and males did not differ in age (23±2 vs. 22±2 years, respectively; *p*>0.99) or BMI (22±1 vs. 22±2 kg/m², respectively; *p*=0.85). There were no differences between groups or conditions for heart rate (Females 59±4 vs. Males 56±7 bpm, sex *p*=0.29; Saline 58±5 vs. L-NMMA 57±7 bpm, condition *p*=0.77), mean arterial pressure (Females 85±6 vs Males 85±6 mmHg, sex *p*=0.91; Saline 86±7 vs 84±4 mmHg, condition *p*=0.42), and end-tidal CO₂ (Females 35±2 vs. Males 37±3 mmHg, sex *p*=0.16; Saline 36±3 vs. L-NMMA 36±2 mmHg, condition *p*=0.65). Gray matter volume did not differ between sexes (Females 790±49 vs Males 830±98 mL; *p*=0.34). Females displayed approximately 20% greater global cerebral perfusion compared to males (43±6 vs 35±4 mL/100g/min, respectively; sex *p*=0.01). L-NMMA reduced perfusion compared to saline infusion (Saline 41±5 vs LNMMA 38±8 mL/100g/min; condition *p*=0.02). The NOS-mediated change in perfusion did not differ between sexes (Females: -10±12 vs Males: -4±5%, respectively; *p*=0.145).

Regional analyses of the frontal lobe, parietal lobe, temporal lobe, occipital lobe, and cerebellum revealed similar patterns of sex differences and L-NMMA effects. These data support previous findings that females exhibit greater cerebral perfusion compared to males. Additionally, NO appears to contribute to basal cerebral perfusion. These data also suggest NO-independent pathways are likely to exist that may explain sex differences in basal cerebral perfusion.

Support provided by the American Diabetes Association grant ADA 1-16-ICTS-099.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C121

The muscarinic cation current in murine ileal myocytes (ml_{CAT}) inhibited by ketamine can be recovered by TRPC4 agonist (-)-englerin A

D.O. Dryn^{2,1}, M.I. Melnyk² and A.V. Zholos^{1,2}

¹*Biophysics and Medical Informatics, ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine and* ²*A.A. Bogomoletz Institute of Physiology, Kyiv, Ukraine*

Ketamine is one of the most widely-used "dissociative anesthetics". It has been primarily known as an NMDA receptor antagonist with a potent anesthetic effect, but it can also interact with many other receptors and ion channels, such as opioid receptors, nicotinic acetylcholine receptors, muscarinic receptors and voltage-gated Ca^{2+} channels. Since the use of general anesthesia during surgical procedures is associated with multiple side effects including postoperative ileus due to inhibition of intestinal motility, we aimed to investigate the effects of ketamine on the muscarinic cation current termed ml_{CAT} , which underlies cholinergic excitation-contraction coupling of visceral smooth muscles. ml_{CAT} is mainly mediated by TRPC4 channels, which are activated in synergy by two muscarinic receptor subtypes, M_2 and M_3 [1, 2]. Experiments were performed on single ileal myocytes freshly isolated using collagenase from the longitudinal layer of the mouse ileum dissected from BALB/c two month-old male mice. ml_{CAT} was recorded in the whole-cell configuration of the patch-clamp techniques at room temperature (22-25 °C) using symmetrical Cs^+ containing (125 mM) solutions with $[Ca^{2+}]_i$ 'clamped' at 100 nM (10 mM BAPTA/4.6 mM $CaCl_2$ mixture). Values are means $\pm$ S.E.M., differences evaluated using Student's *t*-test. Ketamine (100 μ M, clinical concentration) suppressed ml_{CAT} induced by carbachol (CCh, 50 μ M) by $62.4 \pm 2.6\%$ within 3 min. Thus, maximal current densities at -40 mV were -15.1 ± 3.3 pA/pF in control vs. -5.5 ± 0.9 pA/pF in the presence of 100 μ M ketamine ($n=6$, $p<0.05$). The IC_{50} value was estimated at 3.4 μ M. The inhibitory effect was practically irreversible after ketamine wash-out. Remarkably, ketamine-suppressed ml_{CAT} could be completely recovered after application of TRPC4 agonist (-)-englerin A. In these experiments, amplitude of normalized ml_{CAT} activated by 50 μ M CCh at -40 mV was -13.0 ± 2.9

pA/pF in control, it decreased to -7.1 ± 1.5 pA/pF in the presence of ketamine, but increased to -35.7 ± 8.3 pA/pF after 10 nM (-)-englerin A application ($n=5$; $p<0.01$). These data define TRPC4 cation channel and signalling pathways leading to its activation during cholinergic excitation of intestinal myocytes as novel important targets for the inhibitory action of ketamine, while offering a strategy for current recovery by TRPC4 agonist (-)-englerin A.

Zholos AV & Bolton TB (1997). *Br J Pharmacol* 122, 885-893.

Tsvilovskyy VV *et al.* (2009). *Gastroenterol* 137, 1415-1424.

Supported by the Ministry of Education and Science of Ukraine (19BF036-01)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C123

Junctophilin-2 is Necessary for the Formation of Peripheral Ca^{2+} Signalling Domains in Contractile Vascular Smooth Muscle Cells

H.A. Pritchard¹, E. Yamasaki², P. Thakore², C. Griffin², C. Lane², M. Nelson^{3,1}, A. Greenstein¹ and S. Earley²

¹*Division of Cardiovascular Sciences, University of Manchester, Manchester, UK,*

²*Department of Pharmacology, University of Nevada, Reno, Reno, NV, USA and*

³*Department of Pharmacology, University of Vermont, Burlington, VT, USA*

Junctophilin-2 (JP2) is a structural protein that tethers the sarcoplasmic reticulum (SR) to the plasma membrane (PM) within the cardiac dyad, enabling crosstalk between ion channels on the PM and SR, required for excitation-contraction coupling. Contractile regulation of vascular smooth muscle cells (SMCs) also relies on close interactions between the PM and SR, but little is known about the molecular architecture of these peripheral coupling sites (PCS). This study tested the hypothesis that JP2 is critical for maintaining interactions between the SR and PM. C57/BL6 mice were euthanized according to approved protocols by the IACUC of the University of Nevada, Reno, and the UK Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. We found that mRNA encoding JP2 was present in native, contractile SMCs and JP2 protein expression using the Wes capillary electrophoresis-based immunoassay ($N=3$). Confocal and super-resolution microscopy showed co-localization of JP2 and type 2 ryanodine receptors (RyR2) in immunolabeled SMCs ($n=9$ cells, 3 animals). In cerebral artery SMC, RyR2 on the SR are functionally coupled with Ca^{2+} -activated K^+ (BK) channels on the PM, a negative feedback system that buffers vasoconstriction. We employed a molecular interference approach using selective morpholinos to knockdown JP2 expression in isolated cerebral pial arteries. JP2 expression was reduced by ~50% compared with the non-targeting control ($N=3$). 3D live cell deconvolved confocal imaging of the PCS was undertaken using the PM dye (CellMask) and the SR dye (ER-Tracker). SMCs

isolated from JP2 targeting morpholinos treated vessels showed a reduction in the volume of PCS compared to SMCs isolated from control vessels ($0.73 \pm 0.2\%$ vs. $1.84 \pm 0.5\%$, $n=9-17$ cells, 3 animals/group). Ca^{2+} release from RyR2 were recorded in pressurised arteries, treated with associated morpholino. Knockdown of JP2 did not affect the frequency, amplitude or kinetics of these Ca^{2+} events ($n=5-6$ vessels, 3 animals/group). Electrophysiology was performed to record spontaneous transient outward currents (STOCs), which represents the activation of BK channels by Ca^{2+} released from RyR2. These studies found that STOCs were absent from SMCs isolated from JP2-targeted morpholinos vessels, whereas STOCs in SMCs from control vessels were unaffected (0.17 ± 0.12 Hz vs. 1.48 ± 0.31 Hz, at -20 mV, $n=5-6$ cells, 3 animals/group). Finally, the myogenic response was tested between JP2 knockdown and control vessels. JP2 morpholino treated vessels displayed an increased myogenic tone compared to the non-targeting controls ($23.19 \pm 2.28\%$ vs. $14.16 \pm 2.4\%$, at 80 mmHg, $n=5-6$ vessels, 3 animals/group). This study is the first to show the presence of JP2 in contractile arterial SMCs and its role in maintaining functional coupling between RyR2 and BK channels.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C125

Restricted Dietary Intake of Zinc Modulates Parameters of Haemostasis

U. Sumya¹, J. Beattie² and N. Mutch¹

¹School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK and ²The Rowett Institute, Aberdeen, UK

Background

Zinc is an important cofactor in many biological reactions. Zinc is proposed to be endocytosed from plasma into platelets, with pools within the cytoplasm and α -granules, which are subsequently released upon platelet activation. Several studies have shown a direct impact of *ex vivo* manipulation of zinc on platelet aggregation and other haemostatic parameters, including fibrinolysis. The bioavailability of zinc in the body entirely depends on dietary intake, despite the impact of zinc deficiency on haemostatic parameters has not been studied.

Aims

To investigate how changes in the bioavailability of dietary zinc impacts on haemostatic function.

Methods

Healthy volunteers aged above 18 years were recruited and their zinc status on habitual diet recorded. Dietary zinc intake was then significantly restricted for 14 days (1 mg/day), followed by a repletion period for 14 days at various zinc concentrations (4.5-18 mg/day). Blood was collected from the habitual, depletion and repletion phases and platelet rich plasma (PRP) was isolated and haemostatic parameters assessed as detailed below.

Results

Maximal platelet aggregation induced by collagen was significantly decreased in the depletion phase ($45.8 \pm 23.2\%$) compared to habitual diet phase ($95.6 \pm 11.2\%$). Lagtime to thrombin generation during zinc restriction significantly increased (13.6 ± 4 min) compared to habitual phase (18.1 ± 3.2 min). Visualisation of real-time clot formation and lysis were observed using a Hemacore Thrombodynamics Analyzer. Rate of clot growth was significantly slower in depletion phase (33.5 ± 12.2 $\mu\text{m}/\text{min}$) compared to the rate in habitual phase (49.8 ± 11.1 $\mu\text{m}/\text{min}$). Clots formed from PRP during depletion phase showed defective clot retraction (final clot weight 486.2 ± 127.2 mg) compared to the habitual period (403.6 ± 77.8 mg). Fibrinolysis of PRP clots by tissue plasminogen activator (tPA) formed from the restricted phase in an absorbance based assay was delayed (100.6 ± 28.2 min) compared to the habitual phase (83.7 ± 24.5 min).

Summary/Conclusions

Around one-third of the world population currently living under zinc deficiency. Our data indicate that changes in dietary zinc impact its bioavailability and uptake by platelets. Restricted levels of zinc in platelets causes an attenuation in platelet aggregation, slower thrombin generation and clot formation, and delays fibrinolysis. Thus, highlighting how people living with zinc deficiency can experience defective haemostasis due to imbalance in these parameters.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

C126

The antiepileptic agents phenytoin and valproate inhibits oxytocin-induced contractions of myometrium isolated from absence epileptic WAG/Rij rats

A. Kurt, A.K. Salihoglu and A. Ayar

Physiology, Karadeniz Technical University Faculty of Medicine, Trabzon, Turkey

There has been concern about safety of antiepileptic therapy during pregnancy including neurodevelopmental impairments in fetus, possible effects of antiepileptics on natural birth process is less clear. The aim of this study was to investigate possible effects of phenytoin and valproate on oxytocin-induced contractions of absence epileptic WAG/Rij rats. Myometrial strips were isolated from non-pregnant female rats and placed in an organ bath continually bubbled with oxygen and CO₂, at 37 °C, pH 7.4. After equilibrium under 1 g of resting tension, contractions were stimulated by bath application of oxytocin and, effects of cumulatively applied phenytoin or valproate on area under the curve (AUC, normalized data) and frequency of contractions (number of contractions/10 min) were evaluated by 10-minute periods. Application of valproate caused a dose-dependent inhibition of AUC: 1 ± 0 ; 0.94 ± 0.1 ($p > 0.05$), 0.82 ± 0.1 ($p < 0.05$), 0.61 ± 0.18 ($p < 0.05$) and 0.38 ± 0.21 ($p < 0.05$) oxytocin; 100, 300 μM , 1 and 3 mM valproate, respectively ($n=9$ for each

dose). The frequency of oxytocin-induced contractions was $15,9 \pm 1,05$ and reduced to $13,3 \pm 1,9$, $11, 7 \pm 2,2$, $9,6 \pm 3,0$ and $6,3 \pm 4,3$, after respective doses of valproate, ($p < 0.05$, $n=9$, for all). Phenytoin also caused inhibition of AUC values oxytocin-induced contractions: 1 ± 0 ; $0,87 \pm 0,08$, $0,78 \pm 0,15$, $0,33 \pm 0,21$, oxytocin; 10, 30 μ M and 1 mM, respectively ($p < 0.05$ for all tested concentrations, $n=8$ for each). The effects of phenytoin on the mean frequency of oxytocin-induced contractions were significantly inhibited by all the tested concentrations. Although we tested higher concentrations of these agents, considering the increased antiepileptic drug clearance during pregnancy the need for higher doses may be relevant for the tested doses of these anti-epileptics and our findings may be of importance.

Eadie M. J., 2016. Antiepileptic drug safety in pregnancy: possible dangers for the pregnant woman and her fetus. Clinical pharmacist;10.1211/CP.2016.20200320.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC001

Effect of cigarette smoking on the blood pressure, pulse pressure and resting pulse rate in young adults

R.M. Musa¹, L. Kaddam¹, A. Elneel² and H. humeda³

¹physiology, AL-Neelain university, Khartoum, Sudan, ²AL-Neelain university Faculty of medicine, Khartoum, Sudan and ³physiology, International University of Africa, Khartoum, Sudan

Introduction: Cigarette smoking is a major cause of morbidity. Smokers are exposed to about 7,000 chemicals when they inhale cigarette smoke. The damaging effect of smoking involve many organs including the cardiovascular system.

Objective: To evaluate the effect of smoking on the blood pressure, pulse pressure and resting pulse rate among young healthy Males

Methodology: This was a cross sectional study carried out in AL-Neelain university faculty of medicine. The sample involve 65 students who were volunteered to participate they were of 31 smokers and 34 non-smokers.

The data was collected using a self-administered questionnaires and the blood pressure was measured using stander mercury sphygmomanometer and the resting pulse rate was measured using the pulse oximeter.

Results: The result demonstrate a statistically significant difference in the blood pressure reading of smokers when compared to non- smokers (p value of 0.01).

The smokers had higher resting pulse rate compared to non-smokers but the different was not found to be statistically significant (p value of 0.2).

Smokers had a higher pulse pressure compare to non-smokers but the different wasn't statistically significant (p value 0.73).

And when smokers were divided according to the smoking index into light, moderate and heavy smokers no statistically significant difference was detected in the blood pressure, resting pulse rate and pulse pressure among smokers compared to non-smokers (p values 0.8and 0.5) respectively.

Conclusion blood pressure readings among smokers was mainly in two extremes (high blood pressure extreme, low blood pressure extreme) compared to non-smokers.

Smokers had higher pulse rate and pulse pressure compare to non-smokers.

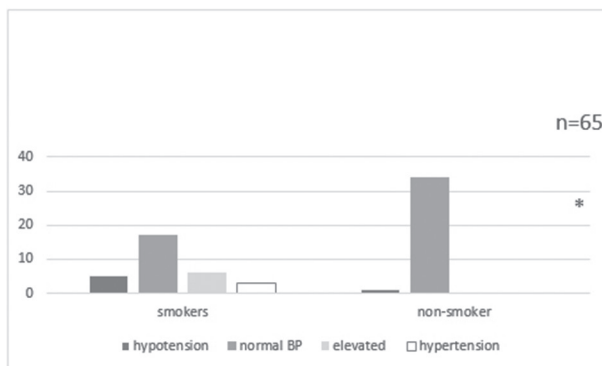


Figure I: Blood pressure among smokers and non-smokes

Parameters	Smokers (n=31)	Non-smokers (n=34)	P value
Age (years)	18-25	18-25	---
Gender	Male	Male	----
Height (m)	1.7 ± 6.39	1.748 ± 5.89	0.320
Weight (kg)	69.29 ± 15.8	70.72 ± 16.45	0.831
Cigarette smoking day-1	7.7 ± 7.2		----
Chronic illness	Not found	Not found	---
Fasting blood glucose (mg/dl)	103±14.3	98±17.0	0.67
Oxygen saturation (percentage)	97.29±1.7	97.25±1.5	0.111
	Mean ± SD		

WHO | Tobacco. WHO [Internet]. 2016; Available from: <http://www.who.int/mediacentre/factsheets/fs339/en/>

Neaton JD, Wentworth D. Serum Cholesterol, Blood Pressure, Cigarette Smoking, and Death From Coronary Heart Disease Overall Findings and Differences by Age for 316099 White Men. Arch Intern Med [Internet]. 1992 Jan 1;152(1):56. Available from: <http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.1992.00400130082009>

Gidding SS, Xie X, Liu K, Manolio T, Flack JM, Gardin JM. Cardiac function in smokers and nonsmokers: The CARDIA study. J Am Coll Cardiol [Internet]. 1995 [cited 2017 Nov 14];26(1). Available from: <http://www.onlinejacc.org/content/26/1/211>

The authors would like to thank the participants at the Al-Neelain University for their cooperation throughout the study. Special thanks for our respectful students Faris Jamal Aldeen, Razaz Musa and Mohammed Yasin at the faculty of medicine who support this research and participate effectively in data collection, Also we would like to thanks our colleagues in the physiology department Tasneem El mekki and tasabeih alnoor who participate effectively in the data collection

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC002

Acute cardiovascular responses to slow and deep breathing in healthy women

M. Felton, V. Hundley and A. McConnell

Bournemouth University, Poole, UK

Background: Daily practice of device-guided slow breathing (DSB) has been shown to decrease blood pressure (Chaddha et al., 2019). However, there is still a lack of understanding of the mechanisms underpinning the improvements in blood pressure. Relatively few studies have characterised the acute cardiovascular responses to DSB, which hold the key to the mechanisms by which DSB might lower blood pressure. This study characterised the acute cardiovascular responses to different DSB protocols. **Methods:** Eighteen healthy, normotensive women completed five 5-minute protocols in randomised order: spontaneous breathing (Sf_R), fixed breathing frequencies of 4, 6 and 8 breaths·min⁻¹ ($4f_R$, $6f_R$, $8f_R$) and a dynamic breathing frequency (Df_R) determined by an algorithm designed to maximise respiratory sinus arrhythmia (RSA). Cardiovascular variables and respiratory airflow were monitored continuously and non-invasively. Data are mean±SD, compared by repeated measures ANOVA with planned pairwise comparisons. **Results:** Average breathing frequency for Df_R was 6.3 ± 1.1 breaths·min⁻¹. Mean heart rate was not significantly different between breathing protocols, but RSA increased significantly between Sf_R and all DSB protocols (Sf_R 0.12 ± 0.05 , $4f_R$ 0.25 ± 0.10 , $6f_R$ 0.25 ± 0.08 , $8f_R$ 0.21 ± 0.07 , Df_R 0.25 ± 0.09 sec; $p < 0.001$). The 'peak-valley' amplitude of intra-breath (inhalation vs exhalation) fluctuations of mean arterial pressure (MAP) were significantly different between $4f_R$ and Sf_R , $6f_R$, $8f_R$ and Df_R (Sf_R 5.1 ± 1.87 , $4f_R$ 7.8 ± 11.81 , $6f_R$ 6.8 ± 10.81 , $8f_R$ 9.1 ± 2.38 , Df_R 4.8 ± 11.30 mmHg; $p < 0.001$). Peak MAP occurred during expiration for all protocols except $4f_R$. Intra-breath fluctuations also increased during DSB for stroke volume, cardiac output, systolic and diastolic blood pressure, and pulse wave velocity ($p < 0.001$). **Conclusions:** DSB induces significant increases in intra-breath fluctuations of haemodynamic variables. It is conceivable that these acute haemodynamic perturbations generate error signal(s) for chronic regulation of blood pressure. Further research is required to understand the acute responses to DSB in people who have hypertension.

Chaddha, A., Modaff, D., Hooper-Lane, C. and Feldstein, D. A. (2019). Device and Non-Device-Guided Slow Breathing to Reduce Blood Pressure: A Systematic Review and Meta-Analysis. *Complementary Therapies in Medicine* [online], In Press. Available from: <https://www.sciencedirect.com/science/article/pii/S0965229918304242> [Accessed 15 April 2019].

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC003

Positive association between initial augmentation of cardiovagal baroreflex sensitivity and onset of arterial pressure decline during passive hyperthermia

E. Ghadhanfar and M. Turcani

Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait, Kuwait

Even in virtually identical conditions, tolerance of hot environment is greatly variable in mammals, including humans. Understanding the mechanisms of this variability may help prevent negative consequences of prolonged hyperthermia. We tested the hypothesis that changes in spontaneous cardiovagal baroreflex sensitivity (cBRS) could be related to hypotension development during passive hyperthermia.

Anesthetized (120 mg.kg⁻¹ ketamine & 6 mg.kg⁻¹ xylazine i.p.) male Wistar-Kyoto rats (12-week old, n = 10) were implanted with telemetric transmitters (HD-S11, Data Sciences, USA) to monitor aortic pressure, ECG, core body temperature (T_c) and locomotor activity. Animals were exposed to hot air (44°C, humidity 13%) in a climatic chamber 1 month after implantation surgery (1). Rats in this hot environment feel discomfort but not pain (2). The transfer function between systolic pressure and R-R interval changes in the low-frequency band (0.2–0.7 Hz) was used to estimate cBRS in the 10-s windows, moved by 1-s steps.

Exposure to hot air immediately, before any change in T_c, doubled cBRS from 1.3 (0.3) ms.mmHg⁻¹ to 3.0 (1.4) ms.mmHg⁻¹, p<0.001 (pairwise comparison with Bonferroni test after repeated measure ANOVA with Greenhouse-Geisser correction; numbers in brackets are standard deviations). High frequency power of heart rate variability, a surrogate measure of vagal drive to the heart, increased simultaneously from 3.9 (1.7) ms² to 47.3 (17.9) ms², p<0.001. The period of high cBRS values ended at a T_c of 38.8 (0.6) °C. During further elevation of T_c, cBRS receded from its maximal values but remained above the reference baseline cBRS. T_c of 40.9 (0.4) °C marked the beginning of the phase with below-baseline cBRS. Cardiovagal baroreflex desensitization was accelerated with increasing T_c, and above a T_c of 42.1 (0.4) °C, the baroreflex was essentially non-operative in heart rate control. Despite progressive desensitization of cardiovagal baroreflex, arterial pressure continuously increased and started to decline only when T_c reached 42.5 (0.3) °C. Thus, it is not likely that hypotension of heatstroke is caused by cardiovagal baroreflex failure. However, we found a positive correlation between the maximal cBRS reached during the initial period of exposure to hot air and the time to onset of arterial pressure decline, r² = 0.5713, p = 0.01144, i.e. the degree of initial baroreflex sensitization explains 57% of the variance in the onset of circulatory failure. In conclusion, exposure of rats to hot air transiently sensitizes cardiovagal baroreflex, probably by increased vagal activity. The strong initial increase in cBRS was associated with delayed appearance of arterial pressure decline suggesting that parasympathomimetic intervention may improve thermotolerance.

Turcani M & Ghadhanfar E (2019). *Scientific reports* 9, 2586.

Sharma HS & Johanson CE (2007). *Prog Brain Res* 162, 459–478

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC004

Divergent effects of moxonidine, clonidine, and metoprolol on the fractal pattern of interbeat interval dynamics

M. Turcani and E. Ghadhanfar

Department of Physiology, Faculty of Medicine, Kuwait University, Kuwait, Kuwait

Progressive increase in sympathetic (SNS) nervous activity and reduction in parasympathetic (PNS) nervous activity may directly contribute to the inevitable lethal outcome of heart failure (HF) syndrome. Partial reduction of augmented SNS activity with β -adrenergic blockade prolongs survival of HF patients. However, this intervention leaves α -adrenergic overstimulation untouched and sympathetic vasoactive adrenergic co-transmitters elevated. Central sympatholysis, which blocks the whole SNS, was postulated as a more efficient approach to limit sympathetic overactivity in HF patients. Unexpectedly, moxonidine (I1-imidazoline receptor agonist) elevated HF mortality. Because alteration of fractal organization of interbeat interval (IBI) variability is associated with raised mortality (1,2), we hypothesized that differences in the effects of central sympatholytics (moxonidine & clonidine), and β 1-adrenergic blocker (metoprolol) on fractal scaling coefficients may provide a clue to understanding the mechanism of moxonidine failure in the HF treatment trial.

During general anesthesia (120 mg.kg⁻¹ ketamine & 6 mg.kg⁻¹ xylazine i.p.), 12-week old male Wistar rats (n = 8) were implanted with telemetric transmitters (HD-S11, Data Sciences, USA). Implants allowed simultaneous monitoring of aortic pressure, ECG, core body temperature, and locomotor activity. Detrended fluctuation analysis was used to assess short-term (α 1) and long term (α 2) scaling coefficients, which characterize the fractal organization of IBI variability. Six increasing doses of central sympatholytics (0 – 12.15 μ mol.kg⁻¹) and metoprolol (0 – 291.1 μ mol.kg⁻¹) were applied s.c.

Moxonidine dose-dependently reduced the α 1 coefficient; IBI intervals became uncorrelated at the highest dose (0.528 (0.116), $p < 0.001$; Tukey HSD after multivariate (Wilks) repeated measure ANOVA; data are mean, S.D. in brackets), which reflects strong PNS activation and SNS inhibition. Sympatholytic effect of high doses of moxonidine was confirmed by the attenuation of the Brownian component in IBI fluctuations (α 2=1.038 (0.118), $p = 0.006$ at 12.15 μ mol.kg⁻¹). Clonidine reduced the α 2 coefficient at all doses making IBI fluctuation less correlated at long scales also (0.657 (0.159), $p < 0.001$), which confirms sympatholytic effects of clonidine. Short-term α 1 coefficient reached white noise values (0.563 (0.1), $p = 0.0005$) at a concentration of 1.35 μ mol.kg⁻¹. In agreement with its β 1-adrenergic blocking

action, metoprolol had no effect on the $\alpha 1$ coefficient, but weakened the Brownian component of IBI fluctuations and brought long-term IBI correlations to 1/f noise (1.059 (0.069), $p=0.002$).

In conclusion, moxonidine and clonidine, but not metoprolol, dose-dependently activate PNS and inhibit SNS, which shift fractal organization of IBI time series toward white noise thus potentially increasing the mortality risk.

Sen J & McGill D (2018). *Chaos* 28, 072101.

Ho KK Moody GB Peng CK Mietus JE Larson MG Levy D Goldberger AL (1997) *Circulation* 96, 842-848

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC005

Rats with heart failure show increased gut-to-blood penetration of trimethylamine, a gut bacterial metabolite.

M. Konop, A. Drapala, K. Jaworska, T. Hutsch, K. Bielinska, M. Gawrys-Kopczynska and M. Ufnal

Department of Experimental Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland

The gut-blood barrier (GBB) controls the passage of nutrients and bacterial metabolites from the intestinal lumen to the bloodstream. Several studies suggest that trimethylamine N-oxide (TMAO), a liver metabolite of gut bacteria-produced trimethylamine (TMA), is a marker of cardiovascular risk. However, the mechanisms of the increase are not clear. The aim of the study was to evaluate the effect of heart failure (HF) on plasma level of TMA and TMAO in rats.

The study was performed on 58-week-old Spontaneously Hypertensive Heart Failure rats (SHHF, $n=9$) and Wistar-Kyoto rats (WKY, $n=7$). Firstly, water-electrolyte and energy balance was evaluated. Subsequently, rats were anesthetized with urethane (1.5 g/kg b.w. i.p.) and underwent echocardiography. Next, the left femoral artery was cannulated for arterial blood pressure measurement, and standard needle electrodes for ECG recordings were connected (Biopac MP160 system). For a direct evaluation of left ventricle pressure, Millar Mikro-Tip SPR-320 (2F) pressure catheter was used. After the hemodynamic recordings, blood from the right ventricle of the heart was taken for biochemical analysis and rats were killed by decapitation. Tissues and the colon content (stools) were harvested for histological and biochemical analysis, respectively.

There was no significant difference in body weight and food intake between WKY and SHHF. There was a trend towards higher water intake in SHHF than in WKY. WKY showed no pathological changes in the circulatory system. In contrast, SHHF showed hypertrophic cardiomyopathy with histopathological picture of transition from hypertrophic to dilated cardiomyopathy, reduced systolic function and

diastolic functions. Specifically, SHHF had a significantly increased heart mass (80% increase), increased diameters of intraventricular septum and posterior heart wall, decreased stroke volume and ejection fraction and increased $+dp/dt$ and $-dp/dt$. Moreover, SHHF had increased plasma NT-proBNP, increased plasma sodium and urea, decreased sodium excretion, significantly increased plasma TNF- α and vasopressin levels. Finally, in comparison to WKY, SHHF showed a significantly higher blood plasma level (mean $\pm$ SE, μ M) of TMA (81.04 $\pm$ 5.19 vs 152.97 $\pm$ 20.26) and TMAO (4.57 $\pm$ 0.39 vs 6.70 $\pm$ 0.68). There was no significant difference in stool concentration of TMA between WKY (273.65 $\pm$ 23.11) and SHHF (284.81 $\pm$ 16.64). However, SHHF showed a significantly higher blood-to-stool ratio of TMA concentration (0.56 $\pm$ 0.11 vs 0.30 $\pm$ 0.03) suggesting a greater gut-to-blood penetration of TMA. This was accompanied by a significantly decreased intestinal blood flow and significantly smaller thickness of the colonic mucosa in SHHF rats.

In conclusion, rats with HF show increased plasma level of TMA and TMAO, which may results from increased gut-to-blood penetration of TMA. Therefore, we would speculate that increased plasma TMAO level in cardioavascular diseases is due in part to alterations in the GBB.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC006

Amelioration of N^o-nitro-L-arginine methyl ester (L-NAME) Induced Hypertension and Cardio-renal Oxidative Stress in Rat Model by Methanol Extract of *Morinda lucida* Leaf.

O.A. Adejumbi¹, S. Oladipo¹, T. Omobowale¹, A. Oyagbemi², A.A. Adedapo³ and M. Yakubu⁴

¹Department of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, ²Department of Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Nigeria, ³Department of Veterinary Pharmacology and Toxicology, University of Ibadan, Ibadan, Nigeria and ⁴Department of Environmental and Interdisciplinary Sciences, Texas Southern University, Houston, UK

The menace of hypertension in man and animal as a risk factor for most cardiovascular complications calls for effective and efficient management protocols. *Morinda lucida* (MOLU) is a medicinal plant with antioxidant potentials that is being used in the management of diseases in Nigeria.

We, therefore, investigated ameliorative effects of *M. lucida* on L-NAME induced hypertension, oxidative stress and cardio-renal complications

Sixty male rats (wistar strain) were used in this study, and they were randomly divided into six experimental groups with ten rats in each group. Group A served as the controls and they received distilled water only for the period of 21 days. Group B was given L-NAME only at 40mg/kg while groups C, D and E were given a

daily dose of L-NAME at 40 mg/kg and methanol root extract at respective dose of 100mg/kg, 200mg/kg and 400mg/kg for 21 days. Group F was given a daily dose of L-name+ Lisinopril (0.25mg/kg) orally for 21 days. L-name was given at 40mg/kg daily. Blood pressure measurements were recorded in conscious animals by tail cuff plethysmography. Biochemical assays were carried out on the serum, cardiac and renal tissues to assess the enzymatic and non-enzymatic antioxidant defense system. Histology was also done on cardiac and renal tissues.

Treatment with L-NAME caused a significant ($p < 0.05$) increase in blood pressure(bp); systolic (181.5 ± 26.93^a), diastolic (172.5 ± 25.09^a) and mean arterial blood pressure (mmHg) (175.25 ± 25.81^a) when compared with those of the control with systolic bp (110.3 ± 16.74), diastolic bp (90.3 ± 21.50) and mean arterial bp (160 ± 10.36). A significant ($P < 0.05$) increase was also observed in the markers of oxidative stress such as H₂O₂; B (49.1 ± 2.37^a) compared with A (30.4 ± 3.30) and Protein carbonyl (PC) B ($39,852.6 \pm 1160.71$) compared with A ($26,606.3 \pm 4732.97$), in cardiac tissues. Histology showed myocardial infarction and severe glomerular damage in group treated with L-NAME alone compared to the control.

Treatment with MOLU caused a significant ($P < 0.05$) decrease in group C in systolic bp (115.0 ± 20.02^b) diastolic bp (81.8 ± 27.89^b) and mean arterial bp ($92.5 \pm 25.01^{a,b}$) and reduction in markers of oxidative stress, H₂O₂ (29.9 ± 1.77^b) and PC ($25,631.0 \pm 343.67$) in the cardiac and renal tissue comparable with that of lisinopril (8.5 ± 2.62^b , 26 and $128.8 \pm 3681.37^{d,e}$ respectively for H₂O₂ and PC). The results obtained from this study showed that MOLU can modulate high blood pressure with associated end organ damage and cardio-renal oxidative stress in L-NAME induced hypertension.

Keywords: Hypertension, oxidative stress, *Morinda lucida* extracts, lisinopril.

Adeneye, A.A. (2013). Profile of *Morinda lucida* leaf fractions on blood glucose and lipids in normal and alloxan-induced Hyperglycaemic rats. *Journal of Pharmacogolia* 4 (5): 408-413.

Edeoga, H.O, Okwu, D.E, Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afri. J. Biotechnol.* 4 (7): 685-688.

Kaur, M., Rana, A.C. and Kumar, S. (2011). Induction of hypertension by various animal models. *International Journal of Pharmacy and Biological Sciences.* 3:335-340.

Zhou J, Xu G, Bai Z, Li K, Yan J, Li F, Ma S, Xu H, Huang K. (2015). Selenite exacerbates hepatic insulin resistance in mouse model of type 2 diabetes through oxidative stress- mediated JNK pathway. *Toxicol Appl Pharmacol* 289:409–418

We acknowledge and appreciate Oba Sansi Foundation for the financial support (Oba Sansi Young Academic staff Development Grant) for this study.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC007

Peripheral Capillary Oxygen Saturation, Mean Arterial Pressure and Pulse Rate Assessment in Young Shisha Smokers at Kano State, Nigeria.

B.I. Waziri, M.A. Yahaya and I. Sani

Department of Human Physiology, Bayero University Kano, Nigeria., Kano, Nigeria

Emerging evidences have shown that shisha (water pipe tobacco) smoking is associated with higher exposure to carbon monoxide and nicotine than cigarette.¹ Despite these evidences, the prevalence and popularity of shisha smoking are rapidly increasing among youth globally including developing countries like Nigeria.^{1,2} Basic assessment of peripheral capillary oxygen saturation (SPO2), mean arterial pressure (MAP) and pulse rate in young chronic smokers is therefore necessary. The study was conducted at Nassarawa local government area of Kano state, Nigeria. 103 young (15 – 30 years) shisha smokers who have been smoking for 3 years or more with at least 1 session of smoking per day and with BMI of 19 – 24 kg/m2 were recruited and classified as group 1 (study group). Likewise, 100 young nonsmokers matched for age, BMI and living in the same environment with group 1 members were recruited as controls (group 2). Participants with known history of cigarette smoking, cardiovascular and respiratory diseases were excluded. Ethical clearance and approval with reference number MOH/Off/797/T.I/527 was obtained from Kano State Ministry of Health prior to the commencement of the study. SPO2 and pulse rate of the participants were assessed at rest and values were taken from middle finger using pulse oximeter. Blood pressure measurement was done at the same time using sphygmomanometer and MAP was estimated as MAP = Diastolic pressure + 1/3 pulse pressure. Values were expressed as Mean ± S.E.M and Data were analyzed with IBM SPSS Statistics for Windows version 23.0. Results were compared between the groups using independent sample t test. P values < 0.05 were considered statistically significant. SPO2 was found to be significantly lower (P < 0.05) among group 1 members (91.98 ± 0.42 %) compared to group 2 (97.94 ± 0.18%). The MAP and pulse rate obtained from group 1 (100.15 ± 0.78 mmHg and 91.32 ± 0.83b/min) were significantly higher (P < 0.05) compared to group 2 with MAP and pulse rate of (92.44 ± 0.79 mmHg and 79.19 ± 1.18b/min respectively). The study implies that shisha smoking is associated with risk of hypoxia, hypertension and tachycardia by causing decrease in SPO2, increase in MAP and increase in pulse rate.

WHO Study Group on Tobacco Product

Regulation (TobReg). Advisory note. Waterpipe

tobacco smoking: health effects, research needs and recommended actions by regulators. Geneva: World Health Organization; 2015.

Maziak W, Taleb ZB, Bahelah R, et al. The global epidemiology of waterpipe smoking. Tob Control 2015 Mar;24 Suppl 1:i3-i12.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC008

Effects of beta-blocker treatment on ventilation in conscious rats before and after exposure to chronic intermittent hypoxia

A. Alzahrani^{1,2}, C.J. Ray¹, L. Cao¹, K. Brain¹, P. Kumar¹, A. Coney¹ and A.P. Holmes¹

¹*Institute of Clinical Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK and* ²*Respiratory Care Department, College of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia*

Obstructive sleep apnoea (OSA) patients have increased risk of cardiovascular and respiratory disease including hypertension, arrhythmia and breathing instability. A key feature of OSA is exposure to chronic intermittent hypoxia (CIH). Carotid body (CB) hyperactivity caused by CIH is central to the development of pathology. However, the mechanisms underpinning CB hyperactivity in response to CIH remain incompletely characterised. We hypothesise that the rise in serum catecholamines in response to CIH could be a key driver of CB hyperactivity.

This study examined the effect of chronic administration of a beta blocker (propranolol) on baseline and hypoxic ventilation, before and after exposure to CIH.

A total of 33 male Wistar rats (11-13 weeks) were split into four groups (Control (N) n=10, Propranolol (PN) n=8, Chronic Intermittent Hypoxia (CIH) n=7, CIH + Propranolol (CIHP) n=8). The CIH paradigm consisted of 8 cycles hr⁻¹, 8 hours day⁻¹, 5% O₂ nadir, for 3 weeks. Propranolol was added to the drinking water (40 mg/kg body weight/day). Ventilation was recorded using whole body plethysmography on conscious rats. The protocol consisted of 5 min baseline ventilation followed by 5 min hypoxia (10% O₂).

Propranolol modified the breathing pattern in normoxia and hypoxia by significantly increasing tidal volume (21% O₂: N 4.3±0.2 vs PN 5.3±0.2 ml/kg, P<0.05; 10% O₂: N 5.8±0.1 vs PN 7.0±0.3 ml/kg P<0.05) and reducing respiratory frequency (21% O₂: N 96±2 vs PN 80±3 bpm, P<0.05; 10% O₂: N 146±6 vs PN 128±5 bpm P=0.1), without altering overall minute ventilation. Propranolol did not significantly impact on the elevation in tidal volume, respiratory frequency or minute ventilation induced by hypoxia. Following CIH, there was an approximately 20% increase in minute ventilation in normoxia (P<0.05) and a trend towards an increase in hypoxia (P=0.09). CIH also significantly increased the inspiratory duty cycle and the Inspiratory:Expiratory time ratio. A similar effect of propranolol on breathing pattern was observed after CIH, as evidenced by a 30% increase in tidal volume and a 10% reduction in respiratory frequency without an overall change in minute ventilation. Following CIH, propranolol did not significantly impact on the elevation in tidal volume, respiratory frequency or minute ventilation induced by hypoxia. These results suggest that propranolol induces a change in the pattern of ventilation before and after CIH, characterised by a switch towards slower and deeper

breathing. This change in pattern is predicted to cause an increase in alveolar ventilation. A role for the CB in mediating these effects is questionable as the hypoxic ventilatory response was not significantly modified by propranolol before or after CIH. Future experiments are required to directly measure CB activity following propranolol treatment before and after CIH.

Holmes, A. P., Ray, C. J., Thompson, E. L., Alshehri, Z., Coney, A. M., & Kumar, P. (2018). Adrenaline activation of the carotid body: Key to CO₂ and pH homeostasis in hypoglycaemia and potential pathological implications in cardiovascular disease. *Respir Physiol Neurobiol*. doi:10.1016/j.resp.2018.05.008

Morgan, B. J., Adrian, R., Wang, Z. Y., Bates, M. L., & Dopp, J. M. (2016). Chronic intermittent hypoxia alters ventilatory and metabolic responses to acute hypoxia in rats. *J Appl Physiol* (1985), 120(10), 1186-1195. doi:10.1152/japplphysiol.00015.2016

Ray, C. J., Dow, B., Kumar, P., & Coney, A. M. (2015). Mild Chronic Intermittent Hypoxia in Wistar Rats Evokes Significant Cardiovascular Pathophysiology but No Overt Changes in Carotid Body-Mediated Respiratory Responses. *Adv Exp Med Biol*, 860, 245-254. doi:10.1007/978-3-319-18440-1_28

Zoccal, D. B., & Machado, B. H. (2010). Sympathetic overactivity coupled with active expiration in rats submitted to chronic intermittent hypoxia. *Respir Physiol Neurobiol*, 174(1-2), 98-101. doi:10.1016/j.resp.2010.08.011

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC009

Obstructive Sleep Apnoea reduces cardiac autonomic function in patients with Type 1 Diabetes

Z. Alshehri^{1,4}, A.A. Tahrani^{2,3}, M. Karamat³, Q. Altaf³, P. Kumar¹ and C.J. Ray¹

¹Institute of Clinical Sciences, University of Birmingham, Birmingham, UK, ²Institute of Metabolism and Systems, University of Birmingham, Birmingham, UK, ³Department of Diabetes and Endocrinology, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK and ⁴Respiratory Therapy Department, Taibah University, Medina, Saudi Arabia

Heart rate variability (HRV) is reduced in patients with Type 1 diabetes (T1D) ¹. Obstructive Sleep Apnoea (OSA) is also associated with alterations in HRV ². However, little is known about the impact of OSA on HRV in patients with T1D and therefore we aimed to investigate this further. Adult T1D patients without past medical history of OSA were recruited from a single secondary hospital in the UK. Apnoea-hypopnoea index (AHI) was measured using polygraphy. OSA was defined as an AHI ≥ 15 or an AHI 5-14.9 with excessive daytime sleepiness (based on Epworth Sleepiness Scale ≥ 11). Cardiac autonomic function (CAF) was assessed using the ANSAR ANX 3.0 system in response to deep breathing, Valsalva, and standing manoeuvres by spectral analysis of HRV (Low Frequency Area (LFa), Respiratory Frequency area (RFa) and the LFa/RFa ratios). 36 patients were included of

which 14 (37%) were OSA positive (6 males), and 22 (61%) were OSA negative (3 males). OSA positive mean (SD) age, diabetes duration, body mass index (BMI), and Hb1Ac were 46.5 (13.6) years, 34.8 (12.0), years, 29.1 (5.8) kg/m², and 8.7% (1.5%). OSA negative mean (SD) age, diabetes duration, body mass index (BMI), and Hb1Ac were 49.5 (11.8) years, 31.5 (13.4) years, 25.1 (2.9) kg/m², and 7.8% (0.9%) respectively. Baseline LFa and RFa were higher in T1D only versus T1D with OSA (Median (IQR)): 0.91 (1.48); 0.82 (2.28); p=0.01 vs 0.41 (0.7); 0.28 (0.31); p=0.01) respectively. However, there was no significant difference in LFa/RFa ratio (sympathovagal balance). LFa was significantly lower in T1D with OSA during baseline, Valsalva and standing manoeuvres (p<0.05). RFa was also significantly lower in T1D with OSA during baseline, deep breathing and Valsalva manoeuvres (p<0.05). LFa, RFa, and LFa/RFa response to standing were better in T1D only than T1D with OSA. As compared to T1D only, T1D with OSA had lower deep breathing ratio (median (IQR)): 1.18 (0.17) vs 1.22 (0.17); p=0.04, standing ratio (1.09 (0.28) vs 1.23 (0.27); p=0.02), and a tendency for a lower Valsalva ratio (1.13 (0.45) vs 1.41 (0.38); p=0.08). Our findings show that CAF is depressed more in T1D with OSA versus T1D without OSA, which indicates sympathetic and parasympathetic withdrawal in the patients with OSA. It has previously been observed that HRV improves with CPAP in patients with OSA³ and therefore irreversible autonomic nerve damage is unlikely to be responsible for the changes observed in this study. Given that there is an increase in oxidative stress and inflammation in both T1D and OSA this is a potential common mechanism that may impact upon neurotransmission or second messenger systems resulting in the worsening of HRV and CAF in patients with T1D and OSA.

1.Jaiswal et al. (2013) *Diabetes Care* 36.1 157-162

2.Wiklund et al. (2008) *Clinical Physiology* 20.3, 234-241

3.Khoo et al. (2001) *American journal of respiratory and critical care medicine* 164.5, 807-812

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC010

The role of the S5 aromatic residue F557 in cisapride inhibition of the hERG channel, probed through mutagenesis and docking to a realistic hERG structure

Y. ZHANG¹, A. Sudol², C.E. Dempsey² and J.C. Hancox¹

¹*School of Physiology Pharmacology and Neuroscience, University of Bristol, Bristol, UK* and ²*School of Biochemistry, University of Bristol, Bristol, UK*

The hERG potassium channel is critical for normal cardiac repolarisation. hERG channel block underlies acquired long QT syndrome. Recent evidence suggests that S5, in close apposition to S6, may enable novel drug binding modes to hERG, in particular F557 in the S5 domain has been suggested to be a significant determinant of binding of some hERG inhibitors and activators [1-3]. Cisapride is a high

affinity hERG channel inhibitor, and binding to the hERG channel of cisapride has been mapped to residues within the channel's central cavity [4]. The aim of this study was to probe whether mutation of F557 influences hERG channel kinetics and cisapride block using a mammalian expression system at physiological temperature, in concert with computational docking to a realistic hERG channel structure. Whole cell patch-clamp recordings of hERG current (I_{hERG}) were made at 37°C from Human Embryonic Kidney (HEK 293) cells either stably expressing WT hERG or transiently transfected with wild-type (WT) or F557L hERG [2]. Docking was performed using the recent cryo-EM open channel structure of hERG (Protein Data Bank code 5VA1) [5].

Using a standard current-voltage (I-V) protocol, mean I-V relations for I_{hERG} tails showed no difference in half-maximal activation voltage ($V_{0.5}$) and k (slope factor) values between WT and F557L, inactivation $V_{0.5}$ and k values from availability plots for WT and F557L I_{hERG} also did not differ significantly from one-another. The time-courses for development of both inactivation and deactivation of WT and F557L I_{hERG} did not differ significantly. The mean time constant of recovery from inactivation showed a modest, but significant reduction for F557L hERG (1.22 ± 0.26 ms ($n=6$) for F557L and 1.77 ± 0.04 ms ($n=7$) for WT; mean $\pm$ SEM; $p < 0.05$, unpaired t test). The potency of cisapride block of I_{hERG} tails at -40 mV following depolarization to +20 mV was evaluated. IC_{50} and n_H values generated from concentration-response relations were 5.19 ± 1.17 nM, 0.68 ± 0.10 for WT and 46.3 ± 10.2 nM, 0.51 ± 0.07 for F557L (right-ward shift of IC_{50} by ~9 fold compared to WT; $n=4-6$ cells for each cisapride concentrations; values are mean $\pm$ SEM). Docking of cisapride to the recent cryo-EM derived hERG structure [5] showed that cisapride can interact directly with F557 on the S5 domain as well as Y652 and F656 on S6.

Our results show that F557L I_{hERG} kinetics are largely similar to those of WT I_{hERG} , although recovery of F557L I_{hERG} from inactivation is somewhat faster. As F557L has a limited effect on hERG channel kinetics, its effect in reducing cisapride potency is most likely to be due to direct interaction of the drug with F557 and/or coordination between F557 and inner cavity high affinity binding sites.

Garg V et al. (2011). Mol Pharmacol 80, 630-7

Saxena P et al. (2016). Sci Rep 6:24182

Helliwell MV et al. (2018). J Biol Chem 293,7040-7057

Mitcheson JS et al. (2000). PNAS 97:12329-33

Wang W & MacKinnon R (2017). Cell 169, 422-430

Funded by the British Heart Foundation (PG/17/89/33414)

Abi Sudol was funded by a Wellcome Trust summer studentship

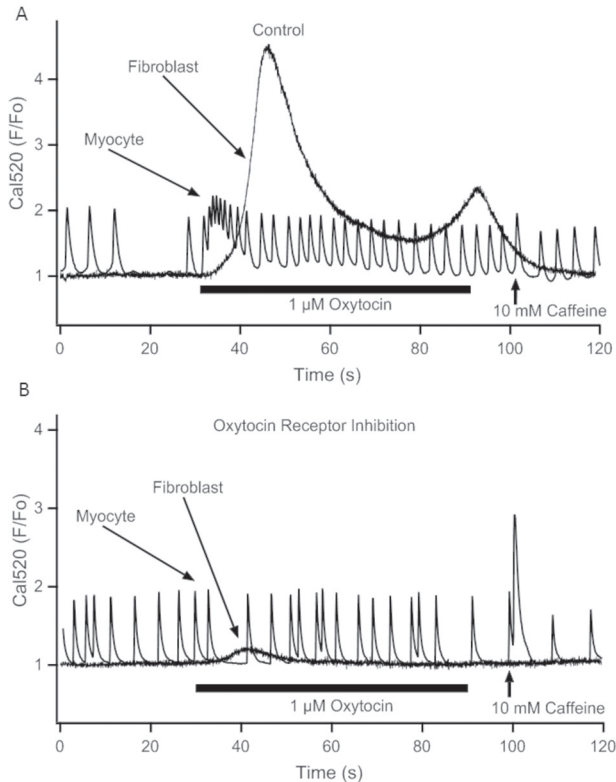
Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Effects of oxytocin on Ca^{2+} signalling in newborn rat cardiac myocytes and cardiac fibroblasts.I.I. Ali¹, S. Al-Salam², F.C. Howarth¹ and A. Shmygol¹¹*Department of Physiology, United Arab Emirates University, Al Ain, United Arab Emirates and* ²*Department of Pathology, United Arab Emirates University, Al Ain, United Arab Emirates*

Oxytocin receptors are expressed in mammalian heart. Systemic injections of oxytocin cause negative chrono- and inotropic effects that were linked to the release of atrial natriuretic peptide [1]. There is a paucity of data describing direct effects of oxytocin on intracellular calcium ($[\text{Ca}^{2+}]_i$) in cardiac myocytes and a complete lack of data in cardiac fibroblasts. Our aim was to investigate the acute responses of cardiac myocytes and cardiac fibroblasts to the application of this neuropeptide. Cells were enzymatically isolated from 1 day old Wistar rat pups and maintained in short-term (up to four days) primary culture. The use of neonatal rats for this study has been approved by the UAEU Animal Ethics Committee (ERA_2017_5681). Spontaneous intracellular Ca^{2+} transients were recorded using the Ca^{2+} sensitive fluorescent dye Cal520 and a fast EM-CCD camera. Sequences of fluorescence images were taken at a rate of 40 Hz. Oxytocin was applied for 60 s after a 30s long baseline recording. The recording continued for an additional 30 s after oxytocin washout. During this time interval, 10 mM caffeine was applied to distinguish the cardiac myocytes from the fibroblasts as the latter cell type does not respond to caffeine. Image acquisition and quantification were performed using the WinFluor software package (J. Dempster, Strathclyde University). Mean fluorescence was measured from ROIs placed over all cells in the entire field of view and presented as self-ratio (F/F_0). Where appropriate, statistical comparisons were made using paired t-test or 1-way ANOVA followed by Dunnet's post-hoc test in IgorPro 7.0 (Wavemetrics, USA).

Within 24 hours, cardiac myocytes developed spontaneous contractions accompanied by transient rises in $[\text{Ca}^{2+}]_i$ while cardiac fibroblasts remained quiescent and maintained low $[\text{Ca}^{2+}]_i$. Application of 1 μM oxytocin to cardiac myocytes caused an increase in the frequency of $[\text{Ca}^{2+}]_i$ transients from 0.13 ± 0.01 to 0.29 ± 0.03 Hz and decreased their amplitude from 2.25 ± 0.1 to $1.73 \pm 0.08 F/F_0$ ($n=21$, $p<0.01$, paired t-test). In the fibroblasts, 1 μM oxytocin induced large $[\text{Ca}^{2+}]_i$ transients averaging $3.59 \pm 1.08 F/F_0$ (illustrated in Fig.1A). The observed effects were abolished in all cells after pre-incubation with selective oxytocin receptor inhibitor L-371.257 (10mM) suggesting that they were mediated by activation of the oxytocin receptors and were not due to the cross-activation of vasopressin receptors that are also expressed in the heart (Fig.1B). Oxytocin induced $[\text{Ca}^{2+}]_i$ transients in cardiac fibroblasts were abolished by a non-selective IP_3 receptor inhibitor 2-APB and disappeared in the presence of the SERCA pump inhibitor thapsigargin.

In conclusion, oxytocin exerts direct positive chronotropic and negative inotropic effects on the neonatal cardiomyocytes and induces IP_3 mediated Ca^{2+} release in cardiac fibroblasts.



Gutkowska J, Jankowski M. Oxytocin Revisited: Its Role in Cardiovascular Regulation. *J Neuroendocrinol.* 2012;24(4):599–608.

Funding from the Al Jalila Foundation (grant AJF201738) is gratefully acknowledged.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC012

Piezo is required to buffer mechanical stress in the heart

L. Zechini¹, M. Diaz¹, P. Hartley² and B. Denholm¹

¹Biomedical Sciences, Edinburgh University, Edinburgh, UK and ²Bournemouth University, Bournemouth, UK

The intrinsic capacity of the heart to respond to stretch with increased contractility, has been described over a century ago^{1,2}. It is known that the response to stretch is biphasic with cardiac myocytes (CMs) showing an abrupt, immediate increase in force followed by a subsequent, slow force response (SFR) over a period of second to minutes. The rapid response is mediated by a change in myofilament affinity to calcium. In contrast, the SFR response is not well characterised. Evidence shows an increase in the magnitude of the systolic calcium transient is associated with the SFR³. However, the mechanistic basis for the SFR is not known.

The stretch-activated ion channels of the Piezo family are possible candidates. Piezo channels are non-selective cation channels with preferential affinity for calcium and have been found to be involved in stretch responses in several tissues and organs. Piezo is expressed in cardiac tissue but its role in the heart has not yet been explored.

This study uses the *Drosophila* as a model to test for a role for Piezo in the stretch response in the heart. We tested heart function from control and from Piezo knock-out (KO) mutants before and after exposure to mechanical stress. To mimic mechanical stretch we exposed the hearts to hypotonic shock, which leads to osmotic swelling and membrane stretch. We measured the magnitude of the systolic calcium transient in control and Piezo KO hearts and observed the effects of hypotonic shock.

In isotonic solution Piezo KO hearts exhibited significantly larger ($p < 0.00001$) systolic calcium transients (235.8 ± 15 SEM, $n=8$) and subsequent contractions than control hearts (86.03 ± 10 SEM, $n=8$). This increased systolic calcium amplitude was associated with a $54.3 \pm 5.5\%$ greater sarcoplasmic reticulum calcium content in Piezo KO hearts ($p < 0.01$, $n=7$). However, Piezo KO hearts failed to respond to hypotonic shock. In contrast to control hearts, which show a significant increase ($67 \pm 10\%$, $p < 0.01$, $n=18$) in systolic calcium transient amplitude in response to hypotonic shock, Piezo hearts failed to respond ($3 \pm 4\%$, $n=19$). Interestingly, the percentage increase of the calcium transient in response to hypotonic shock recorded in control hearts was equivalent to the calcium transient amplitude in Piezo KO hearts under isotonic conditions.

These data show Piezo is required in the calcium release-associated response to mechanical stress in the heart. We suggest that the enhanced calcium transient amplitude recorded in Piezo KO hearts in isotonic solution may result from compensatory mechanisms.

1. Frank, O. Zur dynamik des hersmuskels. *Z. biology* 32, 370-447 (1895).

2. Patterson, S. W. & Starling, E. H. On the mechanical factors which determine the output of the ventricles. *J Physiol* 48, 357-379 (1914).
3. Allen, D. G. & Kurihara, S. The effects of muscle length on intracellular calcium transients in mammalian cardiac muscle. *J Physiol* 327, 79-94 (1982).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC013

Acute effect of volitional breathing without and with minimal inspiratory resistance on heart rate variability

O. Bhagat¹ and K.K. Deepak²

¹Physiology, All India Institute of Medical Sciences, Jodhpur, India, Jodhpur, India and

²Physiology, All India Institute of Medical Sciences, Delhi, Delhi, India

Respiratory modulations can modulate cardiac autonomic tone, which is reflected in heart rate variability. All the respiratory training modules have involved volitional breaths, change in respiratory rate and/or inspiratory resistance. So the question remained that what is the effect of the act of volition has on the heart rate variability? The present study planned to find the acute impact of volitional breathing/ Conscious breathing (VB) and Breathing with minimal inspiratory resistance (IRB) (with no load on respiratory exerciser) keeping rate and depth similar to spontaneous breathing (SB). The novice volunteers (age 30 $\pm$ 4.2 years) participated in the study (n=31 for VB; n=17 for IRB). Standard lead II electrocardiogram (ECG) and respiratory movements were recorded at rest (spontaneous breathing (SB)). After the baseline recording, subjects' Spontaneous Breathing rate (SBR) was calculated, and then they were instructed to do either paced breathing or to breath through respiratory exerciser (POWERbreathe wellness, Southam, UK) with no load to provide minimal inspiratory resistance paced at their SBR using a Human voice-based instruction system (inspiration and expiration duration was kept at a ratio of 1:1). All recordings were done in a comfortable sitting posture in the thermoneutral lab environment. HRV analysis was done using Nevrokard BRS analysis/ver 3.2.0. Data presented in mean $\pm$ SD. Both VB and IRB were compared with respective SB by paired student t-test.

HRV showed a decrease in LF (nu) (50.14 ± 21.41 vs 41.29 ± 20.52 for SB vs VB ($P=0.026$); 55.05 ± 14.91 vs 34.91 ± 19.36 for SB vs IRB ($P=0.028$)) and LF/HF ratio (1.81 ± 1.60 vs 1.13 ± 1.06 for SB vs VB ($P=0.041$); 1.83 ± 1.20 vs 0.75 ± 0.60 for SB vs IRB ($P=0.007$)) with an increase in HF (nu) (42.83 ± 23.32 vs 53.93 ± 20.98 for SB vs VB ($P=0.021$); 38.82 ± 16.35 vs 60.49 ± 19.93 for SB vs IRB ($P=0.004$)) with volitional breathing. The total power, SDNN, RMSSD, and pNN50 did not show significant change.

This data suggests that, volitional breathing shifts cardiac autonomic control towards parasympathetic at the same frequency of spontaneous breathing.

Abbreviations:

HRV: heart rate variability

VB: Volitional breathing/ Conscious breathing

IRB: Breathing with minimal inspiratory resistance

SB: spontaneous breathing

LF (nu): Relative power of the low-frequency band (0.04–0.15 Hz) in normal units

HF (nu): Relative power of the high-frequency band (0.15–0.4 Hz) in normal units

LF/HF ratio: Ratio of LF-to-HF power

SDNN: Standard deviation of NN intervals

RMSSD: Root mean square of successive RR interval differences

pNN50: Percentage of successive RR intervals that differ by more than 50 ms

I acknowledge all the participants of the study

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC014

Cardiac pacemaker dysfunction arising from different pathways of electrical remodelling in the ageing rat

Azzah. M. Algamdi¹, Henggui. Zhang¹

1. School of Physics and Astronomy, University of Manchester, Manchester, UK.

2. School of Physics, Jeddah University KAU, Jeddah, Saudi Arabia.

A.M. Algamdi^{1,2}

¹*Biological physics group, School of Physics and Astronomy, University of Manchester, Manchester, UK and* ²*School of Physics, Jeddah University, Jeddah, Saudi Arabia*

BACKGROUND The function of the sinoatrial node (SAN), the pacemaker of the heart, is known to decline with age, resulting in increased incidence of sinoatrial node dysfunction in the elderly.

OBJECTIVE The aim of the study is to investigate the ionic mechanisms underlying age-associated SND. It is possible that SND with age may be the result of different ionic and molecular mechanistic “pathways”, each of which may result in either up-regulation or down-regulation of ion channel functions.

METHODS A mathematical model of the electrical action potential of rat sinoatrial node myocytes, as developed by (Tao et al. 2011), was modified to simulate the functional impacts of age-induced changes on membrane ion channels and intracellular Ca²⁺ handling on cardiac pacemaking potentials based on experimental data from two independent studies on ageing in Wistar Hannover rat SAN cells. The first data set was from (Tellez et al. 2011) (noted as Ageing Study-1), and the

second from (Huang et al. 2016) and (Jones et al. 2004) and (Hatch. 2012) (noted as Ageing Study-2). In both studies, the roles and relative importance of individually remodelled ion channels were evaluated by inclusive and exclusive methods, in which remodelling of specific currents was considered in isolation.

RESULTS Previously reported age-induced changes in sarcolemmal ion currents result in a marked increase in CL, leading to HR reduction and thus producing bradycardial effects in the SAN. With both Ageing Study-1 and 2's remodelled parameters, there was a 16% and 11% reduction in HR, respectively, where these reductions were comparable to experimental results of 18% and 8% reductions in heart rate in elderly rat SANs as compared to adult rats. The individual method showed that the altered I_{CaL} , either increased or decreased, has notable effects of the AP profiles, modulating the pacemaking rate of APs. The decreased I_{CaL} associated with an decreased amplitude of AP and abbreviated APD50, both of which accelerated repolarisation process, causing an incomplete repolarisation as a consequence of reduced AP amplitude and abbreviated APD50, therefore slowing down the pacemaking rate. Similarly, the increased I_{CaL} produced increased amplitude of AP and prolonged APD50, slowing down the time course of repolarisation and therefore slowing down the pacemaking rate that contributes to ageing bradycardia.

CONCLUSION With age, the changes in the expression of ion channel and Ca^{2+} clock genes in the SAN accounted qualitatively for HR reduction. The various changes in age-related remodelling of I_{CaL} appears to have the most influence on the slowing of APs in rat SAN, which is known as the "Bradycardia effect", indicating that SAN dysfunction could be the result of various mechanisms that come into effect during the ageing process.

KEYWORDS Aging; SA node; Ion channel remodelling; I_{CaL}

Hatch., 2012. Age-associated changes to calcium handling proteins across the whole heart being a thesis submitted for the Degree of Doctor of Philosophy in the University of Hull by Fiona Sara Hatch BSc (Hons) December 2012 Abstract., (December).

Huang, X. et al., 2016. Age-associated expression of HCN channel isoforms in rat sinoatrial node. *Experimental Biology and Medicine*, 241(3), p.331. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/26341471> [Accessed March 14, 2018].

Jones, S.A., Lancaster, M.K. & Boyett, M.R., 2004. Ageing-related changes of connexins and conduction within the sinoatrial node. *The Journal of Physiology*, 560(2), pp.429–437. Available at: <http://doi.wiley.com/10.1113/jphysiol.2004.072108> [Accessed July 26, 2017].

Tao, T., Paterson, D.J. & Smith, N.P., 2011. A model of cellular cardiac-neural coupling that captures the sympathetic control of sinoatrial node excitability in normotensive and hypertensive rats. *Biophysical Journal*, 101(3), pp.594–602. Available at: <http://dx.doi.org/10.1016/j.bpj.2011.05.069>.

Tellez, J.O. et al., 2011. Ageing-dependent remodelling of ion channel and Ca^{2+} clock genes underlying sino-atrial node pacemaking. *Experimental Physiology*, 96(11), pp.1163–1178. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21724736> [Accessed March 27, 2018].

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC015

Changes of osteoprotegerin serum level of diabetes mellitus rat model after exercise

M.D. Pramaningtyas¹ and A.F. Muttaqina²

¹Department of Physiology, Medical Faculty, Universitas Islam Indonesia, Sleman, Indonesia and ²Medical Student, Medical Faculty, Universitas Islam Indonesia, Sleman, Indonesia

Background : Epidemiological studies showed a trend of increased incidence and prevalence rate of diabetes mellitus (DM). Endothelial dysfunction in DM lead to atherosclerosis, high level of osteoprotegerin can lead to endothelial dysfunction. Osteoprotegerin is mainly produced by bones, but is also secreted by cardiovascular structures, lungs, kidneys, hematopoietic and immune cells. Osteoprotegerin acts to prevent osteoclast activation and to neutralizes proapoptotic activity.

Purpose : This study aimed to investigate relation of osteoprotegerin (OPG) serum level in diabetic mellitus (DM) rat model which had given physical exercise.

Method : This study is an experimental research design with a post-test only control group to measure OPG level. Rat model of DM obtained by injecting streptozotocin 35mg/kgbw intra peritoneal Sprague Dawley male rats aged 11-12 weeks. Rats were divided into two groups, physical exercise (PE) and without physical exercise (WPE) group. Physical exercise using a special treadmill for rat, low to moderate intensity, frequency 5x/week for 9 weeks, with a week of adaptation using 5 m/min for 10 minutes, followed 8 weeks using a gradual rise of velocity, 5 m/min to 20 m/min for 60 minutes. Osteoprotegerin serum level was measured by enzyme linked immunosorbent assay (ELISA).

Result : Mean of OPG in PE was lower than WPE. Result of Mann-Whitney test p value = 0,338, there is no significant statistically difference between the two groups.

Conclusion : There is no statistically changes of OPG serum level of DM rat model with physical exercise. Physical exercise had not understand yet to influence OPG level.

Key words: diabetes mellitus, exercise, osteoprotegerin

Nobuyuki U *et al.* (2000). Osteoprotegerin Produced by Osteoblasts Is an Important Regulator in Osteoclast Development and Function, *Endocrinology* 141 (9), 3478–3484

Bjerre M. (2013). Osteoprotegerin (OPG) as a biomarker for diabetic cardiovascular complications. *Springerplus*. 2, 658

Duan P *et al.* (2017). Serum Osteoprotegerin Is a Potential Biomarker of Insulin Resistance in Chinese Postmenopausal Women with Prediabetes and Type 2 Diabetes. *Int J Endocrinol* 2017:8724869

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC016

Inhibition of the human *Ether-à-go-go-Related Gene* (hERG) potassium channels by phenanthrene.E. Al-Moubarak¹, H. Shiels², Y. ZHANG¹, C.E. Dempsey³ and J.C. Hancox¹¹*School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, UK,*²*Division of Cardiovascular Sciences, University of Manchester, Manchester, UK and*³*School of Biochemistry, University of Bristol, Bristol, UK*

Exposure to polycyclic aromatic hydrocarbon (PAH) nanoparticles may contribute to cardiotoxic effects of hydrocarbon-based pollution. Phenanthrene (Phen), comprised of three fused benzene rings, is relatively abundant in polluted air and water. It is lipophilic and can access and accumulate in human tissue *via* skin and mucus membranes. Phen is a constituent PAH of crude oil spills and has been shown to increase ventricular action potential duration of fish ventricular myocytes; this action is associated with inhibition of the rapid delayed rectifier K⁺-current (Brette *et al*, 2017). *Ether-à-go-go Related Gene* encoded potassium channels mediate I_{Kr} in multiple species including human and are known to exhibit marked susceptibility to pharmacological blockade (Hancox *et al*, 2008). Human and fish *ERG* sequences are identical at the known drug binding region of the channel. The purpose of this study was to determine the propensity or otherwise of Phen to inhibit human *ERG*-encoded (hERG) potassium channels.

Whole-cell patch-clamp recordings were made at room temperature from HEK 293 cells stably expressing recombinant hERG channels. Data are presented mean ± SEM. Computational docking was performed using a recent Cryo-EM structure of hERG (Wang and MacKinnon, 2017). A conventional voltage-step protocol, comprised of a 2-second depolarization to +20 mV from -80 mV, followed by repolarization to -40 mV to elicit hERG current (I_{hERG}) tails, was applied in control solution and during exposure to Phen. Five different concentrations between 1 μM and 50 μM were tested, with at least 5 replicates per concentration. Phen was found to inhibit I_{hERG} with a half-maximal inhibitory concentration (IC₅₀) of 16.8 ± 1.7 μM, Hill slope: 1.0 ± 0.1. A modified version of the standard protocol incorporated test depolarizations to a range of voltages. I_{hERG} tail inhibition by 10 μM Phen varied significantly with test voltage over the range evaluated (-40 to +60 mV; p<0.0001, one-way ANOVA; n=16 cells). Phen also shifted half maximal activation voltage (V_{0.5}) for I_{hERG} by -10.1 ± 0.9 mV. Use of an envelope of tails protocol showed that the extent of Phen inhibition of I_{hERG} increased with duration of activating voltage command (to +20 mV). These results indicate that Phen inhibits hERG channels at micromolar levels and that this effect is contingent on the channel gating. In docking simulations Phen could readily be accommodated within the hERG channel pore; its small size means it may be able to interact within the pore in multiple binding configurations.

We conclude that phenanthrene exerts inhibitory effects on the hERG potassium channel.

Brette, F., Shiels, H. A., Galli, G. L., Cros, C., Incardona, J. P., Scholz, N. L., and Block, B. A. (2017) A Novel Cardiotoxic Mechanism for a Pervasive Global Pollutant. *Scientific Reports* 7:41476

Hancox JC, McPate MJ, El Harchi A, Zhang Y (2008) The hERG potassium channel and hERG screening for drug-induced arrhythmias. *Pharmacol Ther* 119: 118-132

Wang and MacKinnon (2017) Cryo-EM structure of the open human Ether-a-go-go Related Gene K⁺ channel hERG. *Cell* 169: 422-430.

Supported by British Heart Foundation Grant PG/17/77/33125.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC017

Mitochondrial succinate metabolism is important in setting carotid body chemoafferent activity in normoxia and hypoxia

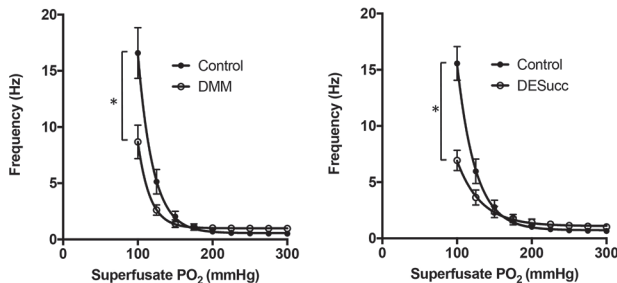
A. Coney¹, C.J. Ray¹, A. Alzahrani^{1,3}, P. Georgiadou¹, A. Swiderska¹, N. Batis², P. Kumar¹ and A.P. Holmes¹

¹*Institute of Clinical Sciences, University of Birmingham, Birmingham, UK*, ²*Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK* and ³*Respiratory Care Department, College of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia*

The mammalian carotid body (CB) is a unique peripheral chemoreceptor that responds to acute hypoxia well before the metabolism of other cell types is impaired [1]. However, the specific O₂ sensing mechanism in the CB remains highly contested. Recently it has been suggested that succinate metabolism at mitochondrial complex II is necessary to cause CB excitation during hypoxia [2]. This has been countered by the finding that CBs with heterozygous *Sdh* knock out display an augmented, rather than depressed basal activity and retain sensitivity to hypoxia [3]. Thus, the role of CB succinate metabolism and downstream signalling during hypoxia requires further investigation. The aim of this current study is to evaluate if CB chemoafferent activity can be modified by changes in succinate metabolism during normoxia or hypoxia.

All animal procedures were approved and carried out in line with the current Home Office (UK) and University of Birmingham guidelines on ethical use of animals. CBs were isolated from adult male Wistar rats (150-250g) and recordings of single fibre chemoafferent activity was performed as previously described [4]. Data was expressed as mean $\pm$ S.E.M and significance determined with paired t-test or one-way repeated measures ANOVA and taken as $P < 0.05$.

Application of cell permeable diethyl succinate (DESucc; 1-10mM) caused a rapid, reversible and concentration dependent increase in chemoafferent activity (e.g. Control 0.5 ± 0.1 Hz, 10mM DESucc 2.4 ± 0.4 Hz ($P < 0.05$)). The peak elevation in chemoafferent activity induced by DESucc measured approximately 15% of that caused by exposure to severe hypoxia. The rise in chemoafferent activity evoked by 5mM DESucc (1.8 ± 0.2 Hz) was significantly reduced by application of 10mM di-methyl malonate (DMM; 0.4 ± 0.2 Hz, $P < 0.05$); a mitochondrial complex II inhibitor, or by mitoTempo (20mM; 0.8 ± 0.3 Hz, $P < 0.05$); a mitochondrial antioxidant. Interestingly, 5mM DESucc also reduced the chemoafferent response to hypoxia (e.g. at superfusate PO₂ of 100mmHg: Control 15.5 ± 1.5 Hz, DESucc 6.9 ± 0.9 Hz, $P < 0.05$, Figure 1 right panel). Application of 10mM DMM caused a small but significant increase in basal chemoafferent activity and depressed the CB response to hypoxia without completely abolishing it (e.g. at superfusate PO₂ of 100mmHg: Control 16.6 ± 2.2 Hz, DMM 8.7 ± 1.5 Hz, $P < 0.05$, Figure 1 left panel). These results suggest that endogenous mitochondrial succinate metabolism in the CB acts to repress basal CB chemoafferent activity. Furthermore, whilst we support the idea that succinate metabolism is required to confer the full CB chemoafferent response to hypoxia, we do not think it is not the sole mediator. Interestingly, excessive succinate metabolism acts to dampen the CB hypoxic sensitivity. Future experiments are required to see if these effects are mediated at the level of the glomus cell or nerve ending.



Changes in mitochondrial succinate metabolism modify the carotid body chemoafferent response to hypoxia. Inhibiting mitochondrial complex II with dimethyl malonate (DMM; 10mM-left) reduces but does not abolish the carotid body chemoafferent elevation during hypoxia. Increasing succinate metabolism, achieved through application of diethyl succinate (DESucc; 5mM-right), also decreases the response to hypoxia.

Holmes, A.P., et al., Is Carotid Body Physiological O₂ Sensitivity Determined by a Unique Mitochondrial Phenotype? *Front Physiol*, 2018. 9:562.

Fernandez-Aguera, M.C., et al., Oxygen Sensing by Arterial Chemoreceptors Depends on Mitochondrial Complex I Signaling. *Cell Metab*, 2015. 22(5): p. 825-37.

Piruat, J.I., et al., The mitochondrial SDHD gene is required for early embryogenesis, and its partial deficiency results in persistent carotid body glomus cell activation with full responsiveness to hypoxia. *Mol Cell Biol*, 2004. 24(24): p. 10933-40

Holmes, A.P., et al., Moderate inhibition of mitochondrial function augments carotid body hypoxic sensitivity. *Pflugers Archiv-European Journal of Physiology*, 2016. 468(1): p. 143-155

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC018

Revealing the dyadic ultrastructure: major differences in ryanodine receptor cluster distributions between the atria and ventricle

L. Toms, J. Caldwell, K. Dibb and A. Trafford

Cardiovascular Sciences, The University of Manchester, Manchester, UK

Calcium-induced calcium (Ca^{2+}) release drives cardiac myocyte contraction. Ca^{2+} release from the sarcoplasmic reticulum is determined by the availability and open probability of ryanodine receptors (RyRs), which are arranged into clusters. Computer modelling suggests that cluster heterogeneity, organisation and the distance to the nearest neighbouring clusters may affect their Ca^{2+} release properties. Currently, there is little known regarding the ultrastructure of RyR clusters in ventricular and atrial myocytes of large mammals, and how this relates to important inter-chamber differences in the spatiotemporal properties of the calcium transient. Therefore, the aim of this study was to determine if there are differences in RyR cluster spatial properties between the atria and ventricle.

Young female adult Welsh Mountain sheep (≈ 18 months) were euthanised with 200 mg.kg^{-1} sodium pentobarbital administered intravenously. Left atrial and ventricular myocytes were isolated and immunocytoologically labelled for RyR. Photo-switchable fluorescent dyes were used and dye-molecule switching events were acquired over 10,000 frames using the Nikon super resolution N-STORM 4.0 setup. RyR cluster distribution was analysed using a custom written MATLAB programme. Protein abundance was quantified by western blot. Data are presented as mean $\pm$ SEM for 27-35 cells from 4 animals for immunocytochemistry and tissue from 8 animals for Western blot with 3 technical repeats. Data were compared using paired t-test where appropriate.

In both the atria and ventricle, RyRs were predominantly arranged along the Z-lines with few longitudinal projections. However, there were distinct inter-chamber differences in the size and distance between these clusters. Atrial RyR clusters are both larger by $49.0 \pm 24.3\%$ ($p < 0.05$) and closer together, with edge-to-edge distances $51.1 \pm 4.2\%$ ($p < 0.0001$) and centroid distances $73.5 \pm 4.6\%$ ($p < 0.0001$) of that in the ventricle. Western blot analysis showed that RyR protein abundance does not differ between chambers ($p = 0.49$).

The use of super resolution microscopy has shown for the first time inter-chamber differences with respect to RyR distribution. This may account for the spatiotemporal differences of triggered Ca^{2+} release seen in the atria and ventricle. Future experiments will further characterise the spatial properties of essential calcium handling proteins and relate the findings to functional Ca^{2+} release.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC019

Sex differences in cardiovascular responses to postural stress among young Black African adults

A.O. Aiku^{1,2}, E. Adagbada¹, S. Ogbona¹ and A. Fasanmade¹

¹Physiology, University of Ibadan, Ibadan, Nigeria and ²Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, UK

Squatting, an active posture manoeuvre imposes one of the most potent ortho-static stresses. Analysis of BP and HR changes during change in posture from standing to squatting position provide information on performance of the baro-reflexes and haemodynamic homeostasis. This study aimed at determining the effect of prompt and sustained squatting on arterial blood pressure and heart rate in young Black Africans and to determine the gender differences in the responses. Method: Sixty five men (32) and women (33) aged 16-30 years were recruited. Following 10 minutes rest in sitting position by stood for 3 mins, then squatted for 2 minutes. Systolic and diastolic blood pressures (SBP, DBP) and heart rate (HR) were recorded using automated blood pressure monitor in sitting, standing and squatting positions. Mean arterial blood pressure (MAP) was calculated using the formula $(1/3 \text{ Pulse pressure} + \text{Diastolic blood pressure})$ and Pulse Pressure (PP) was calculated using the formula; $\text{SBP} - \text{DBP}$. Differences in SBP, DBP, MAP, PP and HR between squatting position and standing were computed. Effect of prompt squat was determined using unpaired T test, effect of squatting at 0s, 60s and 120s was determined using repeated measures ANOVA. Statistical significance was determined at level of $p < 0.05$.

Result:

Prompt squat was associated with increase in SBP (from 103.18 ± 1.50 to 113.13 ± 1.63 mmHg, $p = 0.00$), in MAP (from 78.19 ± 1.08 to 81.45 ± 1.32 mmHg, $p = 0.003$), but DBP did not change (65.69 ± 0.97 vs 66.61 ± 1.29 mmHg, $p = 0.42$), leading to augmentation of PP (from 37.48 ± 0.97 to 44.52 ± 1.08 mmHg, $p = 0.00$), with a decrease in HR (from 81.71 ± 1.53 to 72.26 ± 1.41 bpm, $p = 0.00$). Women showed greater increase in SBP ($F(1,63) = 4.03$, $p = 0.049$), DBP ($F(1,63) = 12.32$, $P = 0.01$) and MAP ($F(1,63) = 10.75$, $p = 0.02$) during squatting compared with men. But HR changes were not statistically significant between the two genders ($F(1,63) = 1.42$, $p = 0.24$) (Figure 1). In women but not in men, sustained squatting was associated with progressive increase in SBP, DBP and MAP.

Conclusion: Squatting evoked increase in blood pressure, augmentation of pulse pressure with decrease in heart rate. Women showed greater blood pressure increases during squatting. These data highlight gender-specific responses to

cardiovascular stress among young black African adults which requires further study to understand the mechanism of the responses.

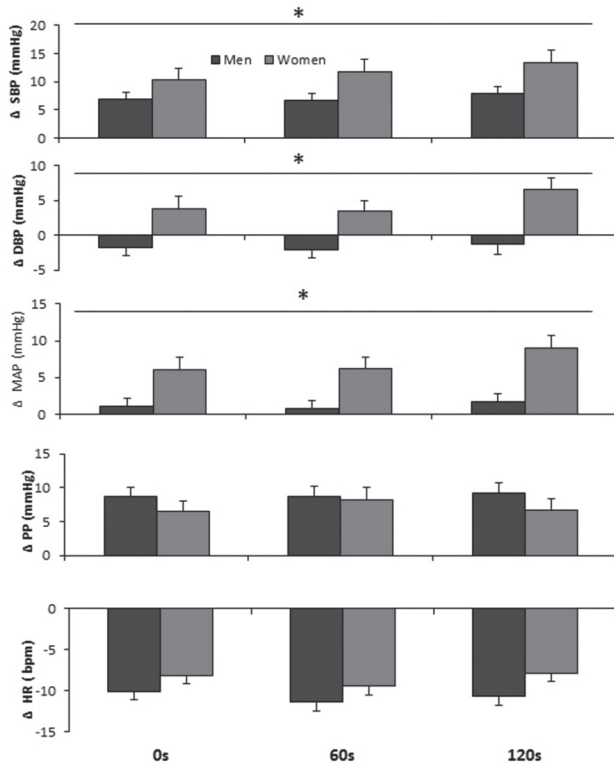


Figure 1: Showing change SBP, DBP, MAP, PP and HR values in mean $\pm$ SEM during squatting from standing position. * $p < 0.05$ men vs women.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC020

Hypoxic ventilatory response is not increased in young adults with stage 1 hypertension during rest or exercise

T. Hinton^{1,2}, Z. Adams¹, K.A. Hope¹, B. Chant¹, J.F. Paton³, A. Nightingale^{1,2} and E.C. Hart¹

¹School of Physiology, Pharmacology & Neuroscience, University of Bristol, Bristol, UK, ²Bristol Heart Institute, University Hospitals Bristol NHS Foundation Trust, Upper Maudlin Street, Bristol, UK and ³Department of Physiology, Faculty of Medical & Health Sciences, University of Auckland, Park Road, Grafton, Auckland 1142, New Zealand, Auckland, New Zealand

Hypertension (HTN) among young adults is a common condition that increases cardiovascular risk and causes end organ damage early in its onset. Muscle sympathetic nerve activity (MSNA) is elevated in young patients with HTN. A potential source of sympathetic activation are the peripheral chemoreceptors; particularly the carotid body. Animal studies indicate that the organ may play a critical role in the onset of hypertension (1). Human studies have been fewer and the results equivocal (2) (3). The aim of this study was to identify whether young adults with primary HTN exhibit elevated carotid body activity as measured by their ventilatory response to hypoxia (HVR) at rest and during exercise. 10 untreated, stage 1 HTN (3 female, mean $\pm$ standard deviation (SD) age; 26 ± 4 years) were recruited from a specialist HTN clinic. 10 normotensive (NTN) controls (5 female, age; 28 ± 2 years) were recruited from the local area. HTN was confirmed using a validated 24-hour ambulatory blood pressure monitor. Each participant underwent a maximal cardio-pulmonary exercise test on an upright cycle ergometer to assess peak oxygen consumption (VO_2 peak). HVR was assessed using the transient method (4). Briefly, participants were exposed to short periods of hypoxia by delivery of between 1-10 breaths of 100% nitrogen titrated into the inspiratory circuit using an electronic switching valve. This was repeated 4 to 5 times to provide a range of hypoxic exposures. Assessment of HVR was repeated in the presence of an intravenous dopamine infusion (2mcg/kg/min) to block carotid body afferent activity and a saline infusion as a control (4). HVR was next assessed during low intensity (30-40% VO_2 peak) exercise during saline and dopamine infusions. HVR was calculated by plotting minute ventilation against $\text{SpO}_2\%$. The gradient of the linear regression line was used for HVR as measured in $\text{L/min}/\%\text{SpO}_2$. A 2-way ANOVA was used to compare HVR during both infusions at rest and exercise. An independent samples t-test was used for non-repeated measures. There was no difference in VO_2 Peak between the two groups (NTN: $38 \pm 5.3 \text{ mL/Kg/min}$, HTN: $32.2 \pm 7.7 \text{ mL/Kg/min}$, $P=0.07$). There was no difference in HVR during saline infusion between groups at rest (NTN: $-0.219 \pm 0.069 \text{ L/min}/\%\text{SpO}_2$, HTN: $-0.275 \pm 0.077 \text{ L/min}/\%\text{SpO}_2$, $P=0.59$). HVR rose significantly during exercise in both groups (NTN: $-0.702 \pm 0.21 \text{ L/min}/\%\text{SpO}_2$, $N=8$, HTN: $-0.665 \pm 0.23 \text{ L/min}/\%\text{SpO}_2$, $n=10$, $P=0.009$), but there

was no difference between groups ($P=0.95$). Infusion of dopamine caused a significant reduction in HVR in both groups ($P=0.0006$) demonstrating that HVR is largely attributable to the carotid body. In conclusion, there is no difference in HVR as mediated by the carotid body between normotensive and hypertensive young adults at rest or during submaximal exercise.

Abdala AP, McBryde FD, Marina N, Hendy EB, Engelman ZJ, Fudim M, et al. Hypertension is critically dependent on the carotid body input in the spontaneously hypertensive rat. *J Physiol.* 2012;590(17):4269-77.

TRZEBSKI A, TAFIL M, ZOLTOWSKI M, PRZYBYLSKI J. Increased sensitivity of the arterial chemoreceptor drive in young men with mild hypertension. *Cardiovascular Research.* 1982;16(3):163-72.

Somers VK, Mark AL, Abboud FM. Potentiation of sympathetic nerve responses to hypoxia in borderline hypertensive subjects. *Hypertension.* 1988;11(6 Pt 2):608-12.

Limberg JK, Johnson BD, Holbein WW, Ranadive SM, Mozer MT, Joyner MJ. Interindividual variability in the dose-specific effect of dopamine on carotid chemoreceptor sensitivity to hypoxia. *Journal of applied physiology* (Bethesda, Md : 1985). 2016;120(2):138-47.

Dr Thomas Hinton is funded by the British Heart Foundation

Dr Katrina Hope is funded by the British Heart Foundation

Zoe Adams is funded by the British Heart Foundation

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC021

Effects of prolactin on ventricular myocyte shortening and calcium transport in the streptozotocin-induced diabetic rat

F.C. Howarth¹, A. Shmygol¹, A. Qureshi¹, O. Mohamed¹, L. Al Kury² and G. Norstedt³

¹Physiology, UAE University, Al Ain, United Arab Emirates, ²College of Natural & Health Sciences, Zayed University, Abu Dhabi, UK and ³Biochemistry, Sultan Qaboos University, Muscat, Oman

Diabetic cardiomyopathy (DCM) is a common cause of heart failure (HF) in diabetes mellitus. Disturbances in Ca^{2+} transport is thought to play a key role in the development of DCM. At an early stage, DCM is manifested by diastolic HF with preserved ejection fraction. In some patients, this may progress to HF with reduced ejection fraction and overt systolic HF. Previous studies have reported that the prolactin (PRL) receptor gene is expressed in rat heart. Little is known about the physiological role of PRL in the heart and in particular, the diabetic heart. It has been shown that PRL produces dose-dependent disturbances in heart rhythm and positive inotropism in isolated rat heart. The aim of this study was to investigate the effects of PRL on ventricular myocyte shortening and Ca^{2+} transport

in the streptozotocin (STZ) - induced diabetic rat. Ethical approval was obtained from the UAE University Animal Research Ethics Committee. Diabetes was induced in adult male rats with a single intraperitoneal injection of STZ/citrate buffer (60 mg/kg body weight). Age-matched controls received citrate buffer alone. Experiments were performed 8-12 weeks after STZ treatment. Myocyte shortening was measured using a video imaging system. Intracellular Ca^{2+} was measured in Fura-2 loaded myocytes by fluorescence photometry (Cairn Research). Measurements were made in electrically stimulated (1 Hz) myocytes at 35-36 °C. The results are expressed as the mean $\pm$ S.E.M. Statistical comparisons were performed using the Independent samples t-test or one-way ANOVA followed by Bonferroni corrected t-tests for multiple comparisons, as appropriate. Diabetes was characterized by a 5-fold increase in blood glucose. Compared to superfusion with normal Tyrode (NT) solution, PRL (50 ng/ml) significantly ($p < 0.05$) increased the amplitude of shortening in myocytes from control (7.43 ± 0.38 vs. $9.68 \pm 0.46\%$) and diabetic (6.57 ± 0.24 vs. $8.91 \pm 0.44\%$) heart ($n = 44-49$ cells). Compared to superfusion with NT solution, PRL (50 ng/ml) significantly ($p < 0.05$) increased the amplitude of the Ca^{2+} transient in myocytes from control (0.084 ± 0.004 vs. 0.115 ± 0.007 Fura-2 ratio units) and diabetic (0.087 ± 0.007 vs. 0.112 ± 0.006 Fura-2 ratio units) heart ($n = 36-50$ cells). Although PRL did not significantly ($p > 0.05$) alter the amplitude of caffeine-evoked Ca^{2+} transient in myocytes from control or diabetic heart, PRL significantly ($p < 0.05$) increased the fractional release of Ca^{2+} (amplitude of the electrically-evoked compared to caffeine-evoked Ca^{2+} transient) in myocytes from control and diabetic heart ($n = 36-50$ cells). The rate of Ca^{2+} transient recovery following PRL treatment was significantly ($p < 0.05$) increased in myocytes from control but not diabetic heart ($n = 36-50$ cells). In conclusion, PRL (50 ng/ml) had similar effects on shortening in myocytes from control and diabetic heart. However, the results suggest differences in the effects of PRL on Ca^{2+} transport in myocytes from control and diabetic heart.

Joint grant from United Arab Emirates University, Al Ain, United Arab Emirates and Sultan Qaboos University, Oman and a grant from Zayed University, Abu Dhabi, United Arab Emirates.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Properties and role of several repolarizing potassium currents in atrial cardiomyocytes isolated from dogs in sinus rhythm and atrial fibrillation

N. JOST^{1,2}, Z. Nagy², V. Juhasz¹, L. Saghy³, I. Bacsko¹, L. Virag¹ and A. Varro^{1,2}

¹Department of Pharmacology and Pharmacotherapy, University of Szeged, Faculty of Medicine, Szeged, Hungary, ²Research Group of Cardiovascular Pharmacology, MTA-SZTE, Szeged, Hungary and ³2nd Department of Internal Medicine and Cardiology Center, University of Szeged, Faculty of Medicine, Szeged, Hungary

Background and aim: Atrial fibrillation (AF) is the most common chronic/recurrent arrhythmia, which significantly impairs quality of life, increases cardiovascular morbidity and mortality. Previous results showed that inhibition of acetylcholine sensitive potassium current ($I_{K,ACh}$) may represent a novel, atrial-specific target for the management of AF in chronic AF (1,2). Therefore, the aim of the present study was to investigate the properties of three repolarizing potassium currents, which contribute to AF-induced electrical remodelling, in isolated atrial myocytes obtained from dogs either with sinus rhythm (SR) or following chronic atrial tachypacing induced AF-dogs.

Methods: Atrial remodelling and AF were induced by chronic (4-6 weeks) right atrial tachypacing (400/min) in dogs. Transmembrane ionic currents were measured by applying the whole-cell patch clamp technique at 37°C. AF was induced by short bursts (800/min).

Results: The transient outward current (I_{to}) current was slightly downregulated in AF cells when compared with that recorded in SR cells. This downregulation was also associated with slowed inactivation kinetics. Inward rectifier potassium current (I_{K1}) current was found to be larger in AF cells. $I_{K,ACh}$ was activated by the cholinergic agonist carbachol (CCh; 2 μ M). In SR, CCh activated a large current either in inward or outward directions. The selective $I_{K,ACh}$ inhibitor tertiapin (10 nM) blocked the outward CCh-induced current by $61.3 \pm 2.3\%$ ($n=5$; $*p<0.05$). In atrial cardiomyocytes isolated from dogs with AF, the presence of a constitutively active $I_{K,ACh}$ was observed, blocked by $59.4 \pm 3.5\%$ ($n=5$, $*p<0.05$) with 10 nM tertiapin. However, in 'AF atrial myocytes', CCh activated an additional, significant ligand-dependent and tertiapin-sensitive $I_{K,ACh}$ current. Tertiapin Q significantly and dose dependently reduced AF incidence and AF episode duration, prolonged atrial effective refractory period (ERP) and action potential duration (APD).

Conclusions: In our dog AF model, I_{to} unlike in human was downregulated only in a slight manner. Due to its slow inactivation kinetics, it seems that I_{to} may play a more significant role in atrial repolarization than in ventricular working muscle myocytes. The presence of the constitutively active $I_{K,ACh}$ in atrial myocytes from AF dogs shows that electrical remodelling truly developed in this model. The $I_{K,ACh}$ current (both ligand-dependent and constitutively active) seems to play a significant role in canine atrial electrical remodelling, and may be a promising atrial selective drug target for suppressing AF.

Jost N, Kohajda Z, Kristóf A, Kovács PP, Husti Z, Juhász V, Kiss L, Varró A, Virág L, Baczkó I. Atrial remodeling and novel pharmacological strategies for antiarrhythmic therapy in atrial fibrillation. *Curr Med Chem*. 2011;18(24):3675-94

Juhász V, Hornyik T, Benák A, Nagy N, Husti Z, Pap R, Sággy L, Virág L, Varró A, Baczkó I. Comparison of the effects of $I_{K,ACH}$, I_{Kr} , and I_{Na} block in conscious dogs with atrial fibrillation and on action potentials in remodeled atrial trabeculae. *Can J Physiol Pharmacol*. 2018 Jan;96(1):18-25

This work was supported by grants from the National Research, Development and Innovation Office (NKFIH K-1 19992, GINOP-2.3.2-15-2016-00006, GINOP-2.3.2-15-2016-00012 and GINOP-2.3.2-15-2016-00047), and EFOP (LIVE LONGER EFOP-3.6.2-16-2017-00006).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC023

Ionised concentrations in Ca^{2+} and Mg^{2+} buffers must be measured not calculated

J.A. McGuigan¹, J.W. Kay² and H.Y. Elder³

¹*Institute of Physiology, Berne, Switzerland*, ²*School of Mathematics and Statistics, Glasgow, UK* and ³*School of Life Sciences, Glasgow, UK*

Modelling intracellular regulation of Ca^{2+}/Mg^{2+} is important in physiology but the validity of conclusions drawn from such studies depends on the accuracy of measured values for resting $[Ca^{2+}]/[Mg^{2+}]$, their changes and the pK^I values for the intracellular binding of Ca^{2+}/Mg^{2+} . The correctness of these parameters depends on the accuracy of Ca^{2+}/Mg^{2+} buffers in which the $[Ca^{2+}]/[Mg^{2+}]$ can either be calculated or measured. Calculation is usual. There are seven freely available software programs; all give different answers for the $[Ca^{2+}]/[Mg^{2+}]$ (by a factor of around four). This is due to: 1) Calculations for changes in ionic strength are based on single ion activity coefficients, which cannot be experimentally determined; 2) pH_a (activity) has to be converted to pH_c (concentration); not possible, because of potential changes at the reference electrode. If values in buffers are calculated, the coefficient of variation cannot be determined as a measure of their accuracy, nor can the calculated values be related to SI units because of unverifiable assumptions. Since calculation is not an option, concentrations must be measured. There are two methods for this, both based on the Ligand Optimisation Method (LOM); Method 1, calibration solutions from 0.5 to 4 mmol/l (McGuigan et al, 2017); Method 2, calibration solutions from 1 μ mol/l to 2 mmol/l (Allen et al, 1977). Both methods can be used to calibrate Ca^{2+}/Mg^{2+} -electrodes. Only Method 2 can be directly used to calibrate fluorochromes and photochromes (e.g. Aequorin). The Excel program (Kay et al, 2008) to calculate $[Ca^{2+}]/[Mg^{2+}]$ in buffers when using Ca^{2+}/Mg^{2+} -electrodes, has been extended using the statistical program "R" for electrodes and now for fluorochromes and Aequorin. Method 1 is routine and laboratory glassware

can be used if adequately washed (Tran et al, 2018). All it requires is a $\text{Ca}^{2+}/\text{Mg}^{2+}$ -electrode, a 3 mol/l KCl reference electrode and a pH meter in mV mode. Method 2 is exact, but requires special laboratory facilities to reduce $\text{Ca}^{2+}/\text{Mg}^{2+}$ contamination to an absolute minimum.

Measurement of buffer $[\text{Ca}^{2+}]/[\text{Mg}^{2+}]$ must be routinely carried out; best done by calibrating $\text{Ca}^{2+}/\text{Mg}^{2+}$ -electrodes with LOM and software written in "R". Such calibrated electrode can be used to calibrate fluorochromes and photoproteins. If $[\text{Ca}^{2+}]/[\text{Mg}^{2+}]$ in buffers are calculated, the parameters used in modelling show the same degree of variability as the software programs. The values for resting $[\text{Ca}^{2+}]$, its changes and the pK^i values for intracellular binding of $\text{Ca}^{2+}/\text{Mg}^{2+}$ based on calculated values in buffers are not only wrong, but have to be re-determined using buffers with measured $[\text{Ca}^{2+}]/[\text{Mg}^{2+}]$. Uncritical acceptance of parameters based on calculated values in $\text{Ca}^{2+}/\text{Mg}^{2+}$ buffers means that conclusions reached from such modelling of intracellular regulation of $\text{Ca}^{2+}/\text{Mg}^{2+}$ must now be critically re-examined.

Allen, D. G., Blinks, J. B. & Prendergast, F. G. (1977). Aequorin Luminescence: Relation of light emission to calcium concentration-A calcium independent component. *Science*, 195, 996–998.

Kay, J. W, Stevens R, McGuigan J. A. S. & Elder H. Y. (2008). Automatic determination of ligand purity and apparent dissociation constant (K_{app}) in $\text{Ca}^{2+}/\text{Mg}^{2+}$ buffer solutions and the K_{app} for $\text{Ca}^{2+}/\text{Mg}^{2+}$ anion binding in physiological solutions from $\text{Ca}^{2+}/\text{Mg}^{2+}$ -macroelectrode measurements. *Computer in Biology and Medicine*, 38, 101-110.

McGuigan, J. A. S., Kay, J. W. & Elder, H. Y. (2017). Ionised concentrations in calcium and magnesium buffers: standards and precise measurement are mandatory. *Progress in Biophysics and Molecular Biology*, 126, 48-64.

Tran, V., Parks, M. C. H. & Stricker, C. (2018). An improved measurement of the Ca^{2+} -binding affinity of fluorescent Ca^{2+} indicators. *Cell Calcium*, 71, 86 – 94.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC024

Differences in atrioventricular conduction time and ventricular depolarization and repolarization time according to age and sex

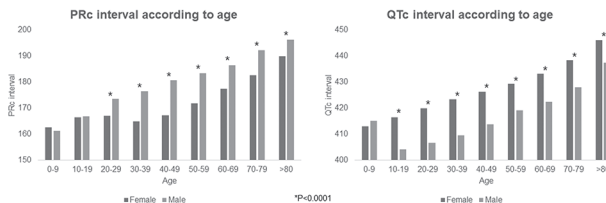
I. OH, J. LEE and Y. CHO

Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Korea (the Republic of)

Long and short PR interval or QT interval are related with cardiovascular outcomes in patients with cardiovascular diseases. This study was aimed to investigate the change and difference of PR interval, an index of atrioventricular conduction time, and QT interval, an index of ventricular depolarization and repolarization time, according to gender and age in healthy people.

We analyzed 121,286 electrocardiograms from 60 to less than 100 beats per minute among people performed for health surveillance at Seoul National University Bundang Hospital for the past 10 years. The mean of PRc interval and QTc interval corrected by RR intervals using Bazett's formula, were compared according to age and sex.

PRc interval was longer in male than female by 11.2 msec (female 170 ± 24 vs male 182 ± 24 , $P < 0.0001$) and QTc interval was longer in female than in male by 11.5 msec (female 428 ± 23 vs male 416 ± 23 , $P < 0.0001$). There was no significant difference in PR and QT interval between sex and age, sex and gender before puberty. There was a difference between men and women after puberty, and this difference was decreased after menopause (sixth decade). In the case of PR interval, there was a difference in the lengthening of the male after the third decade, and in the case of the QT interval, the lengthening of the female was observed after second decade. PR interval and QT interval have a clear difference between men and women. This suggests that there is a gender difference in the atrioventricular conduction time and ventricular repolarization and depolarization time. Therefore, further study on the gender differences in cardiovascular diseases is warranted.



PRc and QTc interval according to sex and age

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC025

Dyadic targeted Ca^{2+} indicator reveals heterogeneity in Ca^{2+} release between dyads that is modified by G-protein coupled receptor (GPCR) signaling

K. Demydenko, K. Sipido and H. Roderick

Cardiovascular Sciences, KU Leuven, Leuven, Belgium

Cardiomyocyte contraction is mediated by electrically-evoked increases in intracellular calcium $[Ca^{2+}]_i$ via the process of excitation-contraction coupling (ECC). During ECC, depolarization of the sarcolemma induces opening of voltage-gated L-type Ca^{2+} channels ($Ca_v1.2$), allowing Ca^{2+} entry into the cell. This Ca^{2+} influx in turn stimulates massive Ca^{2+} release via ryanodine receptor channels (RyR2) on the underlying sarcoplasmic reticulum. $Ca_v1.2$ and RyRs are colocalised in cellular microdomains termed dyads that are distributed throughout the volume of ventricular

myocyte allowing homogenous Ca^{2+} release in response to the action potential. Cardiomyocyte Ca^{2+} handling is influenced by signals downstream of GPCR such as Endothelin (ET-1) and Angiotensin (Ang II) receptors, which promote inotropy and spontaneous Ca^{2+} release events (1). The molecular mechanisms underlying the modulation of dyadic Ca^{2+} signaling by ET-1 and Ang II are not resolved. Experiments were performed in ventricular myocytes isolated from 6 week old WKY rats. Rats were sacrificed in accordance with local ethical and welfare guidelines. Subcellular dyadic Ca^{2+} release following stimulation with ET-1 (100 nM) and Ang II (1 μM) was reported using a genetically encoded dyad-targeted GFP-based Ca^{2+} indicator (GCaMP6f-triadin), expressed via an adenovirus (2). GCaMP6f-triadin fluorescence was recorded at 23°C in field-stimulated myocytes by linescan imaging using a Nikon A1R confocal microscope. Images were processed using ImageJ and Matlab. Data are reported as the mean $\pm$ SEM for n cells. Statistical analysis was by paired Student's t-test or One-way ANOVA. GCaMP was targeted to the dyad and reported electrically-evoked and caffeine-induced increases in $[\text{Ca}^{2+}]_i$. ET-1 increased the rate of Ca^{2+} release ($\Delta F/\text{ms}$) following electrical stimulation (ET-1: 0.30 ± 0.02 vs 0.24 ± 0.02 pre-treatment n=9, $p<0.05$) whereas it was not affected by Ang II (Ang II: 0.25 ± 0.02 vs 0.27 ± 0.02 pre-treatment n=8, $p=0.59$). Neither ET-1 nor Ang II affected the heterogeneity of Ca^{2+} release between dyads (ET-1: 34.72 ± 2.654 vs 31.36 ± 1.0 ms pre-treatment n=9, $p=0.29$, and Ang II: 36.08 ± 1.28 vs 31.06 ± 3.19 ms pre-treatment n=8, $p=0.11$, respectively). Ang II treatment significantly altered the mean latency (L) and mean deviation of the onset of Ca^{2+} release (L_{md}) ($L = 17.59\pm 2.16$ vs 21.94 ± 1.41 ms, $p=0.04$; $L_{\text{md}} = 11.83\pm 1.28$ vs 16.00 ± 0.82 ms for control vs Ang II, n=8, $p=0.0008$). Whereas ET-1 had no effect ($L = 15.63\pm 0.59$ vs 17.27 ± 1.24 ms, $p=0.31$; $L_{\text{md}} = 12.29\pm 0.37$ vs 12.79 ± 0.69 ms, n=9, $p=0.53$, control vs ET-1). Our experiments show that dyads exhibit highly heterogeneous Ca^{2+} release that is further modified by GPCR stimulation. Our data also suggest that despite activating similar signaling cascades, Ang II and ET-1 elicit different effects on the local Ca^{2+} release.

Drawnel FM *et al.* (2013). *Br J Pharmacol* 168(2), 296–317.

Shang W *et al.* (2014). *Circ Res* 114(3), 412–420.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC026

High content screening of adjuvant chemotherapy in breast cancer and cancer treatment-related cardiomyopathy

A.R. Obergrussberger¹, K. Juhasz^{1,2}, O. Reinhardt³, E. Dragicevic¹, U. Thomas¹, M. Beckler¹, S. Stölzle-Feix¹, F. Alves^{3,4} and N. Fertig¹

¹Nanon Technologies GmbH, Munich, Germany, ²Institute for Nanoelectronics, Technische Universität München, Munich, Germany, ³Translational Molecular Imaging Group, MPI of Experimental Medicine, Göttingen, Germany and ⁴Clinic of Hematology and Medical Oncology, University Medical Center Göttingen, Göttingen, Germany

More patients are surviving cancer than ever before and this makes co-morbidity factors such as cardiotoxicity important issues in the quantity and quality of life of cancer survivors. It is important to be able to assess the cardiotoxic risk of new and existing cancer therapies. Changes in electrical impedance of cell-covered electrodes, for example, is one possible method. Alterations in the electrical impedance occur due to changes in morphology, cell contact, confluency and contractility which result from toxic effects on cells. Measurements using electrical impedance are non-invasive and can be monitored over long periods of time.

In this study, electrical impedance was used to monitor cell regrowth in breast cancer *in vitro* following chemotherapy treatment. In order to assess the cardiotoxic potential of this treatment, the same combination therapy was tested on human stem cell-derived cardiomyocytes (hSC-CMs). The combination of cyclophosphamide, adriamycin (doxorubicin) and 5-fluorouracil (CAF) administered for 4 months is a standard treatment for breast cancer patients and although often initially successful, tumor recurrence after this therapy remains a major cause of mortality in breast cancer patients. Using a murine carcinoma cell line (H8N8), we were able to investigate the effect of CAF treatment *in vitro* on untreated cancer cells and on tumor regrowth on H8N8 T3.2 cells previously treated with the regime. In addition, we tested the CAF treatment and paclitaxel on hSC-CMs. Paclitaxel caused a concentration and time-dependent reduction in viability of hSC-CMs as measured by a decrease in base impedance (500 nM paclitaxel caused a $49.2 \pm 3.5\%$ reduction in base impedance after 48 hours exposure compared with control, $P < 0.001$ using a Student's t test) and caused a decrease in mean beat amplitude (500 nM paclitaxel caused a $54.5 \pm 11.9\%$ reduction in amplitude after 48 hrs compared with control, $P < 0.001$, Student's t test) indicating an effect on contractility. Doxorubicin caused a decrease in base amplitude ($>70\%$ decrease compared with control, $P < 0.001$, Student's t test) when present in any combination (alone or with cyclophosphamide or 5-fluorouracil) whereas cyclophosphamide or 5-Fluorouracil did not affect cell viability when applied alone or in combination with each other ($<10\%$ decrease in base impedance compared with control, $P > 0.05$, Student's t test). Doxorubicin also caused a decrease in mean beat amplitude indicating a change in contractility (amplitude decreased $>85\%$ compared with control, $P < 0.001$, Student's t test).

In summary, using electrical impedance, acute and long-term effects of cancer therapy on cell proliferation of tumor cells, and cardiotoxic effects of chemotherapeutics can be identified. Such *in vitro* experiments are important to identify potential novel cancer therapeutics and also to promote cardiovascular health during cancer therapy.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC027

Remodelling of the ryanodine receptor clustering patterns in acute right ventricular heart failure following monocrotaline-induced pulmonary arterial hypertension

M.E. Hurley, T.M. Sheard, R. Norman, E. Pervolaraki, K.K. Narayanasamy, H.M. Kirton, Z. Yang, D. Steele, E. White and I. Jayasinghe

University of Leeds, Leeds, UK

Cytoplasmic calcium signalling is the principal determinant of contractility within cardiac muscle. Within the junctional membrane complex, clusters of the ryanodine receptor (RyR) are located upon the cell's internal calcium store. This forms a structural unit, known as a couplon, which mediates intracellular calcium release. We recently reported structural remodelling of RyR cluster pattern within the couplon in right ventricular heart failure (RV HF). At a local level, the functional implication of this structural remodelling is not fully understood.

Isolated right ventricular cardiomyocytes were obtained from adult male Wistar rats. Rodents were either injected (intraperitoneal) with monocrotaline (60 mg/kg) to induce pulmonary arterial hypertension which led to compensated RV HF (MCT-RV) or with saline (Ctrl-RV) as previously established (Fowler et al. 2015). Cardiomyocytes from MCT-RV and Ctrl-RV were imaged using the new super-resolution microscopy method, DNA-PAINT, exploiting its ability to resolve single RyR *in situ*. All results are displayed as mean $\pm$ SEM.

Remodelling was observed in the form of dispersed RyR array patterns within the peripheral (sub-sarcolemmal) couplons. This was evident by a significant reduction in MCT-RV average peripheral RyR cluster size (MCT-RV 3437 ± 469 nm²; Ctrl-RV 11246 ± 1901 nm², $p < 0.05$ t-test, $n = 3$). Subsequently, MCT-RV had a significant reduction in RyR density per cluster, with a near halving of RyR per cluster compared to Ctrl-RV (MCT-RV 2.31 ± 0.21 puncta; Ctrl-RV 3.83 ± 0.55 puncta, $p < 0.05$ t-test, $n = 3$). Fragmentation of the near-surface RyR cluster pattern was observed within MCT-RV cardiomyocytes. This was represented by a significantly decreased proximity between an RyR puncta and its three nearest neighbours (MCT-RV 61 ± 0.97 nm; Ctrl-RV 64 ± 0.99 nm, $p < 0.05$ t-test, $n = 3$).

Using a correlative imaging protocol, we have examined the functional implication of an altered RyR pattern upon a cardiomyocyte's local calcium signalling

properties. With this, we observe an increased frequency of spontaneous calcium release in the local regions which exhibit a fragmented RyR pattern.

Fowler, E.D. et al. (2015) Decreased creatine kinase is linked to diastolic dysfunction in rats with right heart failure induced by pulmonary artery hypertension. *Journal of Molecular and Cellular Cardiology* 86, 1-8

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC028

The effects of TNF- α and IL-1 β on intracellular calcium handling and contractility in sheep ventricular myocytes

N.E. Hadgraft and D.J. Greensmith

Biomedical Research Centre, University of Salford, Salford, UK

Pro-inflammatory cytokines such as TNF- α and IL-1 β are implicated in the pathogenesis of inflammatory diseases such as sepsis. In sepsis, myocardial depression is a leading cause of death. To design novel therapeutic approaches, it is essential that we understand how these cytokines mediate the cellular basis of this cardiac dysfunction. Previous studies, including Greensmith and Nirmalan (2013), demonstrate that perturbed intracellular calcium handling and contractility can account for many aspects of myocardial depression. However, many of these studies suffer important limitations in that (A) small mammal models were used and (B) it remains unclear how pro-inflammatory cytokines affect certain key aspects of cellular function. To address this, we re-characterised the effects of TNF- α and IL-1 β on intracellular calcium handling using a large animal model then investigated their effects on the ryanodine receptor (RyR) and myofilament sensitivity to calcium.

Sheep ventricular myocytes were used for all experiments. All procedures used accord with the Animals (Scientific Procedures) Act, UK, 1986 and Directive 2010/63/EU of the European Parliament. Intracellular calcium and contractility dynamics were measured by epi-fluorescent photometry and video sarcomere detection respectively. Cells were separately exposed to 50 ng/ml TNF- α and IL-1 β acutely and when required, excited using field stimulation (0.5 Hz). Intracellular Ca²⁺ waves were facilitated using 0.3 mM Ouabain and 5 mM Ca²⁺. SR Ca²⁺ content was estimated using the amplitude of 10 mM caffeine-evoked Ca transients.

TNF- α and IL-1 β decreased SR Ca²⁺ content by (mean $\pm$ SEM) 27 $\pm$ 3 (p <0.05) and 41 $\pm$ 3 (p <0.05) % respectively accounting for a 17 $\pm$ 3 (p <0.05) and 24 $\pm$ 7 (p <0.05) % decrease of systolic calcium. Only with TNF- α did reduced systolic Ca²⁺ translate to reduced systolic shortening (20 $\pm$ 8 (p <0.05) %). We observed no negative effect on the rate of systolic or caffeine-evoked Ca²⁺ decay. TNF- α and IL-1 β decreased Ca²⁺ wave amplitude by 46 $\pm$ 6 (p <0.001) and 34 $\pm$ 7 (p <0.001) % and increased frequency by 38 $\pm$ 8 (p <0.001) and 28 $\pm$ 11 (p <0.001) %. Both cytokines reduced SR Ca²⁺ threshold for waves. In saponin-permeabilised cells the degree of sarcomere shortening over equivalent calcium ranges was attenuated.

These findings confirm that in sheep, TNF- α and IL-1 β decrease SR Ca²⁺ content leading to reduced systolic Ca²⁺. The increase of Ca²⁺ wave frequency and decrease of amplitude suggests both cytokines increase ryanodine receptor open probability; confirmed by a decreased SR Ca²⁺ threshold for waves. So while the reduction of SR Ca content does not appear to be SERCA-dependent, increased RyR leak may contribute. The loss of contractile response to increased Ca²⁺ suggests that both cytokines reduce myofilament Ca²⁺ sensitivity.

These findings advance our understanding of cytokine-mediated cardiac intracellular Ca²⁺ dysregulation and provide additional cellular substrates for myocardial depression in sepsis.

Greensmith DJ, Nirmalan M. The effects of tumor necrosis factor-alpha on systolic and diastolic function in rat ventricular myocytes. *Physiol Rep.* 2013;1(4):e00093.

We would like to thank the labs of Professor Andrew Trafford and Dr Katharine Dibb for provision of sheep myocytes.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC029

CRISPR-based RyR2 gene editing in patient-derived stem cell cardiomyocytes to study and treat rare changes in inherited cardiac arrhythmia

M. Lettieri¹, C. Facchi¹, A. Trafford¹, A. Adamson² and L. Venetucci¹

¹Cardiovascular Sciences, University of Manchester, Manchester, UK and ²Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

Introduction: The advent of next generation sequencing has hugely enhanced our ability to sequence DNA but has also created a new clinical challenge in the variant of unknown significance (VUS). A VUS is a rare change in DNA sequence that based on our current knowledge cannot be classified as pathogenic or benign. The VUS challenge is very prominent in the case of the cardiac ryanodine receptor gene (RyR2). Mutations of RyR2 can cause life-threatening arrhythmias which can lead to sudden cardiac death in young and apparently healthy individuals. Induced pluripotent stem cell derived-cardiomyocytes (iPSC-CMs) from patients carrying the VUS in addition to iPSC-CMs where the VUS has been corrected using CRISPR technology could be used as a novel approach to determine pathogenicity of a VUS. **Objective:** In this work we tested various CRISPR-based strategies to either correct a RyR2 VUS or suppress the expression of the VUS allele in iPSC-CMs. The iPSC-CMs were generated from a patient who suffered a cardiac arrest and carries a nonsense change of the RyR2 gene (R4790*).

Methods and Results: Initially we attempted direct genome editing of the VUS using an endonuclease cut followed by homology directed repair. We used two CRISPR-Cas enzymes: Cas9 and Cas12a. This approach failed because of low cutting efficiency at the site of the mutation. We then tried to induce a premature stop

codon on the VUS allele via a CRISPR-Cas9 mediated DNA cut followed by non-homologous end joining DNA repair. This approach induced an early stop codon as demonstrated by next generation sequencing (5 different clones analysed). An early stop codon is expected to decrease the levels of mRNA by causing nonsense mediated decay; however, this did not occur in our experiments. The expression levels of mRNA carrying the early stop codon and the VUS did not decline as shown by qPCR (n=3 per each clone). Finally, we attempted to reduce VUS allele expression by induction of a large deletion in the *RyR2* VUS allele. Suppression was obtained by inducing a big gene deletion which includes two different gRNAs and insertion of puromycin resistance cassette by homologous directed DNA repair. *RyR2* gene deletion was confirmed by Sanger sequencing of both genomic and coding DNA (12 clones analysed).

Conclusions: This study shows that CRISPR technology can edit the *RyR2* gene; however, the success of the gene editing is dependent on the DNA region targeted. Moreover, allele suppression in the *RyR2* gene can be only achieved by a big deletion of the gene. As recent evidences on mouse models of *RyR2*-derived arrhythmias demonstrated that silencing of *RyR2* mutant mRNA reduces arrhythmia episodes, our approach may develop a novel therapeutic strategy for *RyR2*-derived arrhythmias.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC030

Cardiac Atrial Acidic Organelle Proteomics: A Method to Analyse Lysosomal Proteins

T. Ayagama¹, R. Capel¹, R. Fischer², H. Kramer³ and R. Burton¹

¹Pharmacology, University of Oxford, Oxford, UK, ²Target Discovery Institute, University of Oxford, Oxford, UK and ³Biological Mass Spectrometry and Proteomics Facility, Imperial College London, London, UK

Atrial fibrillation (AF) is a growing public health problem without adequate therapies. Understanding the underlying mechanisms involved in AF is fundamental in developing new therapeutic strategies. Recent research has suggested an important role for calcium-dysregulation in AF ^[1]. Lysosomal calcium signalling is a little-studied pathway in the heart which has been implicated in the adrenergic responses of both atrial ^[2] and ventricular myocytes ^[3]. Published work has suggested that inhibition of the lysosomal Ca²⁺ release pathway is protective against adrenergically-induced arrhythmias ^[4].

Studying the proteomic expression of variously sized acidic organelles relies on the suitable isolation of these organelles in separate density fractions. Here we describe a fractionation protocol which allows us to study organellar proteins in guinea-pig atria (experiments performed in accordance with Home Office Guidance on the

Animals (Scientific Procedures) Act (1986)). Amongst the different density gradient fractions obtained, the three most relevant fractions, confirmed using Western Blot and MS/MS peptide analysis, were yielded from an adapted discontinuous Percoll, and sucrose differential density gradients. The first saved fraction consists of the whole tissue lysate; the second fraction (1.3 g/mL) contains fully mature lysosomes and some sarcoplasmic reticulum (SR) and mitochondrial contamination; the third fraction (1.04g/mL) consists of endo-lysosomes and exosomes with negligible contamination from SR or mitochondria. Western blots for LAMP2, V type H⁺ ATPase, COX IV and Phospholamban were performed to quantify the different organelles (lysosome, mitochondria and SR) in the respective fractions. The organelle purification protocol was first developed on mice and optimised in the guinea pig. MS/MS peptide analysis was performed on a QExec LumosTM mass spectrometer. The proteomic pathway analysis of the wild type guinea-pig atrial tissue fractions was performed using Panther Pathway. Atrial specific proteins such as Myosin heavy chain 7 (MYH7), peptidyl Glycine amidating monooxygenase (PAM) and atrial natriuretic peptide (NPPA), were detected in our whole cell tissue lysate. In the endo-lysosomal and exosome fraction, proteins as such Beta Arrestin - 1 (ARRB1, involved in intracellular trafficking pathway) and autophagy promoting protein BAG3 (intracellular transport chaperone protein which transport parts of the cytoskeleton to lysosomes during cellular stress) were detected. The molecular, biological, functional and protein networks information was extracted using GeneMANIA, UniProt, NCBI databases and protein-hits were cross-checked with the published research literature. This method allows us to explore quantitatively the protein interactions. This will help us to identify differences in protein expression, specifically related to lysosomes and globally which will inform future research.

Heijman, J., et al. (2014) Front Pharmacol 5, 30.

Collins, T.P. et al. (2011). Cell Calcium 50, 449-58

Capel, R.A. et al. (2015). J Biol Chem 290, 30087-98

Nebel, M. et al. (2013). J Biol Chem 288, 16017-30.

RABB is funded by a Sir Henry Dale Wellcome Trust and Royal Society Fellowship (109371/Z/15/Z), and holds a Senior Research Fellowship at Linacre College, Oxford. RAC is a post-doctoral scientist funded by the Wellcome Trust and Royal Society. TA received funding from the RCF, Medical Sciences Division (University of Oxford), the Nuffield Benefaction for Medicine and the Wellcome Institutional Strategic Support Fund (ISSF), University of Oxford.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC031

In vivo non-invasive imaging of inflammation in a preclinical model of myocardial infarction using ^{18}F -LW223 and Positron Emission Tomography

M.G. MacAskill^{1,2}, N. Spath¹, T. Walton^{1,2}, L. Williams³, T. Morgan³, N. Sloan³, C.J. Alcaide-Corral^{1,2}, W. Mungall¹, M.R. Dweck¹, G. Gray¹, D. Newby¹, C. Lucatelli², A. Sutherland³, S.L. Pimlott^{4,5} and A.A. Tavares^{1,2}

¹University/ BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK, ²Edinburgh Imaging, University of Edinburgh, Edinburgh, UK, ³School of Chemistry, University of Glasgow, Glasgow, UK, ⁴School of Medicine, University of Glasgow, Glasgow, UK and ⁵NHS Greater Glasgow and Clyde, Glasgow, UK

Introduction:

For several decades, the 18kDa Translocator protein (TSPO) has been a molecular imaging target for inflammation. Recently, evidence has emerged indicating significant cardiovascular inflammatory events such as myocardial infarction (MI) can lead to subsequent neuroinflammation, all of which can be detected by targeting TSPO¹. We have developed a novel TSPO ligand, LW223, which is the first fluorinated TSPO radiotracer with binding in human tissue independent of the rs6971 polymorphism. This study aims to assess the uptake of ^{18}F -LW223 during MI using the rat permanent coronary artery ligation model.

Methods:

Adult male Sprague-Dawley rats were anaesthetised using 1.5–2.5% isoflurane (50/50 oxygen/nitrous oxide, 1 L/min) before undergoing permanent ligation of the left anterior descending artery to induce MI (n=6). On day 7, PET/CT imaging with ^{18}F -LW223 was performed. Naive male Sprague-Dawley rats were used as a healthy control comparison (n=6). The standard uptake value relative to blood pool (SUVr) of ^{18}F -LW223 in the heart, brain and lungs of MI and healthy controls were analysed using an unpaired t test. Sub regions of the heart were also analysed. Results are shown as mean $\pm$ SEM.

Results:

Day 7 PET imaging demonstrated a significant increase in ^{18}F -LW223 SUVr within the infarcted whole myocardium vs. healthy (3.7 ± 0.4 vs. 2.0 ± 0.1 respectively, $p=0.0020$). The infarcted sub region of the MI hearts had a significantly lower SUVr than a comparable sub region in healthy animals (2.6 ± 0.4 vs. 4.4 ± 0.3 respectively, $p=0.0071$), which was expected due to decreased perfusion. The brains of MI animals had a significantly increased SUVr vs. healthy animals (0.8 ± 0.1 vs. 0.4 ± 0.03 respectively, $p=0.0053$, Fig. 1.B). Finally, the lungs of the MI animals also had increased uptake (4.9 ± 1.3 vs. 2.0 ± 0.1 respectively, $p=0.0470$).

Conclusion:

^{18}F -LW223 SUVr values are increased in the heart, brain and lungs at day 7 post myocardial infarction. Future efforts are now focussed on kinetic modelling of ^{18}F -LW223 uptake in these animals using radiometabolite corrected arterial input functions.

Thackeray JT *et al.* *J Am Coll Cardiol* 2018;71:263–275.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC032

Role Of Reactive Oxygen Species On Fibrotic Elimination And Functional Restoration In Cryoinjured Model Of Myocardial Infarction In Zebrafish

M. Sekar, S. sankar and M. Jayabalan

Department of Anatomy, University of Madras, Chennai, India

Lack of regeneration and formation of permanent fibrous scar are the main hallmarks of post Myocardial infarcted heart in most of the mammals and human, which eventually causes cardiac failure. However, some of the Urodels and Teleosts (zebrafish) have the capacity to regenerate their injured part and attain complete structural and functional recovery. The mechanism of cardiac regeneration and the exclusion of fibrotic scar in zebrafish still remain unclear. Reactive oxygen species (ROS) are important signalling molecules involved in cellular proliferation and migration. **Aim:** The objective of the study is to analyse the role of ROS on dual events (fibrogenesis and fibrinolysis) in the regenerating heart of zebrafish after myocardial injury. **Materials & Methods:** Adult zebrafish of both sex were used in the present study, the entire experiment was approved by Institutional animal ethical committee, Dr. ALM PG IBMS, University of Madras, Taramani campus, Chennai, India. The cryo-injury model was created in adult zebrafish by anesthetizing the animal with 0.25 % of Tricain. The pectoral region was opened and after locating the heart, the injury was created using cryoprobe. The injured animals were grouped into 0day, 3days, 7days, 15days, 30days, 45 days & 60days POP (post-operative period). The animals were subjected to various functional and structural analyses like ECG, morphology, histology, immunohistochemistry, *in-situ* production of ROS and gene expression study by Real time PCR. **Results:** The electrocardiogram analysis showed significant alteration in the ECG pattern with ST elevation in initial post-operative periods (3, 7, 15) and it was reversed towards normal as the POP further progressed. The histology and immunohistochemical studies showed the presence of fibrin in 3, 7 and 15days POP and the amount of fibrin was regressed and was replaced by collagen in 30 and 45 POP; interestingly, the collagen also was replaced by the cardiac myocyte in 60 days POP. The ROS study showed increased production in 0, 3 and 7 POP then showed decline pattern with advanced POP. The gene expression study showed significant increase in *TGFβ* ($p<0.001$), *smad 2* ($p<0.001$), *TIMPs* ($p<0.001$), *MMPs* ($p<0.001$), *Ki67* ($p<0.001$) in 0,3, and 7 days POP when compared to the control and were repressed significantly (0.001) in 30,45 and 60-days POP however it is still at higher level when compared to control. Interestingly, in 15days POP all the above-mentioned genes were significantly repressed when compared to the other groups. Taken together, the present study emphasizes that ROS plays crucial role in regulating the fibroblast on its dual role functioning (fibrogenesis and fibrinolytic) via *TGFβ* mediated signalling.

This study carried out with financial support from SERB-DST (Young scientist scheme), Government of India, India

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC033

Changes in the morphology and density of transverse (t)-tubule networks in sheep atrial myocytes during postnatal development.

C. Smith, C. Quinn, Z. Sultan, H. Najem, D. Eisner, C. Pinali, A. Trafford and K. Dibb
Cardiovascular Sciences, University of Manchester, Manchester, UK

Transverse (t)-tubules are invaginations of the sarcolemma that facilitate excitation-contraction coupling in cardiac myocytes. T-tubules are absent or sparse at birth and develop postnatally, however this process is poorly understood, especially in the atria. Understanding how t-tubules develop normally and influence Ca^{2+} handling could inform strategies to restore t-tubules in disease. Here, we have used serial block-face scanning electron microscopy (SBFSEM) to reconstruct high-resolution 3-D models of t-tubule networks in sheep atrial myocytes from different developmental time points in order to understand how their morphology and density changes throughout postnatal development. Adult (~18 months; AD), 3 month (3M) and newborn (NB) sheep were euthanized using an overdose of pentobarbitone (200mg/kg) with heparin (10,000IU). Hearts were quickly excised and small portions (0.5mm cubes) were fixed in Karnovsky fixative then imaged using an FEI Quanta 250 FEG SEM with Gatan 3view System. Data are reported as means $\pm$ S.E.M using a one-way ANOVA and a Holm-Sadik post hoc test with $n=5-9$ cells. We found that some t-tubules were present at birth and t-tubule density (total t-tubule volume as % of cell volume) increased between NB and 3M myocytes (NB: $0.051 \pm 0.029\%$; 3M: $0.6 \pm 0.1\%$, $p<0.001$). There was no further increase in AD myocytes ($0.41 \pm 0.098\%$, $p=0.10$). We then set out to determine whether increased t-tubule density between NB and 3M was a result of increase in t-tubule size and/or number. There was an increase in t-tubules per unit volume from $4.1 \pm 1.9 \text{ pL}^{-1}$ (NB) to $27 \pm 5.7 \text{ pL}^{-1}$ (3M), ($p<0.001$), but no significant change in AD $22 \pm 7.7 \text{ pL}^{-1}$, $p=0.378$. Similarly, there was a concurrent increase in the average surface area of t-tubules (NB: $2.09 \pm 0.56 \mu\text{m}^2$; 3M: $4.5 \pm 0.63 \mu\text{m}^2$, $p<0.05$) with no difference between 3M and AD myocytes (AD: $2.82 \pm 0.25 \mu\text{m}^2$, $p=0.34$). Unlike ventricular myocytes where t-tubules are relatively straight and uniform, we find that atrial t-tubules are morphologically diverse with branched, dilated or irregular phenotypes. Although present in adult, irregular t-tubules are more common at 3M. Our data indicated the presence of a higher proportion of very short 'stump-like' t-tubules in NB myocytes compared to AD cells (NB = 44%; AD = 23%) suggesting that atrial t-tubules may develop by invaginating from the sarcolemma to the cell centre. In summary, we have characterized the ultrastructure

of atrial t-tubules during postnatal development for the first time. Our data suggest t-tubules develop over the first 3 months of life in the sheep due to both an increase in t-tubule number and size. Finally, although we observed no significant changes in density between 3M and AD atrial myocytes, it appears as though individual t-tubule morphology may still change and become less disordered with age, which warrants further investigation.

We would like to thank all the people involved in this ongoing project for their hard work and the British Heart Foundation for their generous funding.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC034

The organisation of caveolin and cavin proteins within rat cardiac myocyte caveolae

R. Norman¹, T.M. Sheard¹, B. Nichols², I. Jayasinghe¹ and S. Calaghan¹

¹*School of Biomedical Sciences, University of Leeds, Leeds, UK and* ²*MRC Laboratory of Molecular Biology, Cambridge, UK*

Caveolae are flask-shaped invaginations of the membrane (50-100 nm in diameter) found in almost every cell of the body. They are a specialised form of lipid raft with a layer of integral caveolin proteins, covered on the intracellular surface with a filamentous network of cavin proteins. Together this is known as the caveolar coat complex (CCC) [1]. In the cardiac myocyte, caveolae have been shown to contribute to mechanoprotection and the regulation of numerous signalling pathways [2]. However, little is known about the composition and structure of caveolae in these cells. Current knowledge is largely based on experimental evidence from cell lines which express caveolin 1/2 and cavin 1/2/3, but which lack the muscle-specific caveolar proteins (caveolin 3 and cavin 4). Here we examine the organisation of caveolar proteins in the cardiac myocyte using *in situ* crosslinking of caveolin and cavin, alongside a new form of super-resolution microscopy.

Ventricular myocytes were isolated by Langendorff-perfusion of adult rat hearts with a collagenase/protease-containing solution. HeLa cells and cardiac myocytes were incubated with the cross-linker dithiobis(succinimidyl propionate) (DSP) for isolation of the CCC on a sucrose velocity gradient [1]. Myocytes attached to coverslips were dual labelled with caveolin 3 and cavin 1/4. 10X expansion microscopy (10XEX) was performed to achieve ~25nm resolution with Airyscan imaging [3]. As described by others [1], the CCC isolated from HeLa cells was ~80S in size and contained the main ubiquitous caveolar protein isoforms (caveolin 1 and cavin 1). In myocytes, the ~80S complex contained only caveolin 1 and cavin 1/4. Despite being expressed at 5 times the level of caveolin 1, caveolin 3 was absent from these complexes. Indeed, caveolin 3 migration in the sucrose gradient did not change

with DSP. However, using 10XEX, caveolin 3 and cavin 1/4 can clearly be resolved within a single caveola with high levels of co-localisation.

Together these data suggest a form of CCC in myocytes which contains both caveolin 3 and cavin 1/4, but with a different integration of caveolin 3 compared with caveolin 1.

1. Ludwig et al. (2013). Molecular composition and ultra-structure of the caveolar coat complex. *PLoS Biology* 11:e1001640

2. Norman et al. (2018). Caveolae and the cardiac myocyte. *Curr Opin Physiol.* 1:59-76

3. Sheard et al. (2019). Three-Dimensional and Chemical Mapping of Intracellular Signaling Nanodomains in Health and Disease with Enhanced Expansion Microscopy. *ACS Nano* 13:2143-2157

This work was funded by the British Heart Foundation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC035

Spatial differences in atrial calcium release in heart failure

C. Smith, J. Clarke, D. Eisner, A. Trafford and K. Dibb

University of Manchester, Manchester, UK

Transverse (t)-tubules are membrane invaginations that enable close coupling of surface L-type calcium channels and ryanodine receptors of the sarcoendoplasmic reticulum to facilitate triggered calcium release throughout the cell. In diseases such as heart failure (HF) there is disruption to the t-tubule network that contributes to dysynchronous calcium release and thus contractile dysfunction. Compared to the ventricle, atrial t-tubule remodelling in HF is more extensive with an 81% reduction in t-tubule density reported in the HF sheep atria (1). Despite the importance of atrial t-tubules, little is currently known about triggered calcium release in the atria and if this is altered in HF.

Sheep were anaesthetised with isoflurane (2-4% v/v in oxygen) and implanted with a pacemaker and pacing lead for induction of HF by ventricular pacing at 210 bpm. Meloxicam (0.5 mg/kg) and Enrofloxacin (5 mg/kg) were provided perioperatively for analgesia and antibiotics. Control and HF sheep were euthanased with 200 mg/kg intravenous pentobarbitone and cells isolated from the left atrial appendage. Perforated patch clamp experiments were performed in Fluo-3 loaded cells using a confocal microscope to assess spatial calcium release. T-tubules were simultaneously imaged in patched cells using Wheat Germ Agglutinin Alexa Fluor 647. Data is presented as mean $\pm$ SEM for 14-16 cells from 5-6 animals, compared using t-test or two way repeated measures ANOVA where appropriate.

Global calcium transient amplitude was reduced by $60.4 \pm 7.9\%$ in HF ($p < 0.001$), with the transient also $62.8 \pm 7.5\%$ slower to rise compared to control ($p < 0.001$). In control cells, triggered calcium release occurred at discrete sites on the surface

and throughout the cell corresponding to t-tubule locations. In HF, calcium release was primarily triggered at the surface with release in the centre comparatively more reliant on propagation. The loss of t-tubules in HF contributed to increased dysynchrony of calcium release with calcium transients reduced in amplitude by $23.2 \pm 17.6\%$ and $25.43 \pm 15.1\%$ slower to rise in the cell centre vs surface ($p < 0.001$). No spatial differences were observed in control cells.

The loss of t-tubules in HF contributes to reduced central calcium release in the atria. Our data indicates that triggered release occurs in discrete locations on the surface and in the centre of atrial cells, with less central release in HF likely to be associated with reduced t-tubule density.

Caldwell *et al.* (2014). *Circ Res* **115**, 986-996.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC036

Inhibition of Ca^{2+} /calmodulin-dependent protein kinase II protects against ventricular arrhythmias during the awake period

J. Taylor, M. Smith, Y. Wang and M.R. Boyett

Cardiovascular, The University of Manchester, Manchester, UK

Many cardiovascular variables, such as heart rate, cardiac contractility, stroke volume, ECG waveforms (RR, PR, QRS and QT intervals) and blood pressure, exhibit robust day-night (circadian) rhythms. Many cardiac arrhythmias also display a circadian rhythm and occur preferentially at a particular time of day or night. For example, ventricular fibrillation and sudden cardiac death demonstrate a prominent circadian rhythm, being more common in the morning on waking (Black *et al.*, 2019). A local circadian clock in the heart may be ultimately responsible for these rhythms. Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) plays a major role in Ca^{2+} homeostasis in the heart. It also plays an essential role in circadian rhythms by facilitating the heterodimerisation of the circadian clock genes, *Clock* and *Bmal1*. CaMKII overexpression has been associated with ventricular arrhythmias, and knockdown of CaMKII prevents ventricular tachycardia. In an animal model, this study aimed to determine whether CaMKII is responsible for the ventricular arrhythmias seen during the awake period. Experiments were conducted on *ex vivo* Langendorff-perfused hearts from cardiac-specific CaMKII deficient mice (expressing AC3-I inhibitory peptide) and their littermate controls (not expressing the peptide) (Wu *et al.*, 2009). The hearts were dissected at either zeitgeber time (ZT) 0 (start of the sleep period) or at ZT 12 (start of the awake period). A S1-S2 pacing protocol was used to test the vulnerability to ventricular arrhythmias. In control mice, ventricular arrhythmias were inducible at the start of awake period in 4 out of 9 hearts, but not at the start of the sleep period (0 out of 8 hearts; $P = 0.02$, Chi-squared test), which is consistent with the morning peak in ventricular

arrhythmias in the human. In contrast, in the CaMKII deficient mice, ventricular arrhythmias were not observed at the start of the awake or sleep periods (0 out of 5 hearts at ZT 0 and ZT 12). Expression of genes was measured by quantitative PCR. In CaMKII deficient mice, the expression of the circadian genes, *Bmal1* and *Clock*, in the left ventricle was increased at ZT 0 (*Bmal1*, $P<0.001$; *Clock*, $P<0.0005$; two-way ANOVA). Additionally, in the left ventricle of CaMKII deficient mice, there was a reduction in the intracellular Ca^{2+} -handling molecules *Pln* and *Ryr2* at ZT 12 ($P<0.0001$; two-way ANOVA). In summary, this study has shown a circadian rhythm in ventricular arrhythmias in the mouse that is abolished by inhibition of CaMKII. This may be linked to effects on the local circadian clock in the heart as well as on intracellular Ca^{2+} -handling.

Black, N., D'souza, A., Wang, Y., Piggins, H., Dobrzynski, H., Morris, G. & Boyett, M.R. (2019). Circadian rhythm of cardiac electrophysiology, arrhythmogenesis, and the underlying mechanisms. *Heart Rhythm* 16, 298-307.

Wu, Y., Gao, Z., Chen, B., Koval, O.M., Singh, M.V., Guan, X., Hund, T.J., Kutschke, W., Sarma, S., Grumbach, I.M., Wehrens, X.H., Mohler, P.J., Song, L.S. & Anderson, M.E. (2009). Calmodulin kinase II is required for fight or flight sinoatrial node physiology. *Proc Natl Acad Sci U S A* 106, 5972-5977.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC037

An assessment of some white blood cell and platelet parameters amongst normotensive, newly diagnosed hypertensive and known hypertensive subjects in Port Harcourt, Nigeria.

D.V. Dapper and C. Eziuzo

Department of Human Physiology, University of Port Harcourt, Port Harcourt, Nigeria, Port Harcourt, Nigeria

Reports attempting a comparative assessment of values of white blood cells (WBCs) and platelet parameters amongst normotensive, newly diagnosed hypertensive and known hypertensive subjects in Nigeria are relatively scarce. This study sought to compare these haematologic parameters of normotensive and hypertensive subjects in southeastern Nigeria. Fifty each of normotensives, newly diagnosed hypertensives and known hypertensives; age and sex matched, were recruited into the study. Using an automated haematology analyzer, the following hematological parameters were determined in all subjects: total WBC; neutrophil, lymphocyte and platelet counts; percentage value of neutrophil and lymphocytes; mean platelet volume (MPV); platelet distribution width (PDW); and total lymphocyte count (TLC). Significantly higher total WBCs and (absolute) neutrophil counts and lower percentage lymphocyte were observed amongst known hypertensive male subjects, while percentage neutrophil was significantly higher amongst known hypertensive female subjects. Platelet count and MPV were significantly higher in

hypertensive male subjects compared to their normotensive counterparts. MPV was found to be significantly lower in hypertensive female subjects, compared to normotensive females. Present study reports significantly higher leucocytes, platelet counts and MPV amongst hypertensive males and lower MPV amongst hypertensive females. Our results confirm suggestions that significant variations occur in the values of some white blood cell and platelet parameters between normotensive and hypertensive subjects; with a possible sex bias in these variations. Regular assessment of hematological parameters may perhaps be useful indicators of the prognosis of hypertension amongst our population. Our results could be of value in the management of hypertensives, since previous reports in this regard in Nigeria have been relatively scarce.

Drøyvold WB, Midthjell K, Nilsen TIL & Holmen J. (2005) *Int J Obes (Lond)* 29: 650-655

Ighoroje AD & Dapper DV (2005) *Niger J Physiol Sci.* 20: 33-38

Liu X, Zhang Q, Wu H, Du H, Liu L, Shi H, et al. (2015) *Am J Hypertens.* 28: 1339-1346.

Chu SG, Becker RC, Berger PB, Bhatt DL, Eikelboom JW, Konkle B, et al. *J Thromb Haemost.* 2010;8: 148-156.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC038

Aluminum Tainted Drinking Water Impacts Negatively and Differently on Antioxidant Status of Cardio-Renal Systems

P. Akangbou¹, A.N. CHUEMERE¹, D.V. Dapper¹, O. Ilochi² and M. Anyiyeloye³

¹human physiology, university of port harcourt, Port Harcourt, Nigeria, ²Department of Human Physiology, Madonna University, Port Harcourt, Nigeria and ³Department of Pharmacology, University of Port Harcourt, Port Harcourt, Nigeria

Aluminum is one of the most widely distributed elements in the environment and the third most prevalence and bioavailability metal comprising 8% of the earth's crust. Its adverse effect on body organs is incompletely understood. This study aims to evaluate the possible mechanistic-link of prolonged exposure to aluminum tainted drinking water (AlCl₃) on the perturbation of oxidative stress as assessed by alterations in pro-oxidant/antioxidant in the heart and kidney. Five groups of 10 healthy male wistar rats were used for the study. Normal control group received drinking water, while the cases were administered 200-800mg/kg-1 AlCl₃, orally once daily for 28 consecutive days. Thereafter, the heart and kidney oxidative markers were studied. Result showed that AlCl₃ up regulated heart and kidney lipid peroxidation significantly (<0.05) in a dose-response-organ specificity as reflected by marked increased malondialdehyde(MDA) with a concomitant diminution of glutathione peroxidase(GPx), superoxide dismutase (SOD) and catalase (CAT) activities. Correlation studies revealed that oxidative stress contributes to AlCl₃ induced cardio-renal toxicity in increasing other respectively upon comparing in

both $AlCl_3$ and control groups. Our data indicated and confirmed that aluminum tainted drinking water can induce differential pathophysiology risk to heart and kidney oxidative damage.

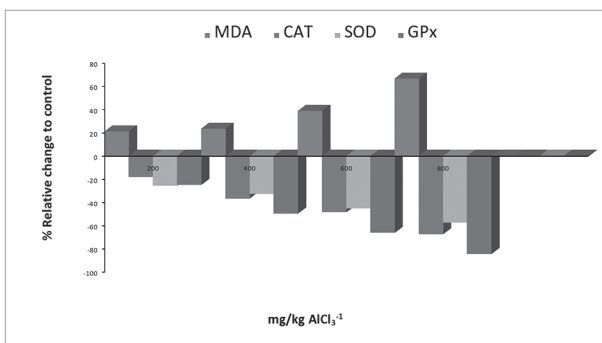


Figure 1: Effect of $AlCl_3$ induced oxidative stress in the heart

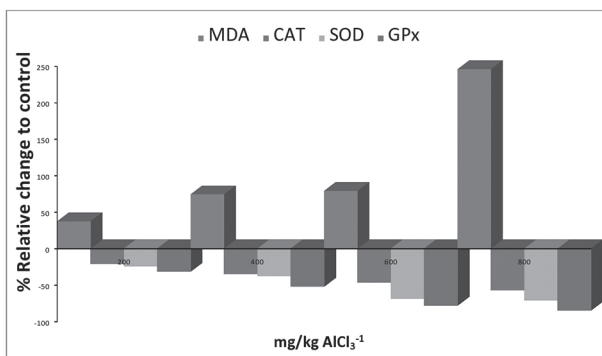


Figure 2: Effect of $AlCl_3$ induced oxidative stress in the kidney

Imam TS et al (2016) Life Sci J 13(1s):93-104

Dennis JM & Writing PK (2017). Nutrient 9(718): 1-25

Exley C (2016) Morphologie 100(329):51-55

Krewski D et al (2007) J Toxicol Environ Health Crit Rev 10(suppl 1):1-269

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC039

Genetic determinants of cardiovascular reactions in humans with different combinations of alleles in Bradykinin receptor B₂ (BDKRB2) and Angiotensin-converting enzyme (ACE) genes in response to the diving reflex

E. Podyacheva and T. Baranova

Saint Petersburg State University, Saint Petersburg, Russian Federation

The diving reflex (DR) is a set of protective functional alterations aimed at economizing oxygen consumption while diving. This effect is achieved through reflexive deceleration of the heart's rhythm, constriction of peripheral vessels (PVs) and selective redistribution of blood to those organs which are most vulnerable to hypoxia (the brain and heart). The effectiveness of DR as a protective mechanism depends on the nature of vegetative and humoral regulation in the cardiovascular system. The aim of this study is to determine whether protective cardiovascular mechanisms when diving vary in people with different polymorphisms of the BDKRB2 and ACE genes. 96 volunteers (students; aged 18-24) were examined. These subjects were not adapted to diving. DR was activated by submerging their faces in cold water (12-14°C) under laboratory conditions. ECG records were taken for the resting state, during diving simulation (DS) and after apnea. The minute volume of blood flow (MVBF) was also determined for these states using rheography, and the blood supply to peripheral vessels was measured indirectly using the pulse wave amplitude (PWA) of the photoplethysmogram (PPG). Vascular tone was calculated from the time of its spread (the pulse transit time, PTT). Blood pressure was recorded. DNA samples from all patients' blood were isolated using phenol-chloroform extraction. I/D polymorphism (rs4340) of the ACE gene was analysed using PCR. The results were analysed using Mann-Whitney nonparametric tests (paired comparisons). According to the results of the ACE polymorphism study, significant differences in PTT were found between I/D (n=27) and I/I (n=23) alleles at the control level (CL) and during DS (p<0.05). They were also found between I/I and D/D (n=21) at CL, during DS and during recovery (p<0.01). When BDKRB2 alleles were studied, significant differences in PWA between T/C (n=35) and C/C (n=24) were found in all dives and during recovery (p<0.05; p<0.01). They were also found between T/T (n=13) and C/C after two dives and two periods of recovery (p<0.01). A significant decrease in MVBF and PWA (p<0.05; p<0.01) occurred in the groups with I/D, I/I and T/C polymorphisms during DS, which is similar to that found in diving animals. In subjects with I/D and T/C polymorphisms, PWA increased in the recovery process when compared with CL (p<0.01). This reflects an increase in blood volume in the PVs and indicates oxygen debt formed during DS. When diving is simulated, constriction of PVs was observed in all subjects examined, but its severity had significant individual differences. Studies have shown that the most effective defence mechanisms against hypoxia are those which develop when diving in people with a heterozygous combination of alleles.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC040

Effects of Tender Coconut Water on The Coronary Artery and Blood Glucose of Male Diabetic Wistar Rats

C. Inneh¹, V. Oigbochie², O. Momodu² and E. Adaniwimowan²

¹Physiology, University of Benin, Benin City, Nigeria and ²Anatomy, University of Benin, Benin city, Nigeria

Diabetes is a major risk factor for cardiovascular disease (CVD). Investigations have shown that diabetics have a two to four fold higher risk of developing CVD (Kannel *et al.*, 1979). Tender coconut water (TCW) is rich in minerals (Potassium, calcium and magnesium), L-arginine, Vitamins (B and C) which are known to reduce the risk of developing Coronary Heart Disease (Anurag and Rajamohan 2003). TCW have been reported to have cardio-protective effect (Nnodim *et al.*, 2013). However, there are very few studies on its cardio-protective effect in diabetes. Therefore the aim of this study was to investigate the effects of Tender coconut water on the coronary artery of male diabetic Wistar rats. Twenty (20) male Wistar rats were selected for this study. The rats in each group were allowed access to feed and water *ad libitum*. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Diabetes mellitus was induced in rats by a single intraperitoneal injection of streptozotocin (STZ) (60mg/kg body weight) dissolved in 0.1M sodium citrate buffer (pH 4.5). Rats found to have blood glucose levels between ≥ 250 mg/dl were considered diabetic and randomly assigned into groups B, C and D. Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period of 72 hours. Before the induction of diabetes, all the rats were fasted for 16-h (overnight), but still allowed free access to water throughout. At the end of the 16-h fasting period – taken as 0 time (i.e., 0h) – blood glucose levels (initial glycemia, G_0) were determined and recorded. Rats in group A served as control; group B served as untreated diabetic group; diabetic rats in group C received 1IU/kg/day of Humulin subcutaneously and group D diabetic rats received 1ml/100g/day of TCW. The experimental period lasted for four weeks after initial glycaemia. The results obtained showed that there was a significant reduction ($P < 0.05$) in the Fasting Blood glucose concentration of diabetic rats treated with Humulin and TCW compared with untreated diabetic rats. Histological studies of the heart of untreated diabetic rats showed coronary artery with focal intimal ulceration, lumen obstruction and hypertrophy of the muscular wall. The wall of the coronary artery of Diabetic rats treated with TCW and Humulin appear normal compared with the control. Histochemistry of elastin fibers showed that there was a strong positive stain for elastin in the wall of the coronary artery of rats in control and those of diabetic rats treated with Humulin and TCW respectively. The wall of the coronary artery of untreated diabetic rats appear

negative to elastin stain. The coronary artery of Wistar rats induced with diabetic and treated with humulin and TCW showed normal vascular wall as compared with control. The results from this study indicate that Tender coconut water has both glucose lowering and cardio-protective properties in diabetic rats.

Anurag, P. and Rajamohan, T. (2003). Cardioprotective effect of tender coconut water in experimental myocardial infarction. *Plant Foods for Human Nutrition*. 58(3) 1–12

Kannel, W. B., Feinleib, M., McNamara, P. M., Garrison, R. J. and Castelli, W. P. An investigation of coronary heart disease in families. The Framingham Offspring Study. *Am J Epidemiol* 110, 281-290

Nnodim, J., Dike-Ndudim, J., Elendu, H. N., Nwagbaraocha, M., Egbuobi, R., and Onyeze, V. (2013). Antioxidant and Cardioprotective Effect of Coconut Water against Doxorubicin Induced Cardiomyopathy. *Journal of Krishna Institute of Medical Sciences*. 2(2). 37-41

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC041

An investigation of small conductance Ca^{2+} -activated K^+ current (I_{SK}) in rabbit and human atrial cardiomyocytes

A. Giommi, A.R. Gurgel, G.L. Smith, A.J. Workman and P. Saxena

University of Glasgow, Glasgow, UK

Background: The small conductance Ca^{2+} -activated K^+ current (I_{SK}) may change the cardiac atrial action potential shape in response to altered intracellular $[\text{Ca}^{2+}]$, and thus may be a potential therapeutic target for treating atrial fibrillation (AF). **Aim:** To measure $[\text{Ca}^{2+}]_i$ -sensitivity of atrial I_{SK} . **Methods:** Myocytes were enzymatically isolated from atrial tissues from patients undergoing elective cardiac surgery and from rabbit hearts. Whole-cell-patch clamp, with voltage ramps (-120 to +50 mV in 7 s), varying $[\text{Ca}^{2+}]_i$ (~100-500 nM; set using 5 mM BAPTA, verified using Fura-2), and conventional (apamin) or putative selective (ICA) I_{SK} blockers. **Results:** A +ve control was required since I_{SK} would be difficult to detect (low density): we tested temporal stability, timing, and reversibility of K^+ current (I_{K1}) block by Ba^{2+} , in rabbit. Ba^{2+} (0.5 mM) significantly decreased inward I_{K1} (at -115 mV) in 15/16 (94%) left ventricular cells, from -38.9 ± 5.9 to -12.9 ± 4.5 pA/pF (by 67%), and in 23/25 (92%) left atrial cells, from -20.3 ± 4.2 to -11.6 ± 2.9 pA/pF (by 43%) ($P < 0.05$ for each; t -test, $n = 11$ -16 rabbits). Ba^{2+} effect onset-time was 30-60 s. All responses were then stable, and reversible in 88% of 24 cells. Ba^{2+} also decreased outward I_{K1} (at -65 mV), e.g. in atrial cells: from $+1.5 \pm 2.7$ to -5.2 ± 4.0 pA/pF ($P < 0.05$). In rabbit atrial cells, with ~100 nM $[\text{Ca}^{2+}]_i$, apamin (100 nM) had no effect on inward current: -5.9 ± 0.6 vs -6.3 ± 0.6 pA/pF control ($P = 0.35$), or outward current: $+0.4 \pm 0.4$ vs $+0.7 \pm 0.3$ pA/pF ($P = 0.35$; 10c, 8r). With ~300 nM $[\text{Ca}^{2+}]_i$, apamin also had no effect on inward (-6.2 ± 0.5 vs -6.5 ± 0.6 pA/pF) or outward current ($+0.6 \pm 0.3$ vs $+0.8 \pm 0.3$ pA/pF; 26c, 11r). With ~500 nM $[\text{Ca}^{2+}]_i$, apamin again showed no effect

(-15.4 ± 4.3 vs -14.8 ± 4.0 pA/pF, and $+2.2 \pm 0.7$ vs $+2.2 \pm 0.7$ pA/pF; 10c, 7r). Furthermore, ICA ($1 \mu\text{M}$) also had no effect on rabbit inward or outward currents at any $[\text{Ca}^{2+}]_i$. In human right atrial cells, ICA was tested at $[\text{Ca}^{2+}]_i$ of ~ 100 nM (5 cells, 3 patients), ~ 300 nM (10c, 4p) and ~ 500 nM (8c, 4p); apamin at $[\text{Ca}^{2+}]_i \sim 300$ nM (7c, 3p). Neither apamin nor ICA significantly affected inward or outward current at any $[\text{Ca}^{2+}]_i$. Considering that I_{SK} might occur in only a small proportion of myocytes, potentially masked by cell-averaging, data were checked for any individual cell I_{SK} -blocker effect, of any magnitude, compatible with the +ve control onset/reversibility profile: a "candidate drug effect" (CDE). Of 84 cells studied from either species/drug, 5 CDEs (6% of cells; 1.2 ± 0.3 pA/pF) occurred, whether or not BSA (to avoid apamin adherence) or BDM (to inhibit cell contracture) were used. CDEs occurred at $[\text{Ca}^{2+}]_i$ of ~ 300 or ~ 500 nM, and not ~ 100 nM; but only with apamin, and were non-reversible. **Conclusion:** I_{SK} is either non-existent or rare, in rabbit or human atrial cardiomyocytes, at $[\text{Ca}^{2+}]_i$ typical of global diastolic-to-systolic values.

Giommi A, Gurgel A, Saxena P, Smith GL, Workman AJ

University of Glasgow, Institute of Cardiovascular & Medical Sciences

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC042

The Role of Human Ether-a-go-go-Related Gene K^+ Channels in Cardiac Arrhythmias induced by Carbon Monoxide

M. Al-Owais, N. Hettiarachchi, D. Steele, C. Peers, A. Holden and A. Benson

University of Leeds, Leeds, UK

Carbon monoxide (CO) is a well-known toxin and exposure to CO has been related to cardiovascular complaints, including arrhythmias¹. Previous studies have shown that CO blocks Ca^{2+} and Na^+ currents and can induce early afterdepolarisations in rat ventricular myocytes². We examined possible mechanisms for CO arrhythmias and the role of hERG in arrhythmogenesis in guinea pig ventricular myocytes and models of human ventricular cells and tissues. Whole-cell patch clamp recordings were made from isolated guinea pig myocytes, as well as from HEK293 cells expressing hERG gene. CO was applied via the CO-releasing molecule, CORM-2 ($1\text{--}10 \mu\text{M}$). The formation of peroxynitrite (ONOO-) in HEK293 cells was monitored fluorimetrically. Values are means $\pm$ S.E.M. The effects of CO on human ventricular myocytes were modelled by -50, +30, -65 and +105 % changes in the maximal conductances for I_{Ca} , I_{K1} , I_{Kr} and I_{Na} in the O'Hara-Rudy³ family of human ventricular cell models. Cell models and tissue models were solved with a space step of $\Delta x = 0.2 \text{ mm}$, an adaptive time step of $0.01 \text{ ms--}0.25 \text{ ms}$. Cell model conductance parameters were Gaussian distributed with a $\pm 5\%$ standard deviation. CO irreversibly inhibited hERG currents in HEK293 cells by $65.2 \pm 4.01\%$; ($3 \mu\text{M}$; $n=6$, $P < 0.001$,

Student's paired t test), the inactive form of CORM-2 had no significant effect. Current amplitudes were restored by the hERG activator, NS1643 (3 μ M, 90.8 $\pm$ 5.5%, P< 0.001, n=7). Currents were also strongly inhibited by E4031 (75 $\pm$ 5.5 % inhibition, 3 μ M, n=4, P<0.001). In guinea pig myocytes action potentials were prolonged and early afterdepolarizations were observed. Inhibition was prevented by ebselen (100nM), antimycin A, or myxothiazol, 1 μ M), suggesting CO-mediated inhibition involves mitochondria-derived ROS. Pre-treating cells with L-NAME (1mM) abolished the inhibitory effects of CORM-2, indicating CO inhibition requires Nitric oxide (NO) formation. CO raised ONOO- levels, an effect reversed by ONOO- scavenger FeTPPS, preventing inhibition of hERG tail currents, and abolishing the CO effects on APs in guinea pig myocytes⁴. Computational modelling by incorporating the CO-induced conductance changes into human cell models showed that with 5% variability, n=500, in all the maximal conductance parameters, the endo- and epi-cardial APD₉₀ and its variability is increased at a BCL of 1000ms, see table 1 for details. Prolongation of the APD₉₀ is seen at all BCL, CO abolishes alternans in the endo-, and induces alternans in the mid-myocardial cell models. The dispersion of repolarisation and the propagation velocity in ventricular tissue and wall models is decreased, and the vulnerable window is increased⁵. These data suggest that CO effects guinea pig cardiac myocytes via the ONOO- mediated inhibition of hERG channels. This inhibition is pro-arrhythmogenic in tissue models, by action potential prolongation, increase in dispersion of refractoriness, and an increased vulnerability to re-entry.

Endo APD ₉₀ /ms				Epi APD ₉₀ /ms			
Normal	CO all channels	CO I _{Kr} only	CO I _{Kr} & I _{Ks}	Normal	CO all channels	CO I _{Kr} only	CO I _{Kr} & I _{Ks}
267.5 $\pm$ 8.7	387.57 $\pm$ 17.8	367 $\pm$ 13.8,	406 $\pm$ 13.1	234.7 $\pm$ 7.3	335.7 $\pm$ 12	320 $\pm$ 11.2	334 $\pm$ 11.9

Bell ML et al. Circulation 2009; 120: 949–955.

Dallas ML et al. Am. J Respir. Crit Care Med 2012; 186: 648-656.

O'Hara T et al. PLOS Comput. Biol. 2011; 7: e1002061.

Al-Owais MM et al, FASEB J. 2017; 31(11):4845-4854.

Al-Owais MM et al, CinC. 2018; 124.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC043

Regulation of the human voltage-gated K⁺ channel Kv1.5 by Gasotransmitters; a role in the cardiovascular system.

M. Al-Owais, J. Lippiat, J. Scragg, D. Steele and C. Peers

University of Leeds, Leeds, UK

Carbon monoxide (CO), hydrogen sulphide (H₂S) and nitric oxide (NO) are now established as enzyme-derived endogenous signalling molecules of widespread physiological importance, providing a range of beneficial effects in the heart and circulation¹. CO is a product of heme oxygenase-1, and 2 (HO-1, HO-2) and accounts for many effects of HO-1². H₂S is produced via cystathionine γ -lyase, cystathionine β -synthetase and 3-mercaptopyruvate sulfurtransferase. H₂S modulates diverse cardiovascular functions³. Many redox-sensitive proteins have been implicated in atrial fibrillation (AF), including the voltage-gated K⁺ channel Kv1.5, encoded by KCNA5. Kv1.5 activates rapidly but inactivates slowly and incompletely⁴. In the heart, expression is largely confined to the atria where it is responsible for the repolarizing ultrarapid outward current, I_{Kur} , active throughout phases 1-3 of the atrial action potential (AP)⁵. Changes in Kv1.5 activity may predispose to AF-associated arrhythmia and it represents an attractive pharmacological target for AF treatment. The aim of this study was to evaluate oxidative modulation of Kv1.5 and how this is regulated by exogenous gasotransmitters. Electrophysiological recordings were made from HEK293 expressing recombinant hKv1.5. CO was applied via the CO releasing molecule CORM-2 (30 μ M), H₂S (300 μ M) was applied via NaHS and NO via an NO donor SNAP (100 μ M). All values are mean $\pm$ S.E.M. CO reduced Kv1.5 by $55.2 \pm 3.78\%$, $n=19$ measured at 50 mV ($P < 0.05$, Student's paired t test). This inhibition was reversible and dose-dependent. Application of the inactive form (iCORM) had no significant effect.

H₂S (300 μ M) irreversibly inhibited Kv1.5 by $31.5 \pm 4.7\%$, $n=8$, $P < 0.05$. When CO and H₂S were tested in combination, H₂S inhibited Kv1.5 and prevented further inhibition by CO, if H₂S was applied before CO. In contrast, if CO was applied before H₂S, a further inhibition occurred; the degree of inhibition caused by NaHS was similar to that in the absence of CORM-2 ($35.9 \pm 6.7\%$, $n=5$, $P < 0.05$). SNAP (100 μ M) inhibited Kv1.5 by $15.77 \pm 5.2\%$, $n=4$, $P < 0.05$; this effect was irreversible after a 5 min wash. Pre-treatment of cells with a nitric oxide synthase inhibitor (L-NAME, 1 mM, 1hr, 37°C) significantly reduced the inhibitory effects of CORM-2 or H₂S on Kv1.5 by $81.8 \pm 7.45\%$, $n=6$, and $91.3 \pm 3.2\%$, $n=8$, respectively, indicating involvement of NO formation.

These data indicate that the redox-sensitive Kv1.5 channel is modulated by the gasotransmitters CO and H₂S, and that both gases inhibit Kv1.5 activity via NO formation. Further studies are required to identify the underlying mechanisms in more detail and establish the potential for related therapeutic interventions that target AF.

Polhemus,DJ & Lefer,DJ. Circ. Res. 114 (2014), 730-737.

Otterbein, LE et al. Nat. Med. 9, (2003), 183-190.
Calvert, JW et al. Circ. Res. 105, (2009), 365-374.
Ravens, U & Wettwer, E. Cardiovasc. Res. 89, (2011), 776-785.
Wettwer, E et al. Circulation 110, (2004), 2299-2306.

Supported by Heart Research UK.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC044

Effects of heart failure-induced electrical remodelling on atrial arrhythmogenesis: insights from a modelling study

N.R. Alshwaira and H. Zhang

Biophysics, The University of Manchester, Manchester, UK

Heart failure (HF) and atrial fibrillation (AF) are the two of major epidemics of cardiovascular diseases behind increasing rate of morbidity and mortality. There is an increased prevalence of AF among HF patients. However, the impact of HF-induced electrophysiological changes in atrial properties on AF genesis has not been adequately studied.

The aim of this study is to study the influence of HF-induced membrane ion channel and the intracellular Ca^{2+} handling on the membrane electrical action potentials, and vulnerability of AF initiation in a response to a premature stimulus at the left atrium and pulmonary veins junction (LA-PVs junction) in an 3D model of the sheep atria.

Experimental data on HF-induced remodelling for different ion channels and Ca^{+2} handling on sheep atrial myocytes were collected from literatures, and then incorporated into the multiscale model of the sheep atria developed by Butters *et al.* (2013) at single cell and 3D organ levels. The 3D model of the sheep atria considered detailed anatomical structures and electrical heterogeneity and tissue anisotropy. In the 3D model, the S1 -S2 pacing protocol was applied at the LA/PVs junction, with two S1 stimuli being applied at a basic cycle length (BCL) of 350 ms, which were followed by four S2 stimuli applied at the same location with a shorter BCL. The vulnerability of the tissue for AF genesis was computed as the time window (VW) during which the S2-evoked excitation wave propagated only in one direction of the LA, leading to the formation of sustained re-entrant circuits. It was shown that the HF-induced electrical and Ca^{2+} handling remodelling abbreviated the action potential duration at 90% of repolarisation (APD_{90}) of both the LA and PVs cells, which is consistent with experimental observations. However, it preserved though reduced the APD_{90} dispersion by 39 ms at the junction of the LA and PVs. Owing to the APD abbreviation and the preserved dispersion, the measured VW at the LA/PVs junction was greater in HF than that in control (CTL)

condition, resulting in an increased tissue vulnerability for AF genesis at the LA/PVs junction by 46%. Further analysis showed that the Dominant frequency (DF) of the re-entry in the HF was greater in both the LA and PVs than those in CTL condition. In conclusion, this study demonstrated that the HF-induced remodelling on different ion channels and Ca^{2+} handling abbreviated atrial APD₉₀, reduced but preserved the APD₉₀ dispersion at the LA/PVs junction, increased tissue's VW for the genesis of sustained re-entry in the sheep atria.

Butters, T.D., Aslanidi, O.V., Zhao, J., Smaill, B. and Zhang, H., 2013. A novel computational sheep atria model for the study of atrial fibrillation. *Interface focus*, 3(2), p.20120067.

This project was funded by Imam Abdulrahman Bin Faisal University, Dammam, Kingdom of Saudi Arabia.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC045

Oligomerisation of the cardiac Nav1.5 channel with and without the auxiliary $\beta 3$ -subunit.

S.C. Salvage¹, J. Rees¹, A. McStea², L. Wang², M. Hirsch², C. Tynan², M.W. Reed³, J.R. Irons¹, R. Butler⁴, A.J. Thompson⁵, M. Martin-Fernandez², C.L. Huang^{6,1} and A.P. Jackson¹

¹Department of Biochemistry, University of Cambridge, Cambridge, UK, ²Central Laser Facility, Research Complex at Harwell, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Oxford, UK, ³Department of Nuclear Physics, Research School of Physics and Engineering, Australian National University, Canberra, ACT, Australia, ⁴Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, UK, ⁵Department of Pharmacology, University of Cambridge, Cambridge, UK and ⁶Physiology Department, University of Cambridge, Cambridge, UK

The $\beta 3$ subunit of the cardiac voltage gated sodium channel (Nav1.5) has been demonstrated to influence both trafficking and gating of Nav1.5 [1]. Indeed, $\beta 3$ mutations or its knock-out have been associated with cardiac arrhythmias such as Brugada Syndrome (BrS) [2]. We have previously demonstrated an ability of $\beta 3$ to form dimers and trimers at the plasma membrane via its extracellular immunoglobulin (Ig) domain [3,4]. However, the stability and importance of this oligomerisation for potential complexes with the Nav1.5- α subunit and the functional implication is unclear.

HEK293F cells $\pm$ stable Nav1.5 expression (HEK and HEK-Nav1.5) were used throughout this study and transiently transfected with tagged $\beta 3$ or Nav1.5 α -subunits, as appropriate. Interactions between $\beta 3$ -subunits was determined by chemical (BS3) cross-linking and between α - and $\beta 3$ -subunits by co-immunoprecipitation (Co-IP) and western blotting. To investigate Nav1.5 channel oligomerisation *in vivo* we used super-resolution STORM imaging with cluster analysis; reporting cluster

radii and number of molecules in each cluster [5]. Comparisons of radii and nearest neighbour distributions for clusters of Nav1.5 $\pm$ β 3 in reconstructed STORM images were tested for statistical significance using the Kolmogorov-Smirnov (KS) test. Whole cell patch clamp was used to measure Na⁺ currents with steady-state activation and inactivation, and recovery from inactivation protocols.

Western-blots of cross-linked β 3-myc or β 3-GFP showed bands at molecular weights indicative of monomeric, dimeric and trimeric forms of β 3. Super-resolution STORM images demonstrated expression of Nav1.5 α at the plasma membrane. However, these were not randomly dispersed; rather, ~40% of Nav1.5 α -subunits assembled into large molecular clusters. Although the proportion of Nav1.5 α in these clusters was unaffected by the β 3-subunit, the proportion of larger-radii clusters was significantly increased (KS test, $P = 4 \times 10^{-9}$) as well as nearest neighbour distances. Co-expression of β 3-GFP did not affect Nav1.5 steady-state activation parameters or peak Na⁺ current. However, it did result in a depolarising shift of V_{1/2} of steady-state inactivation (-96.14 $\pm$ 0.72 mV vs -90.64 $\pm$ 1.02 mV with β 3, $n = 20$ and 27, $P < 0.001$) and an accelerated recovery from inactivation (5.29 $\pm$ 0.92 vs 2.97 $\pm$ 0.82 with β 3, $n = 7$ and 10, $P < 0.001$).

Our results demonstrate that even when expressed alone in HEK cells Nav1.5 α -subunits co-assemble into larger oligomeric clusters. While the β 3-subunit is not required to facilitate this clustering, it does significantly alter the geometry of these complexes, functionally shifting channel inactivation to significantly more depolarised potentials and accelerating recovery from inactivation. This would be expected to increase channel availability, therefore loss of β 3 could contribute to BrS.

Fahmi, A. I., Patel, M., Stevens, E. B., Fowden, A. L., John, J. E., Lee, K., Pinnock, R., Morgan, K., Jackson, A. P., and Vandenberg, J. I. (2001) The sodium channel beta-subunit SCN3b modulates the kinetics of SCN5a and is expressed heterogeneously in sheep heart. *J. Physiol.* 537, 693–700

Hu, D., Barajas-Martinez, H., Burashnikov, E., Springer, M., Wu, Y., Varro, A., Pfeiffer, R., Koopmann, T. T., Cordeiro, J. M., Guerchicoff, A., Pollevick, G. D., and Antzelevitch, C. (2009) A Mutation in the 3 Subunit of the Cardiac Sodium Channel Associated With Brugada ECG Phenotype. *Circ. Cardiovasc. Genet.* 2, 270–278

Yereddi, N. R., Cusdin, F. S., Namadurai, S., Packman, L. C., Monie, T. P., Slavny, P., Clare, J. J., Powell, A. J., and Jackson, A. P. (2013) The immunoglobulin domain of the sodium channel β 3 subunit contains a surface-localized disulfide bond that is required for homophilic binding. *FASEB J.* 27, 568–580

Namadurai, S., Balasuriya, D., Rajappa, R., Wiemhöfer, M., Stott, K., Klingauf, J., Edwardson, J. M., Chirgadze, D. Y., and Jackson, A. P. (2014) Crystal structure and molecular imaging of the Nav channel β 3 subunit indicates a trimeric assembly. *J. Biol. Chem.* 289, 10797–10811

Roberts, S., Hirsch, M., McStea, A., Zanetti-Domingues, L., Clarke, D., Claus, J., Parker, P., Wang, L., and Martin-Fernandez, M. (2018) Cluster Analysis of Endogenous HER2 and HER3 Receptors in SKBR3 Cells. *BIO-PROTOCOL* 8

This work was supported by a British Heart Foundation grant (PG/14/79/31102) to APJ and CLHH and a Medical Research Council grant (MR/K015591/1) to CLF, RAL, STFC.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC046

Differential expression of chloride ion channels between tracheal and articular cartilage.

L. Abdul Kadir, F. O'Brien, K. Whysall and R. Barrett-Jolley

Musculoskeletal Biology, Institute of Ageing and Chronic Disease, Liverpool, UK

Chondrocytes, the single resident cell type of cartilage, common to both articular and tracheal cartilage, express a number of different voltage- and ligand-gated ion channels, together with ion-selective pumps and exchangers. The expression profile and physiological roles of ion channels in articular chondrocytes are well characterised compared to tracheal chondrocytes, where little is known. Recently, the CaCC, ano1 and ano2, were shown to be essential to tracheal cartilage formation; deletion of ano1 resulted in deformed cartilage rings and premature death in mice [1]. miRNA (miR-30c and miR-125b) have been implicated in the deformation of cartilage rings and the pathogenesis of tracheomalacia, which is characterised by the collapsibility of the trachea [1]. Here, we have characterised the ion channel expression profile of tracheal chondrocytes and compared it to that of articular chondrocytes with a focus on chloride ion channels and their interaction with miRNA.

Chondrocytes from rat tracheal and articular cartilage were isolated and the ion channel genes were unbiasedly detected with next generation sequencing (NGS). These datasets was analysed with Ingenuity Pathway Analysis (IPA) and validated with qPCRs. miRNA expression was analysed with qPCR. Patch-clamp electrophysiology was used to characterise the single channel gating of constitutively active ion channel subtypes and pharmacological intervention allowed us to identify VRAC currents as well as CaCC currents.

NGS data revealed significant differences between the tracheal and articular chondrocyte ion channel expression profile, with distinct differences in the expression levels of several members of the CaCC, VGCC and VRAC channels, for example, ano1 (A: 494.5, T: 3816.5; $p < 0.00005$; $n = 8$), ano2 (A: 0, T: 18.7; $p < 0.001$; $n = 8$), cacna1g (A: 17060.9, T: 69224.4; $p < 0.00005$; $n = 8$), lrrc8c (A: 16991.1, T: 49684.7; $p < 0.00005$; $n = 8$) and lrrc8d (A: 10636.4, T: 19263.2; $p < 0.006$; $n = 8$). These findings were validated with patch-clamp experiments, where we identified and characterised the single-channel gating of several ion channel subtypes. Using a pharmacological approach we validated the functional expression of VRAC channels with DCPIB (40 μ M), which blocked VRAC current at ± 100 mV (K_D : 3.28 μ M; $p < 0.02$; $n = 3$). The expression of select miRNAs was also analysed, revealing significant differences between the two chondrocyte types, with miR-30c (A: 0.15, T: 0.23; $p < 0.01$; $n = 6$) and miR-141-3p (A: 0.0034, T: 0.0016; $p < 0.03$; $n = 6$) being significantly different.

Results are given as mean $\pm$ SEM, statistical significance was assessed by one-way ANOVA or Student's t-tests as appropriate.

The ion channel fingerprints between tracheal and articular chondrocytes share several common similarities; yet there were distinct differences observed between the main ion channel families. Future experiments will explore the interplay of the chloride ion channels with miRNA and investigate their impact on chondrocyte function.

1. Rock, J. R. *et al.* (2008). The transmembrane protein TMEM16A is required for normal development of the murine trachea. *Developmental Biology*. 321, 141-149

This work was supported by the Crossley Barnes Bequest.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC047

A role for male, but not female, macrophages in the clearance of endothelin-1

G. Sutton, A. Czopek, T. Krueger, F. Menolascina and N. Dhaun

University of Edinburgh, Edinburgh, UK

Introduction

Hypertension is a major risk factor for cardiovascular and chronic kidney disease. However, its cause remains unclear in most cases. Our recent data suggest that macrophages contribute to, and protect from, hypertension by regulating the endothelin (ET) system [Czopek, *Eur Heart J* 2019]. ET-1 is the most potent endogenous vasoconstrictor with pro-inflammatory properties. We have shown that macrophages remove ET-1 through the ET_B receptor (ET_BR). As cardiovascular risk varies between men and women, here we explored if the expression of the ET system and ET-1 clearance varies between male and female mice and with macrophage phenotype.

Methods

Bone marrow-derived macrophages from 9-12 weeks old male (n=5) and female (n=7) C57B6J mice were cultured *in vitro*. LPS/IFN- γ were used to polarise macrophages to a pro-inflammatory/M1 phenotype and IL-4/IL-13 for anti-inflammatory/M2 polarisation. Gene expression was determined using qPCR. ET-1 concentration was measured by ELISA. Macrophages were then exposed to 2 ng/mL fluorescently-labelled ET-1 (fET-1) and the concentration was measured by fluorescence spectrophotometry after 12 hours. Statistical analyses were performed using a two-way ANOVA with Sidak's multiple comparison method.

Results

Unstimulated macrophages

Male macrophage expressed 2-fold greater ET_BR than females (p<0.01). There was no difference in ppET-1 expression between the sexes. Neither male nor female

macrophages showed net production of ET-1. Whereas male macrophages cleared all extracellular ET-1, females cleared none ($p < 0.05$).

M1 macrophages

Both male and female macrophages expressed ET_BR and ppET-1. Whereas ET_BR levels were similar, male macrophages displayed 3-fold greater ppET-1 than females ($p < 0.05$). Net ET-1 production was similar between male and female macrophages (~20 pg/mL at 24 h). However, male macrophages cleared extracellular ET-1 whereas females did not ($p < 0.05$).

M2 macrophages

Both male and female macrophages expressed ET_BR and ppET-1 similarly. Neither males nor females demonstrated net ET-1 production or ET-1 clearance.

Conclusions

Our data suggest a sex difference in ET system expression and in ET-1 clearance by macrophages. The increased clearance seen with male macrophages is consistent with increased ET_BR expression relative to female macrophages. These differences may be important in the cardiovascular risk difference observed between men and women.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC048

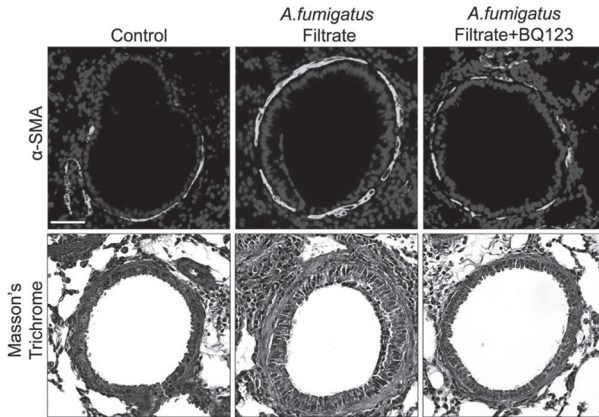
Endothelin 1 mediates *Aspergillus fumigatus* induced airway inflammation and remodelling in mouse inhalation model

S. Namvar^{1,2,3}, B. Labram^{2,3}, T. Hussell^{2,3} and S. Herrick^{2,3}

¹University of Salford, Salford, Manchester, UK, ²FBMH, University of Manchester, Manchester, UK and ³Manchester Academic Health Science Centre, University of Manchester, Manchester, UK

Background : Asthma is a chronic inflammatory condition of the airways and patients sensitized to airborne fungi such as *Aspergillus fumigatus* have more severe asthma. Thickening of the bronchial subepithelial layer is a contributing factor to asthma severity for which no current treatment exists. Airway epithelium acts as an initial defence barrier to inhaled spores, orchestrating an inflammatory response and contributing to subepithelial fibrosis. **Objective:** We aimed to analyse the production of pro-fibrogenic factors by airway epithelium in response to *A. fumigatus*, in order to propose novel anti-fibrotic strategies for fungal-induced asthma. **Methods:** We assessed the induction of key pro-fibrogenic factors, TGF- β 1, TGF- β 2, periostin and endothelin-1, by human airway epithelial cells and in mice exposed to *A. fumigatus* spores or secreted fungal factors. **Results:** *Aspergillus fumigatus* specifically caused production of endothelin-1 (1.5pg/ml rising to 3.5pg/ml) by epithelial cells in vitro but not any of the other pro-fibrogenic factors assessed. *A. fumigatus* also induced endothelin-1 in murine lungs (2pg/ml rising to 7pg/ml), associated with

extensive inflammation and airway remodelling (peribronchiolar matrix staining rising from 1% to 6%). Using BQ-123 a selective endothelin-1 receptor antagonist in *A. fumigatus* exposed mice, we observed a reduction in lung lavage inflammatory cells (240×10^4 to 130×10^4) and prevented the induction of airway remodelling. Conclusion: Our findings are consistent with the hypothesis that elevated endothelin-1 levels contribute to subepithelial thickening and highlight this factor as a possible therapeutic target for difficult-to-treat fungal-induced asthma.



Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC049

Versican upregulation in prematurity-related pulmonary hypertension

Y. Chang^{1,2}, M. Chiang^{1,2} and H. Liu^{1,2}

¹Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan and ²College of Medicine, Chang Gung University, Taoyuan, Taiwan

Background:

Preterm infants with bronchopulmonary dysplasia (BPD) are at risk of pulmonary hypertension (PH), a devastating condition which could lead to right heart failure and mortality. (Hilgendorff et al., 2016) It has been reported that versican, an extracellular proteoglycan, was accumulated in vascular lesions in PH in adults. (Chang et al., 2016) The aim of this study is to test whether versican could be a potential biomarker for prematurity-related PH.

Methods:

This is a prospective study conducted in the neonatal intensive care unit at Chang Gung Memorial Hospital during 2017-2018. The study was approved by

the institutional human research ethics committee (Chang Gung Medical Foundation Institutional Review Board, Taoyuan, Taiwan). Written informed consent was obtained from all parents prior to the study procedure. The preterm infants who were born at less than 32 weeks of gestational age were enrolled. Serum samples were collected at one week of life, one month of age, the time when signs of pulmonary hypertension developed, and at postmenstrual age (PMA) 36 weeks. At each time point, an echocardiography was performed by a pediatric cardiologist. The diagnosis of PH was made by the clinical presentation and echocardiography. Demographic data and clinical characteristics were documented and analyzed. Serum versican level was quantified by an enzyme-linked immunosorbent assay.

Results:

Seventy infants were enrolled in this prospective study. The median (interquartile range) gestational age was 28.8 (26.2-30.1) weeks and median birth body weight was 953 (749-1440) grams. Forty-two infants developed BPD. Eight (11.4%) infants had signs of PH before PMA 36 weeks, and three (4.3%) infants had signs of PH after PMA 40 weeks. Serum versican level was significantly higher in infants with PH (mean $\pm$ SD = 1207 $\pm$ 227 ng/mL) compared to the same age of infants without PH (mean $\pm$ SD = 908.6 $\pm$ 199.7 ng/mL), $p < 0.001$ by Mann-Whitney test. There were no differences in serum versican level between infants with BPD and without BPD.

Conclusion:

Versican expression is increased in prematurity-related pulmonary hypertension. The underlying mechanisms need to be further investigated.

CHANG, Y. T., CHAN, C. K., ERIKSSON, I., JOHNSON, P. Y., CAO, X., WESTOO, C., NORVIK, C., ANDERSSON-SJOLAND, A., WESTERGREN-THORSSON, G., JOHANSSON, S., HEDIN, U., KJELLEN, L., WIGHT, T. N. & TRAN-LUNDMARK, K. 2016. Versican accumulates in vascular lesions in pulmonary arterial hypertension. *Pulm Circ*, 6, 347-59.

HILGENDORFF, A., APITZ, C., BONNET, D. & HANSMANN, G. 2016. Pulmonary hypertension associated with acute or chronic lung diseases in the preterm and term neonate and infant. The European Paediatric Pulmonary Vascular Disease Network, endorsed by ISHLT and DGPK. *Heart*, 102 Suppl 2, ii49-56.

We thank Professor Jing-Long Huang for facilitating this project.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC050

Effect of hyperglycaemia on peptide composition of *in vitro* airway surface liquid.

M.G. Biggart¹, X. Ling², X. Chen², J. Wrobel², R. Tarran³ and D. Baines¹

¹*Infection and Immunity, St George's University Of London, London, UK,* ²*Biochemistry & Biophysics, University of North Carolina, Chapel Hill, NC, USA and* ³*Cell Biology & Physiology, University of North Carolina, Chapel Hill, NC, USA*

The airway surface liquid (ASL), a protective layer secreted by the airway epithelium, represents the first line of defence against inhaled infectious material. It contains a complex array of proteins and peptides that aid the neutralisation and removal of inhaled microbes and toxicants. Diabetic hyperglycaemia has been associated with an increased susceptibility to acute lung infections. We hypothesised that diabetes does this by changing the state of the ASL proteome/peptidome. Therefore, we examined how hyperglycaemia changed the ASL peptide profile of BMI-transformed normal human bronchial epithelial cells (HBEC), cell-lines Calu3 (submucosal adenocarcinoma) and H441 (Clara cell like adenocarcinoma). ASL was acquired by washing the apical surface of epithelial monolayers grown at air:liquid interface with PBS (100ml) at 0, 24 and 120 hours after exposure to hyperglycaemic (25mM glucose) or normoglycaemic (5mM glucose and 20mM mannitol) basolateral medium. The ASL peptides < 10KDa, were subsequently isolated and analysed using the Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. The resulting spectra of uncleaved peptides and their subsequent fragments were analysed using the pNovo sequencing tool. Our preliminary results identified 4765 unique peptides (NHBE-BMI1: 646, Calu3: 3150, H441: 1197). Of these, 37 peptides were common to all samples regardless of glycaemic state. Hyperglycaemia increased the number of peptides observed in Calu3 ASL but not in H441/BMI-HBEC, it also showed an effect on the modifications pattern of peptides. With further analysis we will examine the source and biological significance of these peptides.

Sponsored by the MRC London Intercollegiate Doctoral Training Partnership.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC051

Investigating diabetes-induced changes in lung microbiome using next-generation sequencing in animal models.

S. Vasiljevs and D. Baines

St George's University of London, London, UK

Diabetic hyperglycaemia leads to increased [glucose] in the airway surface liquid (ASL). This has been shown to be associated with an increased risk of developing a pulmonary infection in patients in the intensive care unit and in patients who have diabetes and chronic lung disease¹.

It was recently discovered that the lung is inhabited by resident bacteria and that it possesses its own unique microbiome. Chronic lung conditions, such as cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) shown induced changes to the resident lung microbiota.

The effect of high ASL [glucose] on the lung microbiome was examined using 16S ribosomal RNA amplification. Also compared was the effect of phenotype and environment on the lung microbiome. Preliminary studies used db/db and GK+/- diabetic mice (models of Type 2 and Type 1 diabetes). Animals were subjected to Schedule 1 terminal anaesthesia (i.p. injection of 0.2 mL pentobarbital (60 mg/ml) diluted 1:1 with saline) at 12 weeks. Samples were collected using bronchoalveolar lavage (BAL) technique. Cannulas were inserted via the trachea and lungs were washed using 1ml of sterile PBS. The lung microbiome was analysed using the Illumina MiSeq platform.

Two sets of db/db mice and one set of GK+/- mice were used. One set of each genotype of mice was raised and culled in AstraZeneca's Gothenburg facility, the remaining db/db mice were raised and culled in St George's, London. Mice were kept under similar conditions and were fed a standard rodent chow. The core lung microbiome remained consistent irrespective of the genotype, hyperglycaemia or the housing location. Bacterial orders (o.) such as Actinomycetales, Bacillales (namely family of Staphylococcaceae), Rhizobiales and Burkholderiales dominated lung microbiomes of both diabetic and non-diabetic mice. Bacteria of o. Lactobacillales, o. Sphingomonadales and o. Pseudomonadales have been observed in db/db mice housed in Sweden and the UK, while either absent or in low abundance in GK+/- animals. In contrast, bacterial o. Clostridiales and o. Enterobacteriales represented a major component of GK+/- mice lung microbiome; were absent in the lungs of db/db mice.

Hyperglycaemia was seen to affect the lung's microbiome diversity and species abundance. However, these effects were inconsistent between phenotypes and housing conditions. I.e. there was an abundance of Staphylococcaceae in lungs of diabetic db/db mice housed in Sweden compared to non-diabetic littermates, the opposite trend was observed in db/db mice housed in the UK. Interestingly, increase of abundance in the bacteria of o. Pasteurellales and o. Rhizobiales were observed in both db/db mice housed in Sweden and the UK compared to

non-diabetic littermates, while no changes in the abundance of these species were observed in GK+/- mice. To date, we have demonstrated both hyperglycaemic, genetic and environmental changes to the lung microbiome of mice.

Philips BJ, Redman J, Brennan A, et al. Glucose in bronchial aspirates increases the risk of respiratory MRSA in intubated patients. *Thorax* 2005; 60: 761–764.

Funded by a Medical Research Council.

Work performed in collaboration with AstraZeneca, Gothenburg.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC052

Evaluating the impact of London's Ultra Low Emission Zone on children's respiratory health: Spirometry success rates at baseline - the CHILL Study

H.S. KALSI³, S. JAMES³, R. Dove³, W. Helen³, C. Louise³, I. Tsocheva¹, J. Chavda¹, C. Grainne³, P. Kristian³, C. Newby³, G. Randhawa¹, M. Ian² and C. Griffiths³

¹University of Bedfordshire, Luton, UK, ²King's College London, London, UK and ³Queen Mary University of London, London, UK

Air pollution can cause adverse effects in humans across the life-course, including increases in rates of preterm birth, respiratory infections, asthma attacks, cardio-respiratory disease, COPD, dementia and early death. With lung growth and development continuing until early adulthood, children are particularly vulnerable to the effects of air pollution. Effective interventions are urgently needed to improve air quality.

London's Ultra Low Emission Zone (ULEZ), introduced to central London in April 2019, with proposed extensions to cover the whole of Greater London by 2021 represents a strategy to achieve this objective. Vehicles entering the ULEZ will need to meet minimum emission standards to avoid paying a daily charge. The Children's Health in London and Luton (CHILL) study will test the hypothesis that the introduction of the ULEZ will improve lung growth and respiratory health in primary school children.

We established and carried out baseline health assessments in two parallel cohorts of primary school-children (aged 6-9 years at baseline) in central London (Intervention; n=1606 from 44 schools) and Luton (Control; n=1652 from 38 schools) in the year prior to the commencement of the ULEZ. Annual follow up visits over the next three years will assess changes in lung function (post-bronchodilator forced vital capacity and forced expiratory volume in one second) using spirometry, and other respiratory health outcomes.

Paediatric spirometry is more challenging than in an adult population. Initially assessed spirometry data at baseline (n=943) showed that younger participants were less likely to produce successful spirometry manoeuvres. Overall, 78% of

participants performed acceptable spirometry. When characterised by age, success rates are higher in 8-9 year olds (school year 4; 84%) compared with 7-8 year olds (school year 3; 75%) or 6-7 year olds (school year 2; 76%). No differences were observed between genders (76% male, 77% female). These findings demonstrate a high level of spirometry success in this young cohort, against a rigorous set of QA criteria (Miller et al, 2005), and support maintaining the current approach in future years as the cohort matures.

There is a need to evaluate the effectiveness of air quality mitigation schemes, both in terms of their capacity to improve air quality, but also to improve objective measures of population health. CHILL is designed to achieve this aim by following lung growth in a vulnerable group. The baseline year has demonstrated a high level of engagement by schools and initial analyses have provided evidence of improvements in spirometry performance in older children. The rate of successful spirometry is expected to increase as the cohort ages, leading to a higher overall rate of success across the four years of the study.

Reference:

Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C.P.M., Gustafsson, P., Jensen, R., Johnson, D.C. MacIntyre, N., McKay, R., Navajas, D., Pedersen, O.F., Pellegrino, R., Viegi, G. & Wanger, J. (2005) ATS / ERS Task Force: Standardisation of spirometry. *European Respiratory Journal*, 26, 319 – 338.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC053

3D printed upper airway models with tuneable postural orientation

L. Gorman¹, T. Byrne¹, T. Campbell¹, J.F. Jones¹ and C. McCaul²

¹Anatomy, School of Medicine, Dublin, Ireland and ²Rotunda Hospital Dublin, Dublin, Ireland

Introduction:

Three dimensional printing can provide physiological models with internal complexity that is not possible using traditional subtractive manufacturing or injection moulding [1]. The intricacies of the passages of the upper airway are usually highlighted with CT or MRI and a soft sylgard model of the upper airway has been constructed using a negative dissolvable mold [2]. This model was ventilated with high nasal flow to study the effects of upper airway resistance and resistive work of breathing. At present there is a singular limitation when digital airway models are derived from radiological data sets. This pertains to the restrained posture of the patient when imaged in the confinements of a scanning machine. In this research we present a method for tuning the orientation of many complex anatomical parts in unison and with reference to defined anatomical planes.

Methods:

In order to manufacture the anatomical laryngopharynx, larynx and laryngeal cartilages, trachea and strap muscles of the neck, 3D digital files were downloaded from the open source BodyParts3D project (3) and assembled in Meshmixer as a model in a *neutral position*. Based on previously published papers describing the axial alignment of mouth, pharynx, and larynx obtained by flexion of the neck and extension of the head on the atlanto-occipital and upper cervical joint (4,5), axial planes were incorporated into the mesh. The resulting digital OBJ file was then exported into Blender as *vertex groups* in order to enable minute manipulation of the vertices or angular points of the individual anatomical parts. The angles of anatomic axes were adjusted in accordance with the values given in (4). The various angles between the axes were used as *pivot points* around which the rotation occurs and these were assigned using the Bounding Box Center or 3D Cursor functions within the software. Finally to test the physiological and anatomical accuracy of the bent model, the mesh was exported as an OBJ file into the slicer program Simplify3D and the individual components of the model assigned to different 3D printing filaments. A cross section of the model was saved as an STL file and printed using a Vivodeno T-Rex 3 independent dual extrusion 3D printer.

Results:

It was possible to generate multiple 3D prints with tuneable occipito-atlanto-axial extension (OAA) angles in the sniffing position relative to the neutral position.

Conclusion:

This method shows how a high fidelity 3D model with OAA extension in sniffing and simple head extension positions can be printed. Moreover, the use of soft and hard materials such as FilaFlex and polylactic acid (PLA) and experimental filaments of varying Shore hardness can be combined to produce a high fidelity, anthropomorphic training model for cricothyroidotomy or physiological models for studies of upper airway resistance.

Smith ML, Jones JFX. Dual-extrusion 3D printing of anatomical models for education. *Anat Sci Educ*. 2018;11(1):65–72.

Adams CF, Geoghegan PH, Spence CJ, Jermy MC. Modelling nasal high flow therapy effects on upper airway resistance and resistive work of breathing. *Respir Physiol Neurobiol*. 2018;254:23–9.

Fujieda K, Mitsuhashi N, Kawamoto S, Tamura T, Takagi T, Okubo K. BodyParts3D: 3D structure database for anatomical concepts. *Nucleic Acids Res* [Internet]. 2008 Oct 3;37(suppl_1):D782–5. Available from: <https://doi.org/10.1093/nar/gkn613>

Adnet M.D., Ph.D. F, Borron M.D., M.S. SW, Dumas M.D. JL, Lapostolle M.D. F, Cupa M.D. M, Lapandry M.D. C. Study of the “Sniffing Position” by Magnetic Resonance Imaging. *Anesthesiol J Am Soc Anesthesiol*. 2001 Jan 1;94(1):83–6.

TakeTakenaka I, Aoyama K, Iwagaki T, Ishimura H, Kadoya T. The sniffing position provides greater occipito- atlanto-axial angulation than simple head extension: a radiological study. 2007;129–33.

Acknowledgments:

We would like to thank the Difficult Airway Society, the College of Anaesthetists, Ireland and UCD School of Medicine for their financial support.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC054

Leptin, respiratory parameters and carotid body function in high fat diet fed and chronic intermittent hypoxic rats.

A. Rocher¹, E. Olea², A. Gordillo¹, J. Prieto-Lloret¹, S. Conde³, R. Rigual¹, A. Gomez-Niño¹ and A. Obeso¹

¹Bioquímica y Biología Molecular y Fisiología, University of Valladolid-CSIC, Valladolid, Spain, ²Enfermería, Universidad de Valladolid, Valladolid, Spain and ³CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal

Leptin is a pleiotropic hormone synthesized and secreted mainly by white adipose tissue. It is involved in metabolism and energy balance and cardiovascular and respiratory control, acting as a potent stimulant of breathing in the central nervous system. Recently, the expression of the leptin receptor in the lungs and the carotid body (CB) has been reported and therefore additional peripheral actions of leptin on the respiratory system have been suggested. This finding has been associated to a physiological role of circulating leptin in the regulation of CB function and to the pathogenesis of respiratory disorders. Based on these reports, we have tried to analyze the impact of leptin on the hypoxic ventilatory response (HVR) and on the hypoxic elicited CB response in animal models of two respiratory disorders, obstructive sleep apnea (OSA) and obesity hypoventilation syndrome (OHS). The experiments were carried out on adult male Wistar rats (250-400 g), randomly divided into four groups: Control (C, air breathing, standard diet; n=16), chronic intermittent hypoxia (CIH; 5%O₂ 40s, 80 s 21%O₂, 8h/d, 21 d, standard diet; n=16), high-fat diet induced obesity (O; 21 d, n=16) and CIH/Obesity combination (OIH, both treatment, 21d; n=16), analyzing plasma leptin levels, breathing parameters and effects of leptin on the basal and hypoxia elicited CB secretory response, in anaesthetized rats (sodium pentobarbital, 60 mg/Kg, i.p.) of the four group. At the end of experiments, rats were euthanized by an intracardiac overdose of pentobarbital. We verified that exposure to HCl did not produce significant ventilatory changes, in spite of CB sensitization, that could be related to low plasma leptin level observed (24 ± 10 ng/mL). Diet induced obesity (O) and IH combined treatment (OIH) produced basal hypoventilation and decreased HVR to 7%O₂ (20% decrease vs control rats), with a restrictive respiratory pattern (increased respiratory rate) and very high plasma leptin levels in both conditions (216 ± 41 and 178 ± 37 ng/mL, respectively). In isolated control CB, a high acute dose of leptin (250 ng/mL), but not low doses, potentiated the hypoxia induced catecholamine (CA) release by 190%, while not affecting the CA basal output. Conversely, the effect of leptin on the hypoxic response of CB was abolished after CIH, O or OIH exposure of rats. We conclude that in rats, the effect of leptin on ventilation could also be mediated by

peripheral chemoreceptors, enhancing the CB response to hypoxia. Rat exposure to CIH and high-fat diet, blunts leptin effect on CB activity by different mechanism. Whereas the diet induced obesity, with very high levels of circulating leptin, could abolish the leptin effect on CB by a leptin resistant state, CIH blunts leptin effects with low plasma leptin levels.

Grants: BFU2015-70616R (MINECO-FEDER) and VA106G18 (Junta Castilla y Leon)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC055

The role of microtubules for actin coat formation during the post-fusion phase of surfactant exocytosis in lung alveolar type II cells

T. Müller¹, R. Schempp¹, A. Lutz¹, T. Felder¹, E. Felder¹ and P. Miklavc²

¹Ulm University, Ulm, Germany and ²University of Salford, Salford, UK

Pulmonary surfactant reduces surface tension in lung alveoli and enables inspiration. Lack of surfactant can lead to infant respiratory distress syndrome and can play a role in pulmonary fibrosis. Surfactant is secreted by alveolar type II (ATII) cells in the process of exocytosis where a secretory vesicle fuses with the plasma membrane to release vesicle content. Surfactant extrusion from fused vesicles is facilitated by an actin coat that forms on the vesicle shortly after fusion pore opening (Miklavc et al., 2015). Actin coat compression allows hydrophobic surfactant to be released from the vesicle (Kittelberger et al., 2016). Emerging evidence suggests that actin can interact with microtubules; however, the interplay between actin and microtubules in exocytosis is not understood. Here we study the interaction between actin and microtubules during the post-fusion stage of exocytosis using high-resolution fluorescence microscopy on primary ATII cells. ATII cells were isolated from the lungs of male Sprague-Dawley rats according to the procedure of Dobbs et al. (1986) with minor modifications (Miklavc et al., 2010). Isolated ATII cells were transfected with actin-GFP or actin-DsRed to visualize actin coats on fused vesicles and exocytosis was stimulated with ATP (100 μ M). Microtubules were localized close to actin coats and stayed close to the coats during their compression. Inhibition of microtubule polymerization by colchicine and nocodazole affected the duration of actin coat formation and the extent of actin polymerisation on fused vesicles. Mean $\pm$ SEM time of the peak fluorescence was 75.3 $\pm$ 7.5 s after fusion for control, 112.4 $\pm$ 6.8 s for colchicine and 103 $\pm$ 6.9 s for nocodazole ($p < 0.05$; one-way ANOVA). In actin-GFP transfected cells actin coat fluorescence intensity increase was significantly lower in control (14.48 $\pm$ 1.22%; $n = 50$ actin coats) than in cells treated with colchicine (23.82 $\pm$ 1.60%; $n = 58$; $p < 0.0001$) or nocodazole (20.03 $\pm$ 1.35%; $n = 56$; $p = 0.003$; two-tailed t-test). We also found that microtubule and actin cross-linking protein IQGAP1 localized to fused secretory vesicles. The relative intensity of actin coat

fluorescence in cells transfected with IQGAP1 siRNA (14.62 $\pm$ 1.17%; n=36 actin coats) was significantly lower than actin coat fluorescence intensity in cells treated with control siRNA (26.12 $\pm$ 1.93%; n=37; p<0.0001; two-tailed t-test). These findings indicate that microtubules can influence actin coat formation and actin polymerization on secretory vesicles during exocytosis. This interaction may be mediated by microtubule and actin cross-linking proteins such as IQGAP1.

Dobbs, L.G., Gonzalez, R. and Williams, M.C., 1986. An improved method for isolating type II cells in high yield and purity. *American Review of Respiratory Disease*, 134(1), pp.141-145

Kittelberger, N., Breunig, M., Martin, R., Knölker, H.J. and Miklavc, P., 2016. The role of myosin 1c and myosin 1b in surfactant exocytosis. *J Cell Sci*, 129(8), pp.1685-1696.

Miklavc, P., Frick, M., Wittekindt, O.H., Haller, T. and Dietl, P., 2010. Fusion-activated Ca²⁺ entry: an "active zone" of elevated Ca²⁺ during the postfusion stage of lamellar body exocytosis in rat type II pneumocytes. *PLoS One*, 5(6), p.e10982.

Miklavc, P., Ehinger, K., Sultan, A., Felder, T., Paul, P., Gottschalk, K.E. and Frick, M., 2015. Actin depolymerisation and crosslinking join forces with myosin II to contract actin coats on fused secretory vesicles. *J Cell Sci*, 128(6), pp.1193-1203.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC056

Characteristic markers of diabetes induce a synergistic pro-inflammatory response in porcine aortic valve interstitial cells

J. Zhang and G. Nixon

Medical Sciences and Nutrition, Aberdeen Cardiovascular and Diabetes Centre, Aberdeen, UK

Aortic valve stenosis (AVS) is one of the most common valve diseases, characterised by calcification. Currently the treatment only option is surgical intervention. Inflammation plays a key role in initiating calcification of valve interstitial cells (VICs). Proinflammatory mediators activate VICs leading to osteogenic differentiation. Recent studies have indicated that AVS has a higher prevalence in diabetes patients. Research indicates that cardiovascular disease in diabetes patients is increased due to high glucose and the formation of advanced glycation end products (AGEs) in blood. The current study aims to understand the link between high glucose and AGEs on VICs in an osteogenic environment.

Porcine VICs were isolated from the pig heart obtained from the abattoir and cultured under sterile conditions. Alizarin red stain was used to determine calcium deposition as an indicator of osteogenic differentiation. After 7 days in osteogenic medium, calcification was clearly visible in VICs and this timescale was subsequently used for the study. Inducible cyclooxygenase 2 (COX2) is a key mediator of pro-inflammatory pathways and may play a role in the inflammation of VICs. Immunoblotting was used to determine COX-2 protein expression. COX-2 expression

was not changed in VICs incubated in osteogenic medium for 7 days. However AGEs (100ug/ml) significantly increased COX2 expression in osteogenic medium (7.50 ± 3.11 fold increase vs control, $P < 0.05$, $n = 6$). Interestingly, AGEs incubation for 7 days had no effect of COX-2 expression in non-osteogenic medium.

The effects of high glucose were examined. In non-osteogenic medium, high glucose (4500mg/ml) significantly increased the expression of COX2 (4.06 ± 0.93 fold increase vs normal 1000mg/L glucose control after 7 days, $P < 0.05$, $n = 5$). Furthermore, the combination of high glucose and AGEs for 7 days in non-osteogenic medium had a synergistic effect, significantly inducing COX2 expression compared to high glucose alone or AGEs alone (4.51 ± 1.13 fold increase vs AGE incubation; 2.54 ± 0.46 fold increase vs high glucose incubation, $P < 0.05$, $n = 4$). Finally, all 3 factors were examined together (AGEs, high glucose and osteogenic medium). The combination of high glucose and AGEs in osteogenic medium significantly increased COX2 expression compared to all other factors alone (for example, 4.90 ± 1.49 fold increase compared to AGEs alone, $P < 0.05$, $n = 6$).

In conclusion, this data reveals that although calcification in vitro does not change expression of the pro-inflammatory enzyme COX-2 in VICs, the addition of either AGEs or high glucose under these conditions induces COX2 expression. In combination, these factors have a significant synergistic effect. Thus, diabetes patient with increased may have an increased inflammation in aortic valves, leading to an enhanced progression of AVS.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC057

The application of bioinformatics to investigate the potential for large farm animal tissues in translational research

H. Widmer, B. Adeyileka, S. Cruickshank, A. Strath and I. Rowe

Robert Gordon University, Aberdeen, UK

Large farm animals are used for the investigation of models of human diseases and for study of the underlying physiological signalling (1), yet they make up only 1.5% of the 4 million animals used annually in the UK in scientific procedures (2) while 28 million cattle, sheep and pigs were slaughtered for the food chain over the same period (3). Large animal derived abattoir tissue is viable for research (4) and may be an underutilised resource in both translational research and biomedical education that could reduce our reliance on rodent tissue and models (5).

Homologous protein primary sequences from target proteins in humans, rodents and large farm animals were used to determine the closeness of match as a measure of the appropriateness of an animal model. The location and distribution of predicted and verified post-translational modification (PTM) sites were investigated in the same proteins with a focus on phosphorylation sites to provide a

measure of potential functional differences across the species. Protein primary sequence alignment; phylogenetic analysis; and similar protein searches (UniProt UniRef) were used to establish species ranking with respect to their match to human. All three approaches yielded similar patterns with farm animal homologs (pigs, cattle and sheep) closer to the human homolog than either of the rodent forms (rat and mouse) in 7 out of the 10 proteins. The level of shared identity for individual proteins across all the species analysed ranged from 96% for BKCa to 28% for CD55. PTM sites for phosphorylation were investigated using the NetPhos 3.1 predictive models. Conservation of PTMs across species mirrored the identity data but with a substantial drop in the proportion of shared phosphorylation sites. For example, BKCa shared 96% protein sequence identity and 92% (38 /41) of predicted phosphorylation sites; ABCB1 (a multi drug resistance protein): shared 75% protein identity and 41% (17/41) of phosphorylation sites; and IL23-R shared 53% protein identity but only displayed 6% commonality (1/17) in predicted phosphorylation sites. In addition, some experimentally verified PTM sites were neither reported in databases nor predicted by the relevant software, raising the issue of database curation as a challenge in investigating potential targets across animal models. The investigation shows that functional site and conserved position bioinformatics analysis support large farm animals targets being sound molecular matches to human proteins and frequently better than rodents. Incorporation of a bioinformatics approach and improvement in curation of farm animal data could improve our use of the vast resource of abattoir tissues and may be an ethical and effective route to improvements in translational and pharmaceutical research.

Pinnapureddy *et al.* (2015) *Orphanet J rare diseases* 10,107-114.

Home Office Annual statistics of scientific procedures on living animals Great Britain 2017
Department for Environment, Food and Rural Affairs (DEFRA), UK. Available from: <https://www.gov.uk/government/statistics/cattle-sheep-and-pig-slaughter>

Dospinescu *et al.* (2012) *Am. J. Physiol.* 303, L476-86.

Seok *et al.* (2013) *PNAS* 110(9), 3507-12.

This work was supported in part by NHS Education Scotland

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC058

Does size matter? Effect of screen size on student learning

N.S. Freestone

Chemical and Pharmaceutical Sciences, Kingston University, London, UK

It has been shown (Mangen *et al.*, 2013) that primary school children do better in tests of factual recall when they read printed text material compared to digital text material. We have previously reported a study that looked at higher

education (HE) students at levels 4 and 6 (Years 1 and 3) in a UK HE institution to determine if there were differences manifested in the ability to accurately recall information from paper and digitally-presented content as students progressed through the university system. Students were presented with the same body of information and could choose whether to study that information in digital or paper forms in a time constrained manner. Immediately after this period students were presented with questions relating to the text studied. Analysis of the answers given by the participants in their first year of university studies revealed that there were significant differences between the accuracy of recall of information from the two media sources. Students gained higher marks on average when tested on their recall from paper compared to digital sources of information. However, no such differences were manifested in the final year cohort.

Mangen (2008) has suggested that student learning from any media source is crucially dependent on the nature of the physical interaction between student and media source. We hypothesised that as students progress through the HE system they are increasingly able to efficiently retain information from a variety of media sources.

Here we extend that study to look at the nature of the electronic media used by students to see if that has an effect on learning outcomes. Using a similar testing format as above students undertook a comprehension test immediately after studying a text either using a mobile phone or a lap-top. The decision on what device to use was the students' own with most opting to use a mobile phone (68% of the students). Analysis of answers from the comprehension test after the study period showed a significant decrease in student performance when they used mobile phones compared to lap-tops ($p < 0.05$). This study shows that despite the move by many universities to employ mobile telephone apps to enhance learning and engagement by students, the type of device used may be crucial.

Mangen, A. (2008) Hypertext fiction reading:haptics and immersion. *Journal of Research in Reading* 31:404-419

Mangen, A., Walgermo, B.R., Bronnick, K. (2013) Reading linear texts on paper versus computer screen: Effects on reading comprehension. *International Journal of Education Research* 58:61-68

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC059

Use of clinical teaching simulators by Medical Science students to improve engagement and in depth understanding of Immunology

M. albuhtori and I.J. Crane

Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK

Immunology is a challenging topic to teach due to its complexity of cellular interactions and regulatory mediators involved. Students can find it overwhelming and lose sight of key concepts and there is a need for inclusion of teaching methods which increase engagement and stimulate active learning. Simulation of clinical scenarios using high-fidelity manikins is a teaching method used successfully in clinical teaching. Our aim was to determine whether this method could be adapted to enhance the learning experience of medical science students studying Immunology.

The scenario chosen for this study was sepsis as it provides an example of a real-life problem where immune system responses drive clinical outcome. The class was tested on seven students taking an Applied Immunology course and this was their first exposure to a clinical simulator. They all had fundamental knowledge of the inflammatory response to bacteria from lectures and the class was designed to encourage them to use this knowledge to identify the problem and improve the outcome. The students took part in three iterations of the same five minute scenario in which a 68-year-old male arrived in the A&E department with chief complaints of weakness, dizziness and fever and showing vital signs and lab test results consistent with sepsis. After each iteration there was an interactive discussion. Initially, with discussion, the students were able to offer a primary diagnosis of sepsis and suggested additional tests to confirm the diagnosis. After the second, in which a blood culture was shown to be positive for gram-negative bacteria and the patient had deteriorated, the students suggested the administration of a wide-spectrum antibiotic and high-volume fluid as treatment. These were administered in the third scenario which showed some improvement of the patient. A final review and discussion covered alternative outcomes and the inflammatory mechanisms and cytokines involved.

The students were then asked to complete an anonymous evaluation of the class. They were asked to rate ten statements on a five-point scale where five is the highest. All the students responded that their knowledge of sepsis had improved and six out of seven that their knowledge of inflammation had improved. They all agreed that the class had improved their problem solving skills. They said that it was a 'cool' way to learn and free comments included 'It was good to think of immunology in real life simulation...' and 'this helped seeing how different factors can affect patients which encouraged thinking...'.

We concluded that clinical simulation could be used effectively to improve student engagement in a complex immunology topic. This active learning process improved their understanding and consolidated knowledge. The novel use of

clinical high-fidelity manikins will be helpful for teaching other complex topics in Immunology and could be applied to other medical science subjects.

Issenberg S, McGahgie W, Petrusa E, Gordan D, Scalese R. Features and uses of high-fidelity medical simulations that lead to effective learning: a BEME systematic review. *Medical Teacher*. 2005;27(1):10-28. 2

Maran, NJ Glavin, RJ. Low- to high-fidelity simulation – a continuum of medical education? *Medical Education*. 2003;37:22–28

Ian Morrison, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, United Kingdom

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC060

The benefits of collaboration in hosting a joint physiological research symposium

T. Pocock¹, L. Bagley², E. Sheader¹, D.J. Greensmith³ and I. Kay²

¹Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK, ²School of Healthcare Science, Manchester Metropolitan University, Manchester, UK and ³School of Environment & Life Sciences, University of Salford, Salford, UK

The Physiological Society's Departmental Seminar Scheme provides funding to support members to host seminars about physiological research. This funding covers the cost of transport and accommodation for visiting speakers. Last year we submitted a joint application for funding to organise a one day symposium between the three Universities. The main advantages of this were: we did not have to find a suitable time every month to guarantee a good audience, we were able to invite speakers from three institutions (with reduced travel costs) and our potential audience was much larger. Furthermore, the size of the grant awarded is dependent on the number of members at the institution(s) applying.

Last year we organised a 'Greater Manchester' Physiology symposium at MMU, with 8 speakers from UoM, MMU and UoS. We attracted an audience of 90, which included mostly Society members, but also a few undergraduate students. We combined the symposium with Rachel Tribe's GL Brown Prize lecture (awarded to MMU), thereby boosting her audience. We opted against a specific theme for the symposium, but encouraged speakers from different stages of their research careers (from a PhD student to a Professor with over 40 years' research experience). Audience members benefitted from a day of stimulating 20 minute physiology-themed seminars, a chance to interact with researchers from other institutions and a good lunch and evening reception. This May we organised a similar symposium (hosted by UoM) and included a poster presentation over lunch/coffee for early career researchers to showcase their work.

Attendees were asked to complete a survey about their views of this year's symposium. All 17 respondents agreed that the Symposium had furthered their

knowledge of Physiology, with 88% stating that they had learnt about physiological molecules and pathways they were not previously aware of. 71% stated that they had learnt about physiological techniques, and a further 71% about physiological mechanisms which were new to them. Of the respondents who had also attended the 2018 symposium, 30% stated that they had gained advice from another attendee in the last year. 76% attendees this year would consider collaborating with another attendee.

These findings suggest that hosting a physiology symposium between geographically close institutions is an effective way of sharing findings from current or recent research studies, and may help to instigate collaboration between researchers. Furthermore, it is an economical way of bringing physiologists together and ensuring a good audience for some talented researchers.

We'd like to thank The Physiological Society for their funding.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC061

Novel Cross Faculty Final Year Research Project: Classical and Physiological Perceptions of Sex and Gender.

D. Davies¹, L. Cross¹, E. Duffy¹, M. Lewisohn¹, E. Walton¹, V. Zajko² and F. MacMillan¹

¹*Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK and*

²*Classics & Ancient History, University of Bristol, Bristol, UK*

There is currently much discussion around widening provision for final year undergraduate research projects, due to increasing student numbers and to provide opportunities for students who want to follow alternative career paths. We describe a novel innovative cross faculty project.

The social perception of sex and gender has changed dramatically through the ages. We investigated how our current understanding of the biological basis of these fundamental human traits has influenced social attitudes and traced continuities and differences between ancient Greek medical models and modern perspectives. The project was taken by Physiology and Neuroscience BSc students in collaboration with Classical Studies students studying 'Configurations of Sex and Gender'. Initially, students held a discussion group to explore the issues surrounding the topic from their different perspectives. Following this the science students chose different aspects to pursue. One held a discussion group with A level students, the others made a series of podcasts aimed at raising awareness, primarily in undergraduates.

The focus group with A level Biology, Psychology and Classics students, explored current attitudes to intersex and how these relate to those held in ancient civilisations. Questionnaires indicated that 89% of pupils had not heard of the term intersex before the session but came away with a better understanding and an

interest to find out more. They also felt the topic should be included in the curriculum, with 60% thinking this should be in primary school. Three podcasts, in an interview format, were used to engage undergraduates in 'Greek and contemporary perspectives on gender', 'Discussing intersex' and 'Dissolving the gender binary'. They were launched at a 'silent disco' event thus allowing participants to listen to individual podcasts. Questionnaires were used to gather opinions and these indicated a greater awareness of issues surrounding sex and gender in this age group compared to A level students. It was clear that the podcasts were informative, for example, 50% said their understanding about the gender binary had been altered. Also, 81% of participants indicated they would like to hear more about the topic. To fulfil the requirements of the BSc dissertation students were required to show their understanding of the underlying physiology, despite using little of this content in their resource. This project provided an excellent opportunity for students from different disciplines to influence each other's understanding of a topic. The major challenges faced by the science students were gaining an understanding the ancient Greek and Roman medical, philosophical and literary sources, and distilling their knowledge of the physiology into an approachable format for a non-specialist audience. All the science students reported enjoying the opportunities afforded to them by the project which allowed them considerable freedom in devising resources of their choosing

Bristol Institute for learning and teaching (BiLT) for 'seedcorn' initiative funding to support the development of this project. The Physiological Society for travel funds to attend the meeting.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC062

Simplified organized-structured-practical-examinations to prepare students for examinations involving human subjects

M. Scholz

School of Medicine, Medical Science and Nutrition, University of Aberdeen, Aberdeen, UK

OSPEs (organized structured practical examination) are common in clinical education. In recent years they have been adopted into honours Medical Science and Sport Science teaching at the University of Aberdeen (Scott 2017) and have proven useful to develop skills linked to time management and interaction with volunteers. Student perceive this type of examination as challenging though they admit that it develops these skills. In particular the "role play" component of some examinations involving human subjects such as phlebotomy or ECG produces anxieties and insecurity in students. Interaction with volunteers is particularly important in Sport Science, because it is an integral part of laboratory-based honours projects and of future careers.

A simplified version of an OSPE component to measure volunteer's blood pressures has been introduced in third year Sport Science teaching to give students more and earlier exposure to this type of examinations. Students get an introduction tutorial to blood pressure measurements and are given links to resources of the British and Irish Hypertension Society for self-study. Students are informed about the assessment and its role-play nature. At the practical students get an hour to practice taking blood pressure. After this familiarisation, students are assessed taking blood pressure on each other utilising a simple tick-box scoring system. Proper technique, interaction with the "volunteer" and professionalism are the main scoring criteria.

Students still find the "role-play" component daunting at first at first, but normally lose anxieties quickly during the procedure. One aspect reducing the intensity of the exercise is that there are no time constraints applied, that are normal in an OSPE.

Student feedback from SCEF course feedback questionnaires shows that the mini OSPE is effective in developing student's skills (effectiveness of teaching and development of skill assessed on a 5-point agreement scale, 75% of ratings in 5: "totally agree" or 4: "Agree", n=26 over 2 years). Student comments also illustrate that despite initial concerns and anxieties, the exercise is perceived as useful and helps to build confidence interacting with people. Some students (Class rep feedback at staff student liaison committee meeting) asked to incorporate more exercises like that into the curriculum. Sport & Exercise Science Honours students who participated in the BP pressure mini OSPE before doing the full Medical Science OSPE in fourth year reported that it was a useful preparation and made them feel less anxious and more confident about it (direct communication with author "Was the 3rd year mini OSPE beneficial for doing the OSPEs in year 4", n=11).

In conclusion, the "mini OSPE" is a useful teaching tool to build students confidence to interact with human subjects and improve communication skills.

Scott, DA & Jenkinson, A 2017, 'Developing physiology graduate skills and attributes with Objective Structured Practical Examinations (OSPE's)' IUPS 38th World Congress, Rio de Janeiro, Brazil, 1/08/17

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC063

A novel final year experimental research project: Using objective measures of physical activity to investigate the effect of exercise on wellbeing in undergraduates.

F. MacMillan¹, A.A. Carrie¹, N. Chan¹ and P. Burrows²

¹*Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK and*

²*Centre for Sport, Exercise & Health, University of Bristol, Bristol, UK*

Traditionally final year experimental research projects have involved students undertaking work in a research laboratory supervised by academic or research staff. With increased student numbers and pressure on research time and resources, alternative projects are necessary. Experimental projects are highly sought after by undergraduates particularly those who wish to continue into post-graduate research. Research projects can be run in settings other than research laboratories and they can allow students some degree of autonomy in the design of the experimental questions and procedures. Students can also benefit by gaining experience of different settings, in this case working with the Universities Sport and Physical Activity Team. This experimental project, taken by two Neuroscience students, required no lab space but was a hypothesis driven project, with direct input to the design by the students, and addressed a current and highly relevant issue.

Many studies link an increase in physical activity to improved mental and social wellbeing. These studies have relied on self-reported levels of exercise which may not accurately reflect the actual level or frequency of activity in subjects (1,2). In this project, trackers (Fitbit charge2) were worn by volunteers, recruited from the final year student population at the University of Bristol. Activity trackers were worn for 14 continuous days and at the end of the two weeks participants completed the Warwick-Edinburgh Mental Well-Being Scale. Participants completed a questionnaire at the start of the study to indicate how frequently they exercised. The weight and height of participants were also collected.

Analysis of the data indicated a weak correlation between total minutes exercised and wellbeing score ($r = 0.21$) and between average daily step count and wellbeing ($r = 0.26$). There was no correlation between BMI and wellbeing for all participants but when analysed separately for male and female participants there was a positive correlation for males and wellbeing ($r = 0.69$) and negative for females ($r = -0.33$). Increasing the power of the experiment could be achieved with more students running the project as more experimenters could recruit a greater number of participants and share the collation of data from tracker accounts. Given access to all the data that can be obtained from the trackers (resting heart rate, moderate exercise duration, aerobic exercise duration, step count, sleep quality) students could focus on different aspects of those data for their individual project dissertations. A further extension of the project could be to recruit 2 groups of participants recording normal levels of activity in one group and assessing the impact on wellbeing of participation in additional physical activity sessions during the data

collection period in a second. Students running the project reported enjoying the involvement of shaping the project and the relevance of the research to their own experiences.

Hassmén P et al. (2000) Preventative medicine 30, 17-25

De Moor M H M et al. (2006) Preventative medicine. 42, 273-279

The Physiological Society for travel funds to attend the meeting.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC064

Augmenting Reality in Bioscience Education

D. O'Hare³, W. Hurst², A. Sands³, C. Forker¹ and J. Barrow¹

¹*Institute of Education for Medical and Dental Sciences, University of Aberdeen, Aberdeen, UK,* ²*Department of Computer Science, Liverpool John Moores University, Liverpool, UK and* ³*Imagin3D, Daresbury, UK*

Most early stage undergraduate bioscience curricula cover organ systems physiology, cell physiology, biochemistry and molecular biology. Across all of these disciplines, a vast quantity of fundamental concepts are covered, which are abstract in nature. Based on student feedback, traditional lectures and tutorial based teaching do not always convey these abstract concepts well so do not allow for a full understanding of the material. Therefore, visualisation of cellular processes can allow students to appreciate the concepts and mechanisms being studied in a more effective way than traditional wrote learning styles. Augmented reality (AR) and virtual reality (VR) have a real potential to transform the education of students across many fields, through the creation of visual and immersive learning environments. As an initial pilot study, we have created an AR application for secondary school pupils to enhance their understanding of metabolism, specifically linked to dietary glucose absorption and cellular uptake of glucose in response to insulin signalling. The educational experience couples a mobile device application with traditional wrote learning to give a blended experience of how these dynamic processes work. The study presented here was conducted in the School of Medicine, Medical Sciences and Nutrition at the University of Aberdeen. Ninety participants were self-selected by completing a survey as part of a second year undergraduate biochemistry course that forms part of the core curriculum for the majority of life science degree programmes. Of the ninety questionnaire participants, eight were randomly selected to take part in a focus group and seven volunteered to test the AR application. Questionnaire data showed 40% enjoyed half of their lectures and 49% enjoyed almost all lectures, showing scope for potential improvements in lecture delivery and content. 51% of participants agreed or strongly agreed that they make detailed lecture notes, leaving nearly half of students not making

detailed notes. 38% of participants were neutral and 19% agreed or strongly agreed that PowerPoint slides were boring, signifying there could be enhancements in teaching materials. When asked how lectures could be made more enjoyable, the top five free-text response words were “interactive”, “engaging”, “examples”, “videos” and “interaction”, showing the use of AR in the classroom could be beneficial. 98% of participants own a smartphone, 93% have a laptop and 40% have a tablet, so bringing their own devices to class should minimise expenditure of such technology initiatives. 92% agree or strongly agree that being able to “see” abstract concepts would help their learning. In summary, this research suggests the selective use of AR is one way to embed engaging educational content into the teaching of abstract concepts.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC065

Culturing Enterprise Skills in Bioscience Students

A. Davidson², M. Gardiner², J. Perkins³, P. Marini¹ and J. Barrow¹

¹*Institute of Education for Medical and Dental Sciences, University of Aberdeen, Aberdeen, UK,* ²*Scottish Institute for Enterprise, Glasgow, UK and* ³*Centre for Academic Development, University of Aberdeen, Aberdeen, UK*

Subject benchmark statements in the UK Quality Code for Higher Education state that graduates of bioscience degree programmes should have the necessary skills for enterprise and knowledge transfer. This is a crucial skill set for science graduates, in a world where scientific advancements and technological breakthroughs are occurring at an ever-increasing rate. Furthermore, many university degree programmes state their degrees will prepare their graduates with attributes and life-long learning skills that go beyond subject-specific expertise. Making students aware of their external environment and providing them with an opportunity to express how they may interact with an ever-changing world is therefore an important endeavour and one that we believe should begin as early as possible within university curricula. Therefore, we have developed an enterprise session that runs within a first year undergraduate bioscience course, providing students with the opportunity to explore and practice enterprise skills (such as creative problem solving and team working) demanded by the modern labour market. This aligns with an increasing number of employers requiring graduates to be innovative, adaptable and resilient, and have an enterprising mind-set. The enterprise session supports students to develop these key attributes and skills, enabling them to engage with the pace of change and potentially make effective contributions to the economy following their graduation. The session is run by the Scottish Institute for Enterprise in partnership with the School of Medicine, Medical Sciences & Nutrition and the Careers Service at the University of Aberdeen and takes the

form of a 2-hour on-campus workshop for over 300 first year bioscience students. Working in small groups, students think creatively around a variety of items (e.g. a lab coat, scalpel and safety sign) and then explore their functionality in 10 years' time. A further exercise involves students innovating through drivers of change that range from very focussed recent bioscience ideas to more wide-ranging societal changes, and how these drivers for change may impact on their thoughts and actions as they progress through their degree. At the end of the session, a survey gathered students' perceptions, attitudes and understanding of enterprise education. This poster will highlight key findings and developments brought about from this survey, and explain how they will influence future events and proposed ideas to further skills developments in undergraduate bioscience students.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC066

Impact of a Final Year Skills Workshop Series for Undergraduate Medical Science Students

J. Barrow and D. Scott

Institute of Education for Medical and Dental Sciences, University of Aberdeen, Aberdeen, UK

An ever-increasing number of students are passing through undergraduate science degree programmes with a wide range of skills they are expected to demonstrate for future educational opportunities or to employers. Further to this, students have a diverse range of inherent skills, with more intermediate or advanced skills not directly related to disciplines being neglected and assumed to have been acquired through the course of a degree programme. Whilst it is true that the acquired levels of subject-specific skills will be high in any undergraduate degree programme the softer skills such as professionalism, data and information handling, and scientific writing can vary dramatically between individual students. The study presented here was born out of staff and student feedback, which suggested over recent years due to increasing student numbers and a more diverse student population, the skills attainment gap has widened throughout the student population. This could potentially affect student employability because of a lack of parity in the acquired soft skills that will equip a student for the job market following graduation. Therefore, we aimed to limit this perceived skills attainment gap through the introduction of a standardised workshop series into our final year teaching, with impact being assessed through class feedback surveys. This series of workshops was introduced to all final year undergraduate students in the Medical Science degree programmes and formed a compulsory part of their final year education. We report here that the outcome of the pilot workshop series (academic year 2017-18), which was overall viewed as a positive introduction based on student

feedback. Following the pilot, students ($n = 210$) were asked to rate 15 different skills sets via a feedback questionnaire, with 11 skill sets showing an increase in self-reported reflection of ability following the workshop series, and a significant increase in skills development pre- and post-workshops for 'Graph Drawing' ($p = 0.05$), 'Data Analysis' ($p = 0.01$) and 'Experimental Design' ($p = 0.001$). We have further developed the skills workshop series based on the feedback from the pilot and will report these further findings and how they compare with the original pilot findings to assess sustainability and rate the changes that were introduced into the second skills sessions run in academic year 2018-19. This teaching development has now been embedded into future final year Honours teaching based on their success.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC067

Emerging Ideas for Paperless Laboratory Classes

J. Barrow and D. Scott

Institute of Education for Medical and Dental Sciences, University of Aberdeen, Aberdeen, UK

Using technology in the classroom is not a new idea, with most educational institutions from primary through to tertiary education using technology extensively. Technology in the classroom can enable streamlining of administrative tasks, enhance educational resources and engage students more effectively in their learning. One area where technology can have a huge impact is in science laboratory practical classes, especially in universities where classes can be very large and pose challenges for the delivery of high quality educational and applied learning experiences. In parallel with these developments, students are considered as 'digital natives' with the majority having grown up using technology and devices such as smartphones, tablets and laptops (over 98% of our students have at least one of these) for both personal and professional/educational use. The development of technology and the 'digital native' alongside each other provides a real opportunity to deliver even higher quality educational experiences especially in the laboratory classroom. We have surveyed the paperless classroom landscape to identify potential initiatives that could be used in our own laboratory classes to deliver an interactive, more immersive and enriched educational experience. This review investigated the concepts of cloud computing, the use of various devices, a comparison of different software applications that can be used for paperless teaching, and finally highlights a pilot study we have conducted with several classes that were converted to paperless versions. These pilots all used the Lt cloud-based system from AD Instruments and participants were surveyed via questionnaires and focus groups to ascertain their views and opinions on this new way of teaching their

laboratory classes. Survey results following the pilots were overwhelmingly positive with students (n = 28) seeing the advantages of such a system to streamline and enhance their learning experience. Students particularly liked the use of videos and links to further information that could be accessed through the instructional materials provided in the system. Staff who also used the system highlighted how it can reduce their demonstration time as students can understand equipment setups and background information more directly using videos and links to external content. Some potential downsides of paperless technology suggested by students were difficulties with graph drawing and the use of a single screen to view reports and data. Overall, our experience has been a positive one and the use of such paperless technology offers a real opportunity to fundamentally change the way we teach laboratory classes in the future.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC068

Structure-function relationships, 3D printing and public engagement – developing teaching exercises to integrate core principles and communication skills with a modern technological approach

S.J. Tucker

School of Medicine, Medical Science and Nutrition, University of Aberdeen, Aberdeen, UK

Introduction

Teaching and learning approaches in medical science must keep pace with advances in understanding and technology to support modern and relevant education. With an ever increasing need to engage with the public to develop trust and transparency around medical science, student understanding must translate to improved public engagement to expedite appreciation of science amongst the general public.

The aim of this teaching development project was to create an educational exercise for undergraduate students that would integrate elements of drug design, 3D structural understanding and public outreach.

Process

A new 3D printing practical project for final year pharmacology undergraduates (35 students) was designed, where small groups select and print a drug target and some commonly used drugs that interact with it. Over the course of 4 different class sessions, groups complete the following activities:

- individual justification of drug target choice pitched at a lay audience
- group creation of a short video using the 3D models to demonstrate structure related to function, and aspects of molecular interactions
- group design of a new synthetic drug for the target using molecular modelling software incorporating key chemical modifications

Results

The assessments were challenging and effective at distinguishing between student abilities with a range from 65-90% normally distributed with a mean close to the 1st – 2.1 border ($73.2 \pm 5.3\%$).

Student feedback surveys reported (n = 32):

- 92% rated the project at 4 / 5 or 5 / 5
- 92% strongly or generally agreed that the exercise developed group working skills
- 73% strongly or generally agreed that the exercise developed data handling skills
- 77% strongly or generally agreed that the exercise developed problem solving skills

Free text comments suggested the variety of assessments and inclusion of peer assessment (for video exercises) were beneficial to student skills gain, and the project provided an engaging way to learn about a variety of complex topics in an integrated way.

Discussion

The design of this new teaching exercise was successful in terms of creating a modern, technological way of integrating key topics. The assessments provide a holistic view of student skills relating to specific discipline areas, but also include many transferable skills that fit well into the exercise. The data evidence the success of this project, and the simplicity of the overall design could be easily adapted to any area of medical science where structure and function are related.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC069

Embedding work placed learning within the Medical Science Curriculum through capstone projects: a pilot study

C. Roberts¹, J.C. Bamford¹, A.M. Gillespie¹, D. Haughney¹, J. Morrice¹, E. Poyner¹, K. Stewart¹, K. Watson², K. Bryson² and J.S. Gregory¹

¹University of Aberdeen, Aberdeen, UK and ²Total Endurance Ltd., Aberdeen, UK

Capstone projects (such UK undergraduate honours projects) are important for consolidating previous learning and gaining practical experience. Workplace placements are valued by students, employers and universities but typically occur outside the taught curriculum. This abstract reports and reflects on our experience of placing honours students with a local company.

The primary challenges were in planning. Initial discussions between staff from University of Aberdeen and Total Endurance triathlon coaches, identified areas of mutual interest. We jointly planned a project feasible within the allocated period (10 weeks). Three students were selected each year (2018 and 2019).

Each supervisor adopted complementary roles. Administration, analysis and write up were led by academic staff (JG in 2018-19, JG and CR in 2019), familiarisation

and testing of the equipment was supervised jointly (with support from suppliers Algeos) and day-to-day guidance and coaching expertise by the external supervisors (KB and KW). Regular communication and visits to each site were maintained by supervisors and students throughout, which was key to the placement success. Students worked together to perform the practical work. This included recruiting volunteers and exploring technologies for measuring gait. Technologies ranged from personal trackers (e.g. Garmin Forerunner), to those designed for research or coaching (e.g. OptoGait). The first study (2018) explored all technologies in treadmill running. The second (2019) built on this by investigating changes between treadmill and overground running and used the indoor track at Aberdeen Sports Village as an additional site. Each student had their own role within the project and a slightly different research question.

Reflecting on the experience shows many positive results from this approach. All students reported that the placement had been a key reason for choosing the project. They particularly valued the chance to be in a professional environment and have guidance from the Total Endurance staff as well as their academic supervisors. Academically, students were not compromised by being in this environment as all achieved a mark for their project at least as high as their corresponding exam grades (2:1 or 1st class), though marks were only available for 2018 students at the time of writing. Subject-related work placements can provide a mutually beneficial experience. It enables companies to benefit from dedicated time and resources to investigate a question relevant to their business whilst students gain subject-specific experience in a professional environment. Closer links between the department and the company have also had additional benefits, as we have been able to share expertise and facilities on other occasions when mutually beneficial. Following this success, we aim to build upon this and offer more placement based projects in the future.

We would like to thank Algeos for OptoGait training and support and the loan of additional equipment.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC070

Embedding principles of research ethics and experimental design in a physiology-based curriculum through the systematic review and meta-analysis of clinical trial data.

S.C. Land and D. Booth

School of Life Sciences, University of Dundee, Dundee, UK

Biomedical education aims to equip students with subject-level knowledge that can be combined with critical, analytical and practical skills to evaluate questions relevant to health and healthcare. To develop these skills in combination, we have developed a practical exercise aimed at undergraduate physiology, pharmacology

and biomedical science students that promotes reflection on the post-war history of clinical trial regulation whilst training them in the evaluation of clinical studies using a standardised systematic review, data extraction and meta-analysis method. The activity requires 45 hours of individual effort which involves 3 taught sessions followed by a data analysis and interpretation-style assessed exercise.

The first taught session covers the ethics and history of clinical testing with emphasis on the problem of bias and the role of Sir Austin Bradford Hill in establishing the randomised clinical trial format. This informs a discussion of clinical trial legislation arising from the findings of the Nuremberg trials which led to the Declaration of Helsinki, as influenced by the Kefauver-Harris amendments and the Belmont Report. Students are encouraged to consider ethical implications arising in the modern age and also the significance of the 3R's in minimising use of animals in scientific experimentation. Advanced systematic searching of clinical trial databases is put into practice in a follow-up workshop and a final self-led, mentored IT session provides opportunity to use practice datasets to create forest plots using R-studio and interpret results with reflection on study weighting, heterogeneity and bias containment.

Once confident, students embark on an assessed exercise which requires them to apply these skills to test a suitable clinically-themed hypothesis. A proforma-style report is used to record each student's approach to, i) advanced database searching, ii) eligibility screening of studies using Bradford Hill's Criteria for Causation, iii) reporting of study triage using the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) protocol, iv) extraction of suitable data, v) creation and interpretation of forest plots, vi) assessment of study heterogeneity by funnel plot and, finally, vii) a comment on physiological causes and mechanisms. A marking rubric enables a weighted grade to be awarded for each element of the process together with feedback to explain the basis of the mark. Through this activity, students utilise their subject-level knowledge in combination with understanding of research ethics, experimental design, bias containment and study power to evaluate questions central to disease causation, drug efficacy and healthcare methods. This provides a useful format for advanced study of clinical questions in the upper years of the undergraduate biomedical curriculum.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC071

Easy as 4-AT: Improving Delirium Screening in Acute Elderly Admissions with a Targeted Educational Intervention by Science Students – A Pilot Study.

C. Cooper¹, D. Scott¹, A. Hunter¹, J. Handyside¹ and V. Henderson²

¹*Institute of Education for Medical & Dental Sciences, University of Aberdeen, Aberdeen, UK and* ²*Dr Gray's Hospital, NHS Grampian, Elgin, UK*

Background: Delirium is a common and serious complication of acute illness in elderly patients that frequently goes undiagnosed. Its pathophysiology is often poorly understood by many clinicians, but the abnormalities in physiology are usually transient, reversible and preventable. It is sometimes referred to as 'acute brain failure'. The 4 A's test (4-AT – alertness, age, attention, acute changes) is a simple and fast way of screening for delirium. A Healthcare Improvements Scotland inspection of Dr Gray's Hospital, Elgin in April 2017 revealed that the 4-AT was often being underused and incorrectly completed. 4-AT usage was audited and then a tailored educational intervention aimed at increasing and improving 4-AT usage was delivered.

Methods: The medical and nursing notes of inpatients aged 65 and over on the Acute Care for the Elderly (ACE) ward were assessed for the presence of correctly completed 4-ATs on 15th January 2018. Junior clinicians (n = 11) were surveyed to identify reasons for non-completion before viewing a video demonstrating the quick and easy nature of the 4-AT and an infographic explaining 4-AT scoring. They were then surveyed on this educational intervention. The audit was repeated on 16th March 2018.

Results: 4-AT completion rate increased from 57.7% to 60.9% between January and March (*N.S. p>0.05*). However, clinicians reported that over 80% felt they were using the 4-AT on all elderly admissions. 55% said they had had trouble completing a 4-AT assessment, particularly the final section where they had to judge acute changes in physiology or fluctuating pathophysiology. Junior doctors scored the video and the infographic 7/10 and 6.4/10 respectively but 67% disagreed that it made them more likely to carry out the 4-AT in all elderly admissions as they felt they already completed it regularly. Reminder stickers on patient notes and incorporation of the 4-AT into clerking forms were suggested as further interventions by respondents.

Conclusion: 4-AT completion was not acutely improved by the intervention indicating the need for a more wide-reaching, thorough intervention. The educational intervention seemed to be effective in demonstrating how to overcome issues with 4-AT completion. It did identify that there were major issues with the perceptions clinicians had of how often and effectively they were completing the 4AT documentation, compared to reality. This work is being shared and taken further by NHS Grampian to further enhance use of this screening tool and to minimise the impact of delirium on patients and healthcare providers. The audit will be repeated later this year to check for any long-term improvements in 4AT awareness and completion. This project also demonstrates that science students may effectively

undertake quality improvement work as part of an Honours capstone project to benefit the NHS and the local population.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC072

Subject benchmarks for quality assurance and course enhancement

S.J. Tucker

School of Medicine, Medical Science and Nutrition, University of Aberdeen, Aberdeen, UK

Introduction

Benchmarks are an important tool in higher education to safeguard quality and guarantee the taught materials are appropriate for a given discipline. There are many forms of benchmark including key subject statements, suggested learning outcomes, benchmark statements and more detailed core curricula, e.g. the UG pharmacology core curriculum recently published by the British Pharmacological Society. These guides often originate from individual institutions, but also arise from learned societies or educational bodies with appropriate expertise.

The aim of the project was to compare the pharmacology curriculum at the University of Aberdeen with the British Pharmacological Society core curriculum, and use this as a catalyst for enhancing the programme.

Process

Here, the core curriculum for undergraduate pharmacology programmes, published by the British Pharmacology Society, was aligned with the content of the Aberdeen pharmacology programme and a mapping exercise analysed the extent to which the curriculum was covered across the various pharmacology modules run at the University of Aberdeen. Any shortfalls were then used to help frame development of new teaching blocks and modules to ensure currency and completeness of the programme.

Results

Of the 50 statements that make up the core curriculum:

- 52% were covered across < 3 programme modules
- 60% were covered across < 2 programme modules
- 76% were covered across at least 1 programme module

Particular areas of strength were identified as drug metabolism, toxicology, systems pharmacology and practical approaches to pharmacology. The areas that were covered in several modules were carefully checked to avoid repetition and to ensure progressive development of subject complexity.

Of the remaining 24% of statements:

- 13% of were covered briefly
- 13% were not covered

These areas related to molecular modelling, use of big data, public engagement, societal responsibility and drug development. These were used to inform course and programme redesign and redevelopment, with a new integrated project designed in the Honours year to deliver details of these areas in the context of public engagement and wider society. The module was very well received with 92% of students surveyed (n = 32) evaluating it with a 4/5 or 5/5.

After changes were implemented in 2018, reassessment of the core curriculum coverage was carried out and 96% of statements are now covered by at least one programme module suggesting this exercise has successfully served an important quality assurance and enhancement role.

Discussion

This study demonstrates the importance of reflective practice in considering teaching coverage and approaches in comparison to established standards, outside the context of the institution. This permits not only quality assurance, but also drives innovation, change and improvement to the benefit of the programme and the student experience associated with it.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC073

An Assessment of Students' and Health Professional' Abilities to Site Stomas Accurately on Human Cadavers.

A. Arnott¹, L. Cowpland¹, J. Jury¹, I. Stewart¹, A. McKinley² and D. Scott¹

¹*Institute of Education for Medical & Dental Sciences, University of Aberdeen, Aberdeen, UK and* ²*Aberdeen Royal Infirmary, NHS Grampian, Aberdeen, UK*

A stoma is a surgically constructed opening created to treat disease or damage in a patient. Common examples include colostomies and ileostomies. Macdonald *et al.* (2003) previously indicated that stoma nurses were superior to surgeons at identifying stoma sites, potentially reducing post-operative complications and preventing stoma failure. However, that study did not assess these healthcare professionals under the same conditions, potentially skewing the conclusions.

We aimed to investigate the accuracy of siting stomas between specialist stoma nurses compared with trainee surgeons, students and non-clinical academics with some anatomical background. All groups would site stomas under the same conditions using human cadavers.

Ethical approval was granted by UoA CLSM Ethical Review Board. Students (BSc n=39, MBChB=28), non-clinical academic staff(n=14), stoma nurses (n=3) and surgeons (n=8) were recruited. Each participant assessed stoma locations on ten anonymised cadavers.

A3 Acetate sheets with 1cm² grids indicating XY coordinates covered each cadaver's abdomen. Participants marked the locations of their proposed ileostomy and a colostomy on these acetates. Mean coordinates obtained from different participant

groups were compared against a 'gold standard' established by a group of experienced senior colorectal surgeons/nurses. Statistical analysis using Student's unpaired t-test between sets of coordinates was undertaken to assess whether there were any differences between the 'ideal stoma location' and the mean X and Y coordinates identified by participants. Participants were also asked how they identified their sites and their confidence when doing so.

There was a significant difference between the x coordinates for siting an ileostomy (<0.001) and colostomy (<0.05), but there was no significant difference for the y coordinates when comparing stoma nurses and surgeons. Stoma nurses were nearest the pre-determined 'gold standard' locations. Confusion regarding the height of stoma location on the abdomen dominated when non-clinical academics were assessed, but with physiology staff demonstrating superior accuracy to anatomy faculty. There was no significant difference between the X and Y coordinates for siting both an ileostomy and a colostomy when comparing science and medical students as cohorts.

Participant responses indicated that confidence in siting a stoma was no indication of accuracy. Other comments indicated that those who were most likely to site a stoma accurately had considered how the person would function in real life (i.e. how they would walk, where fat masses would move when upright etc) as well as their anatomy. Those who did less well tended to only observe anatomical landmarks to make their decisions.

Surgical training involving stoma nurses where anatomy, physiology and lifestyle are considered may improve stoma siting technique and post-operative outcomes for patients.

Macdonald, A., Chung, D., Fell, S., Pickford, I. (2003). An assessment of surgeons' abilities to site colostomies accurately. *The Surgeon: Journal of the Royal Colleges of Surgeons of Edinburgh and Ireland*. 1, 347-349.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC074

Healthier nurses? Activity-based learning about physical activity and well-being.

L. Ginesi, P. Goacher and S. Hanson

School of Health Sciences, University of East Anglia, Norwich, UK

Health demographic trends indicate a need for a radical shift in the way we tackle the obesity epidemic. To date, relatively little attention has been directed towards exposing student healthcare professionals to assessment of body composition and adiposity. We introduced a series of interactive applied physiology workshops to engage nursing students who were learning about lifestyle management interventions and their potential for promoting physical health and mental well-being.

Students were asked to prepare for the sessions by completing a flipped learning Physical Activity Questionnaire and Diet survey. The first workshop stations included anthropometric measurements (Goacher Lambert and Moffat, 2012; Grasten and Watt, 2016), blood pressure, total cholesterol and calculation of QRISK3. In the second session, students used personal response system (clickers) to respond to 20 True/False statements about exercise physiology and diets, which revealed many myths and misconceptions. Students then undertook a timed walk in small groups (up to 30 minutes) recording pulse, blood pressure and oxygen saturation (% O₂) before and after. Assessment of student learning was formative through analysis of QRISK3 for patient cases supported by action plans for each person. Students' feedback about these workshops has been positive, reporting that they prefer the "bite-sized" format and feel they learn more than in lectures.

Since qualified nurses are more likely to be overweight, have higher levels of stress and get less sleep than average (Trossman, 2009; American Nurses Association, 2017) there is a need to engage student nurses with the aim of deepening their understanding of positive health impacts of regular physical activity (Hanson and Jones, 2015). Activity-based workshops provided opportunities for co-operative learning; intermittent probing questions prompted dialogue and discussion about a wide range of related topics such as perceptions of obesity, metabolic syndrome, obesogenic environments and sedentary lifestyles.

Further evaluation is required, but interactive workshops clearly offer potential for powerful and engaging learning about causative factors of obesity and short- and long-term consequences including vascular disease and type II diabetes. Although our aim was to focus on prevention through improved levels of physical activity, the workshops revealed that it was important to emphasise that students were not expected to share personal measurements or discuss their body compositions with others. Although they appear to be inclined towards adopting healthier lifestyle behaviours, we also found that a number of students chose not to engage with a short "star-jump" session or the group walking activity. There appears to be an additional need to support student nurses' learning about sustaining own well-being and modelling optimal health behaviours.

American Nurses Association (2017) Health Risk Appraisal Findings. Available from *Healthy nurse, health nation challenge*[online : <http://www.healthynursehealthynation.org>; Accessed 11th April, 2019]

Goacher, PJ, Lambert R, Moffatt PG (2012) Can weight-related health risk be more accurately assessed by BMI, or by gender specific calculations of Percentage Body Fatness? *Medical Hypotheses* 79 (2012) 656–662

Grasten, A; Watt, A. (2016) Perceptions of Motivational Climate, Goal Orientations, and Light- to Vigorous-intensity Physical Activity Engagement of a Sample of Finnish Grade 5 to 9 Students *International Journal of Exercise Science* 9(3): 291-305, 2016.

Hanson S, Jones A (2015) Is there evidence that walking groups have health benefits? A systematic review and meta-analysis. *Br J Sports Med*; 49:710-715.

Trossman, S (2009) *Permission slip. RNs should consider their own needs for a healthier-living agenda.* *The American Nurse*, Jan/Feb.

Thanks to the Module 3 BSc Nursing team at UEA.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC075

Assessing Geriatric Proton Pump Inhibitor Prescribing and Polypharmacy in General Practice with an Educational Intervention by Physiology Students.

M. Pollock, B. Roddy and D. Scott

Institute of Education for Medical & Dental Sciences, University of Aberdeen, Aberdeen, UK

Polypharmacy is a rising concern in the Scottish elderly population. Proton Pump Inhibitors (PPIs) are among the most widely sold and distributed drugs in the world and are overprescribed in primary health care settings. The older population are at a greater risk of side-effects when taking PPIs due to changes in their physiology during the ageing process. As part of the approach to widen the range of Honours projects undertaken by Honours Physiology students, we initiated a partnership with a GP practice to help them assess whether they were prescribing PPIs appropriately to their older population and to improve their understanding of deprescribing of PPI's.

We aimed to assess polypharmacy and PPI prescribing in the >75 year old population of Oakley Health Centre, in Fife, Scotland. This project also aimed to observe the efficacy of an intervention in reducing polypharmacy and promoting PPI deprescribing and to evaluate the attitudes of clinicians towards the use of PPIs and polypharmacy.

A two cycle audit and an educational intervention were carried out between the 17th of January and the 7th of March 2019. The first audit undertaken was pre-intervention, and the second post-intervention, to assess effectiveness of intervention. An oral presentation, demonstration videos and an infographic algorithm were created for the educational intervention, presented to staff on the 6th February 2019. Two surveys were distributed, one assessing attitudes of staff towards deprescribing and polypharmacy, and one collecting opinions of the intervention and educational resources.

Results: The first audit found 32.6% of patients >75 years old were on a repeat PPI prescription, and the second audit found 32.7% (Not significant at $p < 0.05$). The number of these patients on >5 additional medications was 89.8% in first audit, and 89.7% in second audit (Not significant at $p < 0.05$). 60% of clinicians said PPIs were overprescribed, and 100% considered polypharmacy to be an issue within the local area. 83% agreed the intervention highlighted issues regarding PPI prescribing and made them more likely to deprescribe in appropriate patients. Respondents also reported that they had had very little formal education on deprescribing, and that they felt there was a lack of resources to help them in this regard.

Conclusion: Despite no significant change seen acutely post intervention in this short, time-limited project, the resources created were well received. A further

re-audit has been scheduled for a date six months after initiation of this project to investigate longer-term effects of this intervention. Results demonstrate the scale of local PPI overprescribing and polypharmacy, and a longer-term audit/ intervention has the potential to improve these issues more significantly. This project also demonstrates how physiology students may usefully contribute to quality improvement work with healthcare professionals.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC076

Assessment of Attitudes of First Year Medical Undergraduate Students towards Communication Skills Training

K. Badaam and S. Shaikh

Government Medical College, Aurangabad, Aurangabad, India

Background: There is a lack of communication skills training in current Indian medical curriculum leading to medical professionals coming out without proper attitude & communication skills which often lead to unclear & disturbed doctor patient relationship. Medical Council of India is coming up with Attitude, Ethics and Communication (AETCOM) modules for undergraduate medical students & faculty development programs are being conducted on the implementation of the modules. Initially, there is a need to assess attitudes of students towards such communication skills training programs. Communication Skills Assessment Scale (CSAS) is a validated & accepted scale to measure attitudes of students towards communication skills training in diverse student populations [1-3]. Present study was done to assess the attitudes of 1 year medical undergraduates towards communication skills learning using CSAS.

Methods: Cross-sectional study at Department of Physiology, GMC, Aurangabad. All 1 year medical undergraduates present for the activity were included. Ethics Committee approved the protocol & consent was taken from participants. Questionnaire was filled by students along with details like demographic information, how do they rate themselves as a speaker, whether they would like formal communication skills training courses during clinical years. The questionnaire has 26 items using five-point Likert scale which include 13 positive & 13 negative statements about communication skills training labelled as PAS & NAS respectively. Both scales range from 13 to 65 with higher scores indicating stronger positive or negative attitudes. The scores were compared for gender groups by Independent student's t test. Descriptive data was described as Mean $\pm$ Standard Deviation (SD). Cronbach's alpha was calculated for PAS & NAS to test internal consistency.

Results: Sixty students were present. Response rate was 100%. Mean age was 18.4 ± 0.8 years. There were 34 females & 26 males. Twenty nine students rated themselves as good speakers, 16 as average, 10 as excellent & 5 students rated

themselves as poor speakers. When asked whether they would like formal courses in communication skills training, an overwhelming 53 students mentioned yes, only 5 students mentioned no whereas 2 students did not answer the question. Cronbach's alpha for PAS was very good at 0.87 whereas it was lower for NAS at 0.62. Overall for 60 students, PAS was 53.5 ± 7.8 and NAS was 32.2 ± 6.1 . Among 26 male students, PAS was 52 ± 10.7 & among 34 female students it was 54.7 ± 4.4 . There was no statistically significant difference ($p=0.18$). The NAS in males was 34.2 ± 6.3 whereas in females was 30.7 ± 5.6 . It was significantly lower in females with $p=0.026$.

Conclusions: The findings indicate the acceptability of communication skills training among our institute 1 year students and reflect the potential for success of AETCOM module if the implementation is proper.

Lee YM, Lee YH. Evaluating the short-term effects of a communication skills program for preclinical medical students. *Korean J Med Educ* 2014; 3: 179-187.

Leslei K and Prosper T. A study of university of Zimbabwe medical students 'attitudes towards the teaching and learning of communication skills. *IJOAR* 2013; 1(9) 35-42.

Tor Anvik, Tore Gude, Hilde Grimstad, Anders Baerheim, Ole B Fasmer, Per Hjortdahl, Are Holen, Terje Risberg and Per Vaglum. Assessing medical students' attitudes towards learning communication skills – which components of attitudes do we measure? *BMC Medical Education* 2007, 7:4 doi:10.1186/1472-6920-7-4

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC077

Clarifying Renal Clearance: An educational approach using visualisation of virtual volumes via student-developed video resources.

K. Bradshaw and D. Scott

Institute of Education for Medical & Dental Sciences, University of Aberdeen, Aberdeen, UK

There are many aspects of physiology which seem harder to teach and learn than others. One such aspect is renal clearance. Whether this is due to its perceived complexity or whether it is genuinely a more complicated topic to navigate, it appears to be difficult to for some students to conceptualise. Attempts have been made to try and create a less daunting approach which will help students grasp the basic principles and retain information whilst aiding tutors in the teaching process. One such method of helping to clarify renal clearance was adapted from Guevara & Milanick (2017). This simple demonstration was easily replicable using inexpensive resources such as bottles, clear mineral oil and coloured water. Different volumes of coloured fluid were used to represent change in concentrations before and after renal clearance had occurred to aid understanding of virtual volumes. Instructional videos were developed by an undergraduate science student using basic camera

and editing resources and placed on the virtual learning environment (VLE) of a second year organ system physiology course. A voluntary quiz was made available to students before and after the video resources were released to determine if the teaching had benefitted the students. The second quiz had additional renal questions, plus some optional questions about the video resources on the VLE. When rating the helpfulness of video resources, students ($n=59$) rated them positively (8.5 ± 1.5 out of 10 on a Likert scale). In terms of audio-visual quality, they were rated 9.6 ± 0.7 , with ease of viewing via the VLE being scored at 9.2 ± 0.8 . Most students preferred delivery of the videos and associated quizzes online. 65 students voluntarily took Quiz 1 before the videos became available, and 59 students took Quiz 2 after they could watch the resources. The correct answers were only provided to the students in a feedback session after Quiz 2 had closed. Students rated Quiz 2 as being easier than Quiz 1 (8.5 ± 1.6 vs 7.9 ± 1.9 , $P = 0.0015$, Mann-Whitney test). There were substantial increases in the number of students answering questions correctly in Quiz 2, with some questions that had previously been answered incorrectly ~80% of the time changing to 100% correct. Free text comments indicated that the resources were valued by students with special learning requirements or those for whom English was not their first language. This project shows that relatively simple video resources developed in partnership with students can prove useful in enhancing students' understanding of renal physiology concepts and in helping them improve their knowledge and understanding. Given the positive response to the video resources this year, we intend to keep these resources permanently on the VLE so that future cohorts of students can use them to enhance their studies. We hope to adapt this idea to other areas of the curriculum so that we can cater for a wider range of learning strategies used by students.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC078

Perceptions of using infographics for physiology poster projects – a comparison between cohorts.

F. Coombey and D. Scott

Institute of Education for Medical & Dental Sciences, University of Aberdeen, Aberdeen, UK

We have previously reported the use of infographics to revitalise a physiology poster research project. Infographics are progressively being used to demonstrate key scientific concepts in simple graphical form. Developing students to effectively communicate with both a scientific audience and the general public is increasingly important within an academic curriculum. We had a very positive response to this innovation during the initial year it was introduced, but it was not yet clear whether this opinion would be sustained across cohorts. We decided to compare

the opinions of students across two different cohorts to investigate whether the positive opinions of infographic projects was sustained or merely a transient response due to the novelty of the activity.

Students were asked to undertake a 11 week project on a physiological topic of their choice and present it in printed format at a traditional poster session, and online. Staff and peers graded the infographics at the poster session. In addition, students had to submit an abstract reporting their findings as if they were submitting it to a Physiological Society meeting. Students could explore any area of physiology they wanted to as long as they were excited about the topic, and they could communicate the important messages or concepts involved in their project clearly and concisely. A range of free software/websites that could be used to produce infographics were demonstrated, and students could choose whichever one they felt was most suitable for them.

Students were invited to complete an anonymous questionnaire on the delivery and outcomes of the exercise. This considered how easy it was to simplify the scientific material and how infographic presentation compared with a standard scientific poster. Comparison between online and printed delivery was also evaluated, along with the impact on the overall learning experience. Students rated various aspects of the project on Likert scales from 0-10.

46 students completed the questionnaire in cohort 2016-17 and 50 in cohort 2018-19. Both cohorts preferred online infographic posters rather than printed ones. Other consistent findings included that students felt that infographics could communicate complex scientific concepts more effectively than traditional scientific posters. Students found the science easier than trying to pitch the content at the correct level for the audience. They appeared to give more thought as to how information should be presented and delivered to different audiences and became more critical of their own work. There seemed to be no significant differences between cohorts in terms of their responses for most questions.

This initiative has now been running for 3 years and has rejuvenated a physiology project assignment that had previously received poor ratings in student feedback. This approach has now been adopted by other courses to enhance student communication skills and to increase graduate skills.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC079

Na⁺-Taurocholate cotransporting polypeptide (Ntcp) mediates thyroxine transport in sheep choroid plexusN.A. Kassem^{1,3}, K. Zibara², M. Sabra¹, Y. Fares¹, J. Preston³ and R. Deane⁴

¹Neuroscience Research Center (NRC), Medical School, Lebanese University, Hadath, Beirut, Lebanon, ²Biology, Lebanese University, Beirut, Lebanon, ³Institute of Pharmaceutical Science, King's College London, London, UK and ⁴Medical School, Rochester University, New York, NY, USA

Thyroid hormone (TH) is essential for normal development and growth of the central nervous system (CNS), and neuronal regeneration in adults after traumatic brain injury (TBI). The entry of thyroxine (T₄) into brain is via two main barriers, the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barrier through the choroid plexus (CP). We have previously demonstrated that transport of T₄ from CSF into CP and other brain tissues is carrier-mediated and dependent on the presence of transthyretin and on organic anion transporting polypeptides. However, the transport route of T₄ from the CSF compartment into blood across CP is still poorly investigated. The aim of this research was to characterize the mechanism by which T₄ is transported from CSF to blood across the lateral ventricle CPs *in situ*. We investigated the effect of taurocholate and of sodium replacement with choline in artificial CSF (aCSF) on ¹²⁵I-labelled-T₄ transport at the apical (CSF) side of the CP using the steady-state method to calculate ¹²⁵I-T₄ efflux from the CSF to blood (R%). Sheep (Clun Forest strain, either gender) weighing 20-45 kg were anaesthetised with sodium thiopental (20 mg/kg, iv), heparinized (10,000 U, iv), exsanguinated, and head was removed. The brain was extricated from the skull and the CPs perfused with a modified Ringer solution via internal carotid arteries at a flow rate of 1.5 ml/min. Both ventricles were then opened, and the CP was superfused with aCSF containing 0.018MBq ¹²⁵I-labelled T₄ and 0.37MBq ¹⁴C-labelled mannitol (as a reference molecule) per 100 ml of aCSF. After 1 h, steady state uptake of ¹²⁵I-labelled T₄ was achieved, then the CP was perfused with low Na⁺ aCSF (contained 10 mM Na) or aCSF containing low Na⁺ and 1.0 mM taurocholic acid (TA) for 40 min, followed with normal aCSF. Values are means ± S.E.M., compared by *t* Test. Results showed that the R% in the presence of low Na⁺-aCSF (1.8±0.1%) was significantly attenuated (n=3, p<0.05) compared to control (3.1±0.6%) (n=3). On the other hand, this efflux decreased even further after the addition of TA (0.54±0.11%), an inhibition of > 70% in comparison to low Na⁺ (n=3, p<0.01). We suggest that the reduction in the R% of T₄ efflux from the CSF to blood across the CP epithelial cells in the presence of low Na⁺ and TA might be mediated by Na⁺-Taurocholate cotransporting polypeptide (Ntcp) functioning at the apical side at of the CP by controlling T₄ concentration in the CSF compartment and monitoring the efflux toward the blood. This is consistent with our previous finding which demonstrated the presence of Organic anion transporting polypeptides dependent on sodium gradient at the CSF face of the CP *in vivo*. This work

is in progress to determine the mechanisms of T_4 efflux from CSF to blood across blood-CSF-barrier.

All procedures accorded with current UK legislation and approved by Lebanese University, NRC, internal board.

The authors would like to acknowledge the considerable help and contribution of Dr Malcolm B Segal who was involved in the study conception and design.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC080

Urea-induced increases in glycosylated UT-B urea transporters

A. Farrell and G. Stewart

Biology & Environmental Science, University College Dublin, Dublin, Ireland

Facilitative UT-B urea transporters play a crucial role in the human bladder (1), protecting urothelial cells from the damaging effects of urea (2). Allelic variation in these UT-B transporters has been linked to the risk of developing bladder cancer (3), so it is imperative to understand the physiological regulation of the UT-B proteins. Our previous studies utilised the RT4 cell line model of human urothelial cells to show that NaCl-induced, but not mannitol-induced, increases in external osmolality upregulated the abundance of UT-B transporter protein (4). In this current study, we further investigated the effects of urea-induced changes in external osmolality using the same cell line. RT4 cells were cultured for 48 hours in media with a range of additional urea concentrations and then membrane-enriched protein samples were prepared from treated flasks of cells. Next, these samples were used for semi-quantitative western blotting experiments and then densitometry analysis was performed (with data given as mean values $\pm$ SEM). Compared to control experiments (50 ± 7 , $N=4$), no significant change in UT-B protein abundance occurred with 10mM urea treatment (56 ± 6 , $N=4$, NS, ANOVA), but significant increases did occur with both 50mM (67 ± 4 , $N=4$, $P<0.05$, ANOVA) and 100mM urea (66 ± 1 , $N=4$, $P<0.05$, ANOVA) treatments. Importantly, while there was significant specific increase in glycosylated UT-B protein with 50mM urea treatment (86 ± 5 versus 59 ± 8 , $P<0.05$, $N=4$, ANOVA), there was no change in NaKATPase protein (27 ± 4 versus 32 ± 6 , NS, $N=4$, ANOVA). Overall, these data show that urea-induced changes in external osmolality specifically upregulated levels of glycosylated UT-B urea transporter in the RT4 cell line. As glycosylation is crucial for UT-B function, we suggest that the urea-induced changes observed may be of important physiological significance.

Walpole et al., 2014, *AJP Renal* 307(9):F1088-94

Dong et al., 2013, *PLoS One* 8(10):e76952

Garcia-Closas et al., 2011, *Hum Mol Genet.* 20(21):4282-9

Farrell et al., 2018, *Proc Physiol Soc* 41

This work was funded by The Jack Pickard Research Foundation and University College Dublin.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC081

Effect of vape carriers propylene glycol and vegetable glycerine on epithelial permeability and glucose uptake in airway cells.

J.G. Jacob¹, M. Woodall¹, I. Khan¹, R. Tarran² and D. Baines¹

¹*Institute of Infection & Immunity, St George's University of London, London, UK and*

²*Cell Biology and Physiology, University of North Carolina, Chapel Hill, NC, USA*

Electronic nicotine delivery systems (ENDS), more commonly known as e-cigarettes, are used as a tobacco free nicotine delivery system. ENDS utilise a liquid solution which is drawn over a heating element to produce a vapour which is then inhaled giving rise to the term 'vaping'. The solution normally contains propylene glycol and vegetable glycerine to generate the vapour together with nicotine and flavourings. Evidence has indicated these 'carriers' may affect the function, growth and survival of airway epithelial cells [1][2]

H441 or primary bronchial epithelial cells (HBEC) were grown on plastic or cultured on permeable supports at air-liquid interface. PG (0-3%) or PG:VG (55:45, 0-3%) were applied to the medium, directly to the apical (luminal) surface, or the cells were exposed to vapour for 10 minutes.

Transepithelial electrical resistance was measured using an electrovoltmeter and apical to basolateral permeability was assessed using sodium fluorescein. Glucose uptake across the cell membrane was measured using the Uptake Glo luminescent assay (Promega, UK) or ¹⁴C D-glucose. Cytotoxicity was measured using an LDH cytotoxicity assay (Peirce, UK). Mannitol was used as an osmotic control in some experiments.

Exposure to 3% PG and 3% PG:VG decreased TEER relative to PBS at 15 minutes, 4hrs and 24hrs compared to PBS (3% PG: $p < 0.001$, 3% PG:VG $p < 0.05$, $n = 5$). At 24 hours 3% PG and 3% PG:VG exhibited more fluorescein permeability compared to PBS and mannitol and the permeability coefficient (Papp) was larger for cells exposed to 3% PG than 3%PG/VG.

PG elicited a dose dependent inhibition of glucose uptake in proliferating H441 and HBEC. 3% PG inhibited glucose uptake to a similar level as the glucose transport inhibitor cytochalasin B ($35 \pm 5\%$ and $32 \pm 7\%$ of control $p < 0.001$, $n = 9$ respectively). PG did not elicit any changes in LDH release. Treatment of air-liquid interface cultures with PG:VG (3%, 15ml apical) inhibited basolateral but not apical glucose uptake ($p < 0.01$, $n = 4$) in H441 cells and the effect was similar in HBEC when compared to a PBS control. Inhibition was less than that induced by the GLUT transport inhibitor phloretin. No effect of PG:VG on uptake was determined when

compared to 3% mannitol. These data indicate that exposure to PG:VG reduces TEER, increases permeability and decreases glucose uptake in airway cells.

As glucose uptake is a key metabolic requirement for cell growth, survival and is critical for the normal function of the airway epithelium, we hypothesised that propylene glycol (PG) and propylene glycol mixed with vegetable glycerine (PG:VG) inhibited glucose uptake and modified barrier function human airway epithelial cells.

Sassano MF, Davis ES, Keating JE, et al. Evaluation of e-liquid toxicity using an open-source high-throughput screening assay. *PLoS Biol* 2018;16:e2003904.

Morshed KM, Jain SK, McMartin KE. Acute toxicity of propylene glycol: an assessment using cultured proximal tubule cells of human origin. *Fundam Appl Toxicol* 1994;23:38-43.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC082

Effects of bile acids on human epithelial sodium channels in *Xenopus* oocytes and H441 airway epithelial cells

Y. Ling^{1,2}, S.M. Gettings¹ and M. Althaus¹

¹*School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK* and ²*School of Animal Science, Anhui Agricultural University, Hefei City, China*

The epithelial sodium channel (ENaC) mediates transepithelial sodium absorption across airway epithelia and contributes to volume homeostasis of airway surface liquid (ASL). Increased ENaC activity leads to ASL depletion and impairment of mucociliary clearance. Bile acids (BA) directly activate ENaC in heterologous expression systems. In cystic fibrosis patients, BA appear in ASL due to gastro-oesophageal reflux and aspiration. We hypothesise that BA contributes to enhanced ENaC activity and ASL depletion in cystic fibrosis airways. We measured the effects of BA on heterologously expressed human ENaCs and endogenous ENaCs in human H441 airway epithelial cells.

Human $\alpha\beta\gamma$ - and $\delta\beta\gamma$ -ENaCs were expressed in *Xenopus* oocytes and channel activity was measured by the two-electrode voltage-clamp technique at -60 mV. ENaC-mediated current fractions were determined using the inhibitor amiloride (100 μ M). Sodium salts of the BA cholic acid (CA), chenodeoxycholic acid (CDCA), taurocholic acid (t-CA) and taurochenodeoxycholic acid (t-CDCA) were applied to a final concentration of 100 μ M. Amiloride-sensitive currents (ΔI_{ami}) before and after application of BA were analysed. All data (n = 6-7) were normally distributed and analysed with Student's paired t-test. Human H441 airway epithelial cells were cultured to polarised monolayers at air/liquid interface and sodium absorption was measured as transepithelial short-circuit current (I_{SC}) in Ussing chambers. I_{SC} signals were baseline corrected (100 μ M amiloride) and normalised to values before application of BA. Data (n = 4-5) are reported as means $\pm$ standard error.

CA significantly increased ΔI_{ami} in $\alpha\beta\gamma$ -ENaC expressing cells from 3.32 ± 0.55 to $3.61 \pm 0.59 \mu A$ ($p < 0.05$) and from 8.63 ± 1.42 to $11.33 \pm 1.68 \mu A$ in oocytes expressing $\delta\beta\gamma$ -ENaC ($p < 0.05$). CDCA had no effect on $\alpha\beta\gamma$ -ENaC ($p = 0.15$) but increased ΔI_{ami} from 8.45 ± 2.03 to $11.21 \pm 2.60 \mu A$ in oocytes expressing $\delta\beta\gamma$ -ENaC ($p < 0.05$). t-CA significantly increased ΔI_{ami} from 3.68 ± 0.66 to $4.72 \pm 0.77 \mu A$ ($p < 0.001$) in $\alpha\beta\gamma$ -ENaC and from 6.8 ± 0.95 to $8.54 \pm 1.12 \mu A$ in $\delta\beta\gamma$ -ENaC expressing cells ($p < 0.001$). t-CDCA significantly increased ΔI_{ami} in $\alpha\beta\gamma$ -ENaC expressing cells from 4.87 ± 0.48 to $6.37 \pm 0.71 \mu A$ ($p < 0.05$) and from 6.21 ± 0.57 to $8.64 \pm 0.76 \mu A$ in oocytes expressing $\delta\beta\gamma$ -ENaC ($p < 0.001$). In H441 monolayers, t-CDCA significantly increased I_{SC} by $9 \pm 0.02 \%$ after 2 min of application ($p = 0.036$; RM one-way ANOVA/Dunett's multiple comparisons test), whereas no other BA had any effect on I_{SC} . After 5 min of exposure to any BA, there were no differences in normalised ΔI_{ami} ($p = 0.25$; one-way ANOVA).

These data indicate that BA have the capacity to activate human ENaC in heterologous expression systems. There is no such effect on endogenous ENaC in human H441 airway epithelial cells. This discrepancy might be due to differences in ENaC maturation in heterologous expression systems compared to cultured airway epithelial cells.

YL is supported by the China Scholarship Council.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC083

Increased blood-to-stool ratio of short-chain fatty acids as a new marker in inflammatory bowel disease.

K. Jaworska¹, M. Konop¹, K. Bielinska¹, T. Hutsch¹, M. Dziekiewicz², A. Banaszekiewicz² and M. Ufnal¹

¹Department of Experimental Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland and ²Department of Pediatric Gastroenterology and Nutrition, Medical University of Warsaw, Warsaw, Poland

Background. Impaired gut-blood barrier has been noticed in a variety of diseases, including cardiovascular and metabolic diseases (1, 2). Especially intestinal diseases such as inflammatory bowel disease (IBD) are characterized by "leaky gut" (3, 4). Therefore, functional evaluation of the gut-blood barrier is a promising diagnostic marker. We hypothesized that gut bacteria-produced short-chain fatty acids (SCFA) may serve as a marker in IBD.

Methods. Animal study. Male, Sprague Dawley rats were anaesthetized i.p. with a mixture of ketamine (90 mg/kg) and xylazine (5 mg/kg). 24h after induction of IBD with acetic acid (IBD group, n=9), 1 week after induction of IBD (recovery group, n=9) and in sham controls (n=9) stool, portal blood and systemic blood samples

were collected. The gut-blood barrier permeability was determined by the ratios of: (i) portal blood concentration of SCFA (Cp) to fecal concentration of SCFA (Cf) and (ii) systemic blood concentration of SCFA (Cs) to fecal concentration of SCFA (Cf) as well as (iii) Cp and Cs of FITC-dextran administered into the colon. Clinical study. We evaluated Cs, Cf and Cs/Cf ratio of SCFA in 6 pediatric patients with IBD assessed as mild/moderate/severe in PUCAI and PCDAI at the time of sample collection, and 9 age-matched healthy controls.

Results. Rats with histologically confirmed IBD had significantly increased ratios of Cp/Cf (direct permeability) and Cs/Cf (indirect permeability) for SCFA (Fig.1). This was positively correlated with plasma FITC-dextran concentration. Likewise, IBD patients showed a significantly higher Cs/Cf ratio for SCFA including acetic, valeric, isocaproic, caproic and propionic acids in comparison to healthy controls (Fig.2). Conclusions. In both animal model and in pediatric patients with IBD we found an increased blood-to-stool ratio of SCFA, suggesting an increased gut-to-blood penetration of SCFA. These findings pave the way to a new, non-invasive diagnostic tool in IBD and other diseases accompanied by intestinal barrier malfunction.

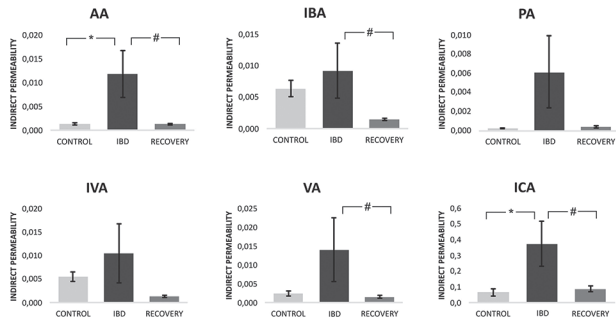


Figure 1. Indirect permeability (Cs/Cf) to SCFA in acute IBD, control and recovery groups. AA - acetic acid, PA – propionic acid, IBA - isobutyric acid, IVA - isovaleric acid, VA - valeric acid, ICA - isocaproic acid/4-methylvaleric acid. Values are means, + SE, * - $p < 0.05$ control vs IBD, # - $p < 0.05$ IBD vs recovery, by ANOVA followed by Duncan's test.

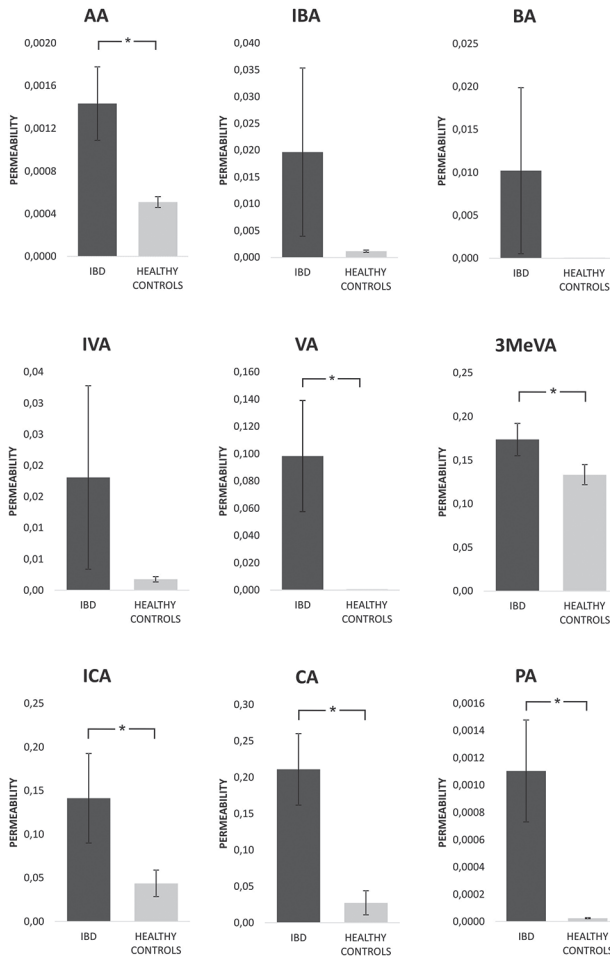


Figure 2. Gut-blood barrier permeability to SCFA in patients with IBD and healthy controls. AA - acetic acid, IBA - isobutyric acid, IVA - isovaleric acid, VA - valeric acid, ICA - isocaproic acid/4-methylvaleric acid, CA – caproic acid, PA – propionic acid, 3MeVA – 3-methylvaleric acid. Values are means, + SE, * - $p < 0.05$, by T-test.

Jaworska K, Huc T, Samborowska E, et al. Hypertension in rats is associated with an increased permeability of the colon to TMA, a gut bacteria metabolite. *PLoS One* 2017;12:e0189310.

Ufnal M, Pham K. The gut-blood barrier permeability - A new marker in cardiovascular and metabolic diseases? *Med Hypotheses* 2017;98:35-37.

Bischoff SC, Barbara G, Buurman W, et al. Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol* 2014;14:189.

Welcker K, Martin A, Kolle P, et al. Increased intestinal permeability in patients with inflammatory bowel disease. *Eur J Med Res* 2004;9:456-60.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC084

Paracellular water and ion transport is unaffected in ILDR1 knockout mice

W. Hempstock¹, S. Sugioka¹, N. Ishizuka¹, M. Furuse^{2,3} and H. Hayashi¹

¹University of Shizuoka, Shizuoka, Japan, ²Cellular structure, National Institute of Physiological Sciences, Okazaki, Japan and ³Physiological Sciences, The Graduate University for Advanced Studies (SOKENDAI), Okazaki, Japan

Water is important for all living things. In the intestinal tract, more than 10 L of water is absorbed per day, including water that is ingested and secreted into the intestinal lumen. The pathway of water transport in the intestinal epithelium is thought to be mediated by both transcellular and paracellular routes. Permeability of the paracellular pathway is characterized by tight junctions, which are composed of many proteins (1). It has been shown that claudin family proteins, which are mainly expressed in bicellular tight junctions, are important to permselectivity (2). However, in claudin knockout (KO) mice, changes in water absorption in the intestine were not observed, suggesting another mechanism is involved (3). Recently it was shown that knock out of ILDR1, a tricellular tight junction protein, results in water transport abnormalities in the kidney (4). ILDR1 KO mice had impaired urine concentration (390 vs 1460 mOsm/Kg H₂O), polydipsia (34 mL H₂O/day) and polyuria (7.8 mL urine/day)(4). Since ILDR1 KO mice have impaired water transport in the kidney, the goal of this research was to examine the effect of ILDR1 knockout on the paracellular transport of water and electrolytes in the large intestine. ILDR1 KO mice (*Illdr1^{tm1(KOMP)Wtsi}*; Wellcome Trust Sanger Institute Mouse Genetics Project) and their controls (age- and sex-matched C57Bl/6J mice) were put into metabolic cages for 72 hours and daily food and water intake was recorded and urine and feces were weighed and analyzed. To examine the effect of ILDR1 on paracellular transport, ²²Na and ³H-mannitol fluxes were measured with the Ussing chamber method. Urine for osmolarity quantification was extracted directly from the bladder. All animal experiments were approved by the University of Shizuoka Animal Care and Use Committee (Permit #175179) and carried out in accordance with the rules for animal research at the University of Shizuoka. Anaesthesia (30 µg/mL medetomidine, 0.4 mg/mL midazolam, and 0.5 mg/mL butorphanol) was administered by intraperitoneal injection (10 µL/g body weight). There was no significant difference between wild type and ILDR1 KO mice in urine and feces Na⁺ and K⁺ concentration. Water and food intake as well as urine and fecal output did not differ in ILDR1 KO mice and controls. Large intestinal luminal Na⁺ and K⁺ concentration did not vary between wild type and ILDR1 KO mice. In addition, there was no difference in ²²Na and ³H-mannitol flux in wild type and KO mice and conductance was also unchanged. Since there was no difference in urine osmolarity with free access to food and water, wild type and ILDR1 KO mice were

subjected to 24 hour water restriction. ILDR1 KO mice were able to concentrate urine to a lesser extent than wild type (2620.5 ± 167.3 vs 3673.5 ± 87.7 mOsm, $n=4$, $p=0.0014$, independent t-test). Based on these results, ILDR1 knockout does not significantly impact water transport in the large intestine or kidney.

Furuse M. Molecular basis of the core structure of tight junctions. *Cold Spring Harb Perspect Biol* [Internet]. 2010 Jan [cited 2019 Mar 29];2:a002907. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2827901/>

Tsukita S, Tanaka H, Tamura A. The claudins: from tight junctions to biological systems. *Trends Biochem Sci* [Internet]. 2019 Feb [cited 2019 Mar 29];44(2):141-152. Available from: [https://www.cell.com/trends/biochemical-sciences/fulltext/S0968-0004\(18\)30193-2](https://www.cell.com/trends/biochemical-sciences/fulltext/S0968-0004(18)30193-2)

Rosenthal R, Günzel D, Theune D, Czichos C, Schulzke J, Fromm M. Water channels and barriers formed by claudins. *Ann NY Acad Sci* [Internet]. 2017 Jun [cited 2019 Mar 29];1397(1):100-109. Available from: <https://nyaspubs.onlinelibrary.wiley.com/doi/full/10.1111/nyas.13383>

Gong Y, Himmerkus N, Sunq A, Milatz S, Merkel C, Bleich M, Hou J. ILDR1 is important for paracellular water transports and urine concentration mechanism. *PNAS* [Internet]. 2017 May [cited 2019 Mar 29];114(20):5271-6. Available from: <https://www.pnas.org/content/114/20/5271> DOI: 10.1073/pnas.1701006114

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC085

Physiological relevance of intestinal paracellular Na⁺ pathways for Na⁺-coupled glucose transport in mouse small intestine

H. Hayashi, M. Nakayama and N. Ishizuka

University of Shizuoka, Shizuoka, Japan

Nutrient absorption in the small intestine is essential for assimilation of nutrients required for energy and growth. To meet energy requirements, nutrients are efficiently absorbed from the luminal side against their chemical gradients. Many nutrients such as amino acids and glucose are actively absorbed across the brush border membrane of enterocytes via transporters that couple their movements to that of luminal Na⁺. Na⁺ coupling allows nutrients to be transported against their concentration from low luminal to higher intracellular concentration. It is therefore envisaged that Na⁺-dependent nutrient absorption mechanisms require a large amount of luminal Na⁺. However, little is known about how the intestine meets the needs of Na⁺ for nutrient absorption. It is thought that Na⁺ diffuses back into the lumen via paracellular pathways to support nutrient absorption. However, direct experimental evidence in support of this idea has not been shown. To investigate the relationship between paracellular Na⁺ movements and Na⁺-dependent glucose absorption, we took advantage of claudin 15 deficient (cldn15^{-/-}) mice,

which have been shown to have decreased paracellular Na^+ permeability in the small intestine. Mice were anaesthetized with a mixture of drugs (10 $\mu\text{l/g}$, i.p.) consisting of medetomidine (30 $\mu\text{g/mL}$), midazolam (0.4 mg/mL) and butorphanol (0.5 mg/mL) and the small intestine was excised. The isolated segment was opened and mounted in an Ussing chambers. To investigate whether paracellular pathways are involved in Na^+ recycling, we measured glucose-induced currents (ΔI_{sc}) under open and short-circuit conditions and simultaneously measured changes in unidirectional $^{22}\text{Na}^+$ fluxes (ΔJ). Under short-circuit conditions in wild-type mice, luminal application of glucose resulted in an increase in ΔI_{sc} ($13.7 \pm 0.8 \mu\text{mol/cm}^2/\text{h}$) and an increase in unidirectional mucosal-to-serosal $^{22}\text{Na}^+$ flux ($\Delta J_{\text{MS}}^{\text{Na}}$, $12.5 \pm 0.8 \mu\text{mol/cm}^2/\text{h}$) but not $\Delta J_{\text{SM}}^{\text{Na}}$. However, under open-circuit conditions, equivalent glucose-induced ΔI_{sc} ($12.1 \pm 0.8 \mu\text{mol/cm}^2/\text{h}$) was observed but ΔJ^{Na} ($1.7 \pm 1.3 \mu\text{mol/cm}^2/\text{h}$) was strongly inhibited. In the presence of phloridzin, both glucose-induced ΔI_{sc} and ΔJ increments were totally abolished, suggesting that glucose-induced $\Delta J_{\text{MS}}^{\text{Na}}$ is mainly mediated by Na^+ -dependent glucose transporter SGLT1. Under short-circuit conditions, in *cldn15^{-/-}* mice, luminal glucose induced an increase in ΔI_{sc} ($23.9 \pm 3.8 \mu\text{mol/cm}^2/\text{h}$) and $\Delta J_{\text{MS}}^{\text{Na}}$ ($21.4 \pm 2.4 \mu\text{mol/cm}^2/\text{h}$) similar to that in wild-type mice. Unlike wild-type mice, though there was large negative luminal potential difference (V_{te} -20 mV), and a robust glucose-induced $\Delta J_{\text{MS}}^{\text{Na}}$ increment was observed ($14.5 \pm 1.9 \mu\text{mol/cm}^2/\text{h}$). These observations suggest that the Na^+ which is absorbed by Na^+ -dependent glucose cotransport is rapidly recycled back into the lumen via paracellular pathways which are driven by increased luminal negativity.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC086

The mode of action of the NHE3 inhibitor tenapanor in intestinal Na^+ absorption

N. Ishizuka, S. Koido and H. Hayashi

University of Shizuoka, Shizuoka, Japan

Na^+/H^+ exchanger isoform 3 (NHE3) is predominantly expressed in the apical membrane of intestinal and renal epithelia, where it plays a pivotal role in NaCl absorption and acid-base homeostasis. Transport activity of NHE3 is sensitive to intracellular pH (pH_i). At resting pH_i , a large fraction of transporters resides in an inactive state. When the H^+ concentration of the cytosol rises, the transporters are converted into an active state. There are two proposed models to explain this kinetic regulation of transporters, one is the H^+ sensor model and the other is the symmetrical dimer model. We have previously shown that NHE3 is slowly activated over the course of minutes, implying involvement of a conformational change of NHE3 such as dimerization in heterogenous expression systems (1). Under physiological conditions, NHE3 is thought to be coupled with other transporters in a concerted

manner. One example is with $\text{Cl}^-/\text{HCO}_3^-$ exchanger in electroneutral NaCl absorption in the intestine. Another is with H^+ -coupled peptide transporter whose driving force is supported by NHE3 (2). To gain insight into the activation mechanism of NHE3, we used the NHE3 specific inhibitor tenapanor, which has a symmetrical structure with two proposed binding sites, and compared it with another NHE3 inhibitor, S3226. We examined the effect of inhibitors on NHE3 activity in different NHE3 transport modes, which involve coupling to other transporters, in NHE3 expressing cell lines and isolated mouse intestinal tissue. NHE3 was stably expressed in PS120 cells, which are deficient in endogenous NHE activity. pH_i was measured by microfluorometry of the emission of BCECF. The activity of NHE3 was determined as the rate of Na^+ -induced pH_i recovery after acid loading. Mice (C57Bl/6jJcl) were anaesthetized with a mixture of drugs (10 $\mu\text{l/g}$ B.W., i.p.) consisting of 30 $\mu\text{g/ml}$ medetomidine, 0.4 mg/ml midazolam and 0.5 mg/ml butorphanol. The isolated intestinal segments were mounted on Ussing chambers. In NHE3 expressing cells, tenapanor inhibited NHE3 activity dose-dependently with IC_{50} values of 1.26 ± 0.40 nM ($n=4$) and 3.02 ± 0.53 nM ($n=3$) for 1 min and 10 min acidification, respectively. In native epithelia, peptide-induced short-circuit current was inhibited dose-dependently by tenapanor and S3226 with an IC_{50} value of 6.3 ± 3.4 nM ($n=3$) and 5.9 ± 1.0 μM ($n=3$), respectively. However, S3226 completely inhibited transepithelial $^{22}\text{Na}^+$ flux but tenapanor did not. It is of interest to note that pretreatment of tenapanor (no tenapanor during NHE3 activity measurement) dose-dependently inhibited acid activated NHE3 activity. Together, these results suggested that tenapanor may irreversibly bind to NHE3 and recognize the different NHE3 transport modes.

Hayashi H, Szász K, Coady-Osberg N, Orłowski J, Kinsella JL, Grinstein S. A slow pH-dependent conformational transition underlies a novel mode of activation of the epithelial Na^+/H^+ exchanger-3 isoform. *J Biol Chem.* 277(13):11090-6, 2002

Ishizuka N, Nakayama M, Watanabe M, Tajima H, Yamauchi Y, Ikari A, Hayashi H. Luminal Na^+ homeostasis has an important role in intestinal peptide absorption in vivo. *Am J Physiol.* 315, 799-809, 2018

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC087

Anion species confounds measurement of intracellular Ca^{2+} in murine white fat adipocytes.

P.A. Smith and N. Akaniro-Ejim

Life Sciences, University of Nottingham, Nottingham, UK

White fat adipocytes have a V_m , membrane potential, of -30 mV under physiological ionic conditions which is shown to be mainly due to Cl^- permeability (1). Flux and imaging studies demonstrate that their intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$, is influenced by a Ca^{2+} window current flowing through spontaneously active L-type

voltage-gated Ca^{2+} channels (2,3); an observation consistent with their depolarised resting membrane potential. Given these facts, we have investigated if changes in extracellular Cl^- concentration, $[\text{Cl}^-]_o$, can affect $[\text{Ca}^{2+}]_i$ via V_m mediated changes in voltage-gated Ca^{2+} influx.

White fat adipocytes were isolated from subcutaneous fat of male CD-1 mice. After plating onto coverslips, $[\text{Ca}^{2+}]_i$ was imaged using convention epifluorescent microscopy as described (1). Cells were continuously perfused at 28°C in a Hanks salt solution, control, with a $[\text{Cl}^-]_o$ of 152 mM. The effect of serial equimolar substitution of bath NaCl with either Na gluconate or Na glutamate to decrease $[\text{Cl}^-]_o$ on $[\text{Ca}^{2+}]_i$ was explored. Data are given as mean $\pm$ S.E.M. Statistical significance, $p < 0.05$, was determined by ANOVA with Holm-Sidak's multiple comparison test.

Substitution of NaCl with Na gluconate ($n = 39$) to give 113 mM $[\text{Cl}^-]_o$ did not affect $[\text{Ca}^{2+}]_i$. However, further reduction of $[\text{Cl}^-]_o$ to 53 mM, and then to 18 mM significantly decreased $[\text{Ca}^{2+}]_i$ from 127 ± 9 nM to 117 ± 8 nM and then to 113 ± 7 nM respectively. $[\text{Ca}^{2+}]_i$ only partly recovered to 115 ± 7 nM on return to normal control Hanks. Repetition of these experiments but with Na glutamate ($n = 32$) instead also decreased $[\text{Ca}^{2+}]_i$; substitution of $[\text{Cl}^-]_o$ to 113 significantly decreased $[\text{Ca}^{2+}]_i$ from 165 ± 9 nM to 142 ± 9 nM after which it increased back to 150 ± 9 nM at 53 mM. By 18 mM $[\text{Cl}^-]_o$ $[\text{Ca}^{2+}]_i$ had returned to control levels. To explore the mechanistic differences in the $[\text{Ca}^{2+}]_i$ response to the different anions we looked at their physical chemistry. Since gluconate is reported to chelate Ca^{2+} (4) we analysed the free Ca^{2+} of our solutions.

Gluconate, but not glutamate, was found to chelate free Ca^{2+} , the former with a K_d of 33.1 ± 0.7 mM ($n=3$). So the effect of gluconate substitution was repeated but with $[\text{Ca}^{2+}]_o$ titrated back to that measured in control Hanks solution: 2.7 ± 0.3 mM ($n=3$). Under these conditions, substitution of bath Cl^- with gluconate had no effect on $[\text{Ca}^{2+}]_i$. Possible mechanisms for the effects with glutamate were not forthcoming and have yet to be investigated.

In conclusion, the physiochemical properties of the anion used for chloride substitution confounded measurement of $[\text{Ca}^{2+}]_i$ and attempts to gate voltage-dependent Ca^{2+} influx in white fat adipocytes. Consequently, the free $[\text{Ca}^{2+}]$ of gluconate containing salt solutions should always be checked. Overall, changes in $[\text{Cl}^-]_o$, and so V_m , do not appear to affect voltage-gated Ca^{2+} entry and $[\text{Ca}^{2+}]_i$ of white fat adipocytes.

Bentley DC, Pulbutr P, Chan S, Smith PA. Etiology of the membrane potential of rat white fat adipocytes. *Am J Physiol - Endocrinol Metab.* 2014;307(2).

Martin BBR, Clausen T, Gliemann J. Relationships between the Exchange of Calcium and Phosphate in Isolated Fat-Cells. 1975;152:121–9.

Pershad Singh HA, Lee LY, Snowdowne KW. Evidence for a sodium/calcium exchanger and voltage-dependent calcium channels in adipocytes. *FEBS Lett.* 1989;244(1):89–92.

Vavrusova M, Munk MB, Skibsted LH. Aqueous solubility of calcium L-lactate, calcium D-gluconate, and calcium D-lactobionate: Importance of complex formation for solubility increase by hydroxycarboxylate mixtures. *J Agric Food Chem.* 2013;61:8207-8214.

This work was supported by the Schlumberger Stitching Fund and a Leverhulme Trust Research Project Grant

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC088

Development of human and rat PTC monolayers as in vitro models of nephrotoxicity

A. Obaid^{1,2}, C. Brown¹ and G. Chung¹

¹ICaMB, Newcastle University, Newcastle, UK and ²Umm Al Qura University, Mecca, Saudi Arabia

Nephrotoxicity is a serious side effect of many drugs due to the kidney being one of the major sites for their excretion. In vitro models of nephrotoxicity are therefore paramount in drug development process, which would be used to detect early stages of drug-induced toxicity. This study investigates the use of human and rat primary proximal tubule cells as in vitro models of nephrotoxicity.

Human and rat proximal tubule cells (PTCs) were isolated and cultured from cortical tissues of the kidney. The cells form highly polarised monolayers that were exposed to polymyxin B, gentamicin and cisplatin – all well-known nephrotoxins. Cell viability and LDH-based cell death were measured, alongside the production of renal toxicity biomarkers, KIM-1, NGAL and clusterin.

As expected, cell viability of the monolayers after polymyxin B, gentamicin and cisplatin challenge was decreased. The proportion of cells alive was dependent on the concentration and period of exposure of the nephrotoxin. For example, the cell viability of rat PTCs treated with gentamicin for 48h decreased by 43% than control cells ($P < 0.01$). Higher levels of KIM-1, NGAL and clusterin secretion were detected when human and rat PTCs were treated with nephrotoxins. All three biomarkers were predominately secreted across the apical membrane of the PTCs monolayers. For instance, KIM-1 level across the apical membrane was 0.9 ± 0.05 ng/ml, which was significantly higher than across the basolateral membrane at 0.35 ± 0.04 ng/ml. The levels of biomarkers secretion were also found to be dependent on concentrations of nephrotoxin and period of exposure. For example, rat PTCs treated with $250 \mu\text{g/ml}$ polymyxin B for 24h produced 5.5 ± 0.7 ng/ml of KIM-1, which increased to 19 ± 0.1 ng/ml with 48h challenge, an increase of more than 3-fold ($P < 0.01$).

The mechanisms of toxicity were also investigated by pre-treating the PTCs with supposedly nephron-protectant, rosuvastatin, cilastatin and cimetidine, prior to nephrotoxin exposure. While $50 \mu\text{M}$ rosuvastatin did not change the cell viability nor KIM-1 expression levels in human and rat PTCs, the pre-treatment of rosuvastatin with polymyxin B increased cell viability by 15 % when compared to polymyxin-B only treatment. Similarly rat PTCs treated with $250 \mu\text{g/ml}$ gentamicin and $40 \mu\text{M}$ cilastatin showed KIM-1 at levels of 7.5 ± 0.3 ng/ml, compared with 11.5 ± 1.2 ng/ml in

cells treated with only gentamicin, giving a decrease of 35 % ($P < 0.05$). These data suggest mechanisms of the aminoglycoside uptake could be via megalin/cubilin receptors, which were inhibited by rosuvastatin and cilastatin.

These data show the utility of human and rat PTCs as in vitro models for the study of nephrotoxicity, and their potential in elucidating the mechanisms of action of polymyxin-B, gentamicin and cisplatin induced toxicity.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC089

The ameliorative effect of methanol stem extract of *Moringa oleifera* in glycerol-induced acute kidney damage in rats through Kim-1 and NF- κ B signaling pathways

A.A. Adedapo¹, U. Etim¹, O. Falayi¹, B. Adeoye¹, A. Oyagbemi³, T. Omobowale², M. Yakubu⁴ and B. Ogunpolu²

¹Veterinary Pharmacology and Toxicology, University of Ibadan, Ibadan, Nigeria,

²Veterinary Medicine, University of Ibadan, Ibadan, Nigeria, ³Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Nigeria and ⁴College of Science, Engineering and Technology, Texas Southern University, Houston, TX, USA

Moringa oleifera is popularly referred to as wonder plant because it has remarkable variety of therapeutic purposes (Sushma et al 1989). It is traditionally used for the circulatory system disorder and other diseases hence many studies have been carried out to ascertain the diverse medicinal properties of the leaves, stem, bark, fruits, stalk, root and even the seeds of the plant *Moringa oleifera* (Tan et al 2003). The aim of this study was to ascertain the ameliorative effect of *Moringa oleifera* on glycerol-induced acute kidney injury in rats. Glycerol (50% v/v in sterile saline, intramuscular) was used to induce acute kidney injury. A control group received distilled water alone, another group received glycerol alone on the 8th day, two groups were given 50 mg/kg and 100 mg/kg of methanol stem extract respectively for seven days and glycerol on the 8th day. Another group received 100 mg/kg of methanol stem of *Moringa oleifera* alone. To ascertain renal damage and amelioration, serum BUN, creatinine, myeloperoxidase, advanced oxidative products, malondialdehyde, superoxide dismutase, reduced glutathione, and protein carbonyl were assayed for. Histopathological analysis of kidney tissues was done while further, expression of KIM-1 and NF- κ B was done on kidney tissues using immunohistochemistry. The results showed that methanol stem bark extract of *Moringa oleifera* ameliorated glycerol-induced acute kidney injury by inhibiting markers of inflammation, oxidative stress and renal damage by down regulating expressions of KIM-1 and NF- κ B in their signaling pathways. KIM-1 has become one of the key biomarkers for diagnosis, disease activity monitoring and prognosis of acute kidney injury (Malhotra and Siew, 2017). It should be noted that Acute kidney

injury (AKI) is becoming increasingly common and is linked to progressive loss of kidney function, cardiovascular disease, and death (Singbartl and Joannidis, 2015). Though it may often be reduced to its rudimentary manifestations such as a change in serum creatinine), AKI is comprised of several diseases, thus highlighting a need to improve phenotyping beyond the circumstantial. Thus, developing therapies will require a timely description of the onset, location, and severity of parenchymal injury, its underlying mechanisms, and prognosis (Nadkarni and Coca, 2016). The ability of the methanol stem bark extract to down regulate KIM-1 in this study is a pointer to its nephroprotective property. Since the extract also down regulate NF- κ B expression, it should be paid a closer attention. NF- κ B NF- κ B regulates multiple aspects of innate and adaptive immune functions and serves as a pivotal mediator of inflammatory responses. NF- κ B plays a critical role in regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells, its upregulation will contribute to the pathogenic processes of various inflammatory diseases showing that the plant exhibit anti-inflammatory properties.

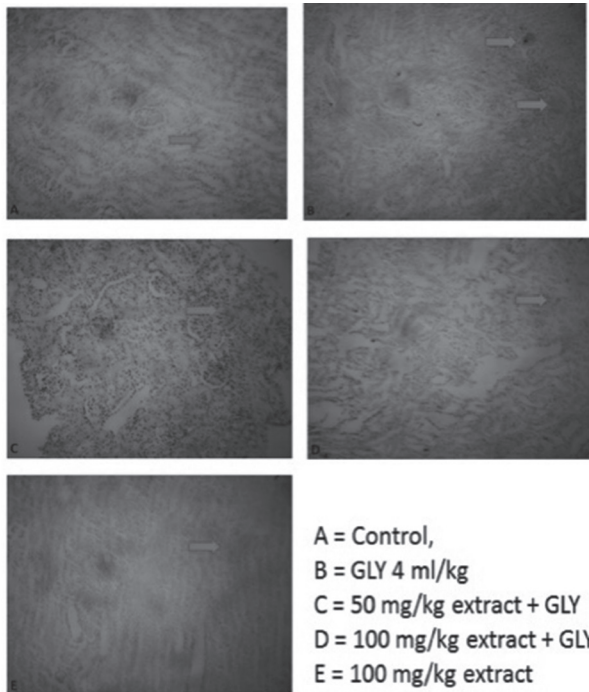


Figure 1: A shows least expression of KIM-1 in the kidney section. B shows highest expression of KIM-1 when compared with A, C, D, and E.

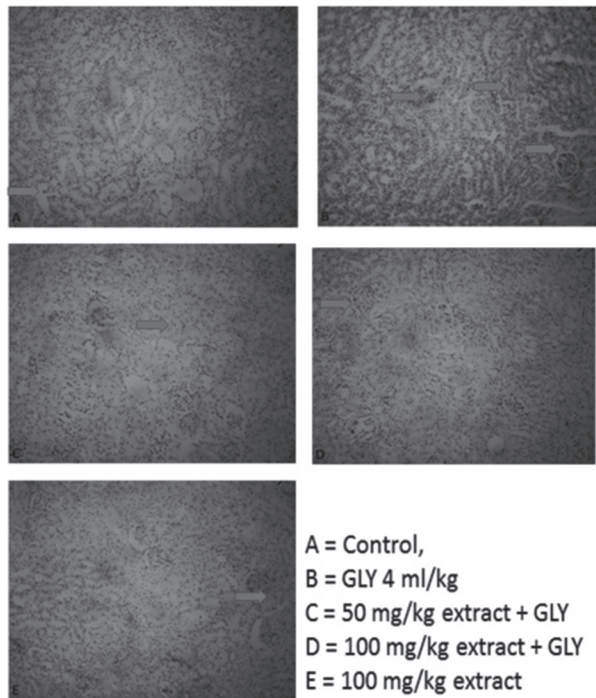


Figure 2: A shows least expression of NF-KB in the kidney section. Group B shows highest expression of NF-KB when compared with A, C, D, and E.

Maholtra R, Siew ED. Biomarkers for the early detection and prognosis of acute kidney injury. Clin J Am Soc Nephrol 2017; 12 (1): 149-173.

Sushma KS, Jaydeep A, Kumar JSS. Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. Indian J. Expt. Bio. 1989; 27: 134-137

Tan L, Burniston JG, Clark WA, Ng Y, Goldspink DF. Characterization of Adrenoceptor Involvement in Skeletal and Cardiac Myotoxicity Induced by Sympathomimetic Agents: Toward a New Bioassay for β -blockers. J. Cardiovasc. Pharmacol. 2003; 41(4):518-525

Singbartl K, Joannidis M. Short-term Effects of Acute Kidney Injury. Crit Care Clin 2015; 31: 751-762.

Nadkarni GN, Coca SG. Temporal Trends in AKI: Insights from Big Data. Clin J Am Soc Nephrol 2016; 11: 1-3.

This study was supported with a grant (TETFUND/DESS/NRF/UI IBADAN/STI/VOL. 1/B2.20.11) received from the National Research Foundation of the Tertiary Education Trust Fund (TETFUND), Abuja, Nigeria.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC090

Gastroenteritis Management: Interplay of chemotherapy and Electrolytes Equilibration

H.M. Ambali¹, J.O. Aiyedun³, K.T. Biobaku⁴, O.M. Azeez² and S.A. Ameen⁵

¹Veterinary medicine, University of Ilorin, Ilorin, Nigeria, ²Vet Physiogy and Biochemistry, University of Ilorin, Ilorin, Nigeria, ³Department of Vet public Health, University of Ilorin, Ilorin, Nigeria, ⁴Department of Vet Pharmacology, University of Ilorin, Ilorin, Nigeria and ⁵Department of Vet Medicine, University of Ilorin, Ilorin, Nigeria

Background

Cases of bloody vomitus and smelling bloody diarrhea come to the small animal clinic unit of University of Ilorin Veterinary Teaching Hospital, Nigeria. Ilorin is a gate way city between the North and Southern parts of the Country. Cases are often presented with the mentioned symptoms. Many breeds are affected such as Boerboel, Caucasian and Rottweiler and even the local breeds. This condition is neither restricted to any breed nor restricted to a particular time of the year. However presentation of the condition occurs more in the February and March
Diagnosis: were carried out using standard microbial technique and clostridium was isolated which was sensitive to neomycin sulphate and colistin.

Treatment: The cases were managed therapeutically using neomycin sulphate and supportive therapy. Neomycin suphate was administered at dose rate of 40mg/kg for 5days orally. Fluid therapy such as lactated ringers solution and 5% dextrose were instituted intravenously. 5% dextrose was based on its pharmacological basis of correcting dehydration and supplying energy. Its use had also been associated with prevention of ketosis due to starvation, vomiting, diarrhea among its other uses. Ringers' lactate which physiological basis includes physiological fluid rapidly expands intravenous volume, providing buffering capacity.

Conclusion: Aminoglycosides are thus becoming drug of choice in management of gastroenteritis in conjunction with fluid therapy and high fibre diet irrespective of breeds and season. Its cost and availability is also added advantage. Veterinary care is core for the survival of pets, livestock and other animals generally though nutrition and housing play important roles in their management. The Veterinarian should advice the client on balanced diet, appropriate food, hygiene and routine checkups.

Key words: Gastroenteritis, neomycin Sulphate, breeds and season.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC091

Heat treatment improves the exaggerated exercise pressor reflex in rats with peripheral artery disease and engagement of P2X

J. Li and C. Yang

Heart and Vascular Institute, Penn State University College of Medicine, Hershey, PA, USA

Background: Femoral artery occlusion in rats has been used to study human peripheral artery disease (PAD) (1-5). The responses of arterial blood pressure (BP) and sympathetic nerve activity are exaggerated during static exercise in PAD rats with femoral artery occlusion (3-5). **Aims:** The purpose of the current study was to examine if heat treatment (a prior heat exposure) has beneficial effects on the exaggerated exercise pressor reflex in PAD rats. We further determined if temperature sensitive P2X receptor was engaged in the effects of heat treatment. **Methods:** The pressor response to static muscle contraction was recorded to determine the exercise pressor reflex. Also, $\alpha\beta$ -methylene ATP was injected into the arterial blood supply of the hindlimb muscles to stimulate P2X receptors in the muscle afferent nerves. **Results:** Basal muscle temperature (T_m) was 35.6 ± 0.1 °C in control rats ($n=3$); and 34.2 ± 0.1 °C in PAD rats ($n=5$; $P < 0.05$ vs. control rats). As T_m was increased by ~ 1.5 °C and this was maintained for 30 min, core temperature was not altered in control group and PAD group. The heat treatment was performed 2 times daily for 3 days. Then we examined the BP response to static muscle contraction. The greater mean arterial pressure (MAP) response was observed in PAD rats (31 ± 5 mmHg in PAD rats/ $n=6$ vs. 22 ± 3 mmHg in control rats/ $n=8$; $P < 0.05$). Heat treatment attenuated amplification of BP response in PAD rats (MAP response: 24 ± 3 mmHg in PAD rats with heat treatment/ $n=5$; $P < 0.05$ vs. no heat treatment). Note that no significant difference in developed muscle tension during muscle contraction was seen in control rats and PAD rats with heat treatment or without heat treatment ($P > 0.05$). In addition, heat treatment attenuated enhancement in MAP response induced by $\alpha\beta$ -methylene ATP injected into the arterial blood supply of the hindlimb muscles of PAD rats (20 ± 4 mmHg in control rats/ $n=5$; 41 ± 4 mmHg in PAD rats/ $n=6$ and 26 ± 5 mmHg in PAD rats with heat treatment; $P < 0.05$, PAD vs. control and PAD with heat treatment). **Conclusion:** A prior heat exposure plays an inhibitory role in modifying exaggeration of the exercise pressor reflex in PAD and P2X is likely a part of mechanisms engaged in the heat treatment.

Xing J, Lu J, Li J. Acid-sensing ion channel subtype 3 function and immunolabelling increases in skeletal muscle sensory neurons following femoral artery occlusion. *J Physiol* 590: 1261-1272, 2012.

Xing J, Lu J, Li J. Augmented P2X response and immunolabeling in dorsal root ganglion neurons innervating skeletal muscle following femoral artery occlusion. *J Neurophysiol* 109: 2161-2168, 2013.

Copp SW, Kim JS, Ruiz-Velasco V, Kaufman MP. The mechano-gated channel inhibitor GsMTx4 reduces the exercise pressor reflex in decerebrate rats. *J Physiol* 594: 641-655, 2016.

Harms JE, Kuczmarski JM, Kim JS, Thomas GD, Kaufman MP. The role played by oxidative stress in evoking the exercise pressor reflex in health and simulated peripheral artery disease. *J Physiol* 595: 4365-4378, 2017.

Xing J, Lu J, Li J. Role of TNF- α in regulating the exercise pressor reflex in rats with femoral artery occlusion. *Frontiers in Exercise Physiol*. 9: 1461, 2018.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC092

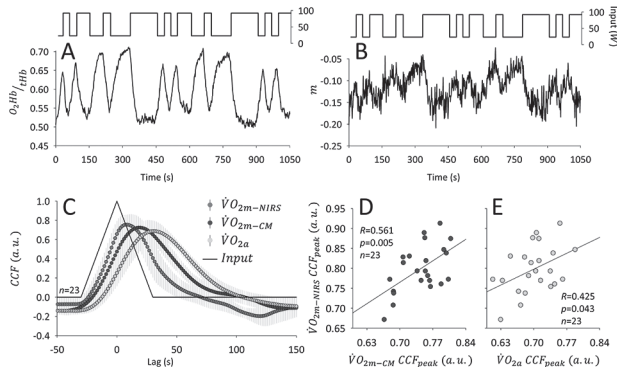
NIRS-derived Muscular Oxygen Uptake During Exercise in Humans

T. Beltrame^{1,2,3,4}, J. Koschate², U. Hoffmann², M. Gois³, R. Hughson⁵, M. Frade³, S. Linares³, R. Torres¹ and A. Catai³

¹University of Campinas, Campinas, Brazil, ²German Sport University Cologne, Köln, Germany, ³Federal University of Sao Carlos, Sao Carlos, Brazil, ⁴Universidade Ibirapuera, So Paulo, Brazil and ⁵University of Waterloo, Waterloo, ON, Canada

Aerobic system evaluation has many clinical applications. Commonly, the aerobic system is investigated by the analysis of muscular oxygen uptake (VO_{2m}) dynamics through the modeling of the alveolar oxygen uptake (VO_{2a}) responses during exercise. However, VO_{2m} dynamics differ from VO_{2a} due to circulatory distortions. Therefore, new methods are needed to estimate VO_{2m} during exercise. The goal of this study was to develop a method for VO_{2m} estimation based on near-infrared spectroscopy (NIRS, $VO_{2m-NIRS}$). The $VO_{2m-NIRS}$ dynamics were then compared with VO_{2a} and VO_{2m} dynamics estimated by the circulatory method (VO_{2m-CM}) [1]. Twenty-three participants (12 men and 11 women, 27 ± 4 years old, 69 ± 11 kg and 170 ± 9 cm) performed a pseudo-random binary sequence exercise protocol (PRBS). Changes in concentrations of the vastus lateralis oxy- and deoxy-hemoglobin (O_2Hb and HHb , respectively) were measured by the OxyMon device (ARTINIS, The Netherlands). The VO_{2a} was measured by a metabolic cart while VO_{2m-CM} was estimated following a previously published circulatory model [1]. The $VO_{2m-NIRS}$ was estimated from the O_2Hb/tHb signals (Figure 1A), where $tHb = O_2Hb + HHb$. The NIRS data were filtered and detrended, and then a peak detection algorithm was used to identify each contraction/relaxation phase. The slope of the O_2Hb/tHb data for each contraction was computed (m in Figure 1B) and interpreted as VO_{2m} since it calculates the O_2Hb decay rate normalized by the local blood volume (i.e., tHb). The VO_{2a} , VO_{2m-CM} and $VO_{2m-NIRS}$ dynamics were analyzed by a cross-correlation function (CCF in Figure 1C) between the time-series of these variables and the PRBS workload ("Input" in Figures 1A and 1B). The CCF peak (CCF_{peak} , or the response speed) and lag (CCF_{lag} , or the response delay), as aerobic system evaluation indexes, were compared between VO_{2a} , VO_{2m-CM} and $VO_{2m-NIRS}$ by repeated measures ANOVA. The correlation level was calculated by Pearson's correlation coefficient. The statistical significance level (p) was set at 0.05 (two-tailed). As expected from a local VO_{2m}

measure, the CCF_{lag} of $VO_{2m-NIRS}$ occurred statistically ($p < 0.05$) before VO_{2m-CM} and VO_{2a} . In addition, the $VO_{2m-NIRS}$ CCF_{peak} was statistically ($p < 0.05$) correlated with the CCF_{peak} of VO_{2m-CM} and VO_{2a} (Figure 1 D and E, respectively). The NIRS-derived VO_{2m} (i.e., $VO_{2m-NIRS}$) seemed to be reliable for the investigation of VO_{2m} dynamics during exercise, therefore, the aerobic system can be evaluated directly from NIRS data.



Method for muscular oxygen uptake estimation from near-infrared spectroscopy during exercise. Please see text for further details.

Hoffmann U et. al. (2013) Skeletal muscle VO_2 kinetics from cardio-pulmonary measurements: Assessing distortions through O_2 transport by means of stochastic work-rate signals and circulatory modelling. Eur J Appl Physiol 113:1745–1754

This study was supported by Sao Paulo Research Foundation - FAPESP, grants # 2017/09639-5 and 2018/19016-8 to Thomas Beltrame, and grants #2014/12236-1, #2015/24494-8, #2016/50250-1, #2017/09639-5, and #2017/20945-0 to Ricardo da Silva Torres.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC093

Osteoprotegerin (OPG) as a potential therapeutic target to combat inflammation associated with ageing and other comorbidities.

J. Piasecki¹, N. Al-Shanti² and J. McPhee²

¹Sport Science, Nottingham Trent University, Nottingham, UK and ²Department of Sport and Exercise Sciences, Manchester Metropolitan University, Manchester, UK

Purpose: The present study was designed to, firstly, investigate the relationship between whole-body lean mass (WB Lean mass) and levels of circulating factors that have established roles in bone remodelling during ‘healthy’ ageing (Coulson et al., 2017). Then, secondly, investigate the potential protective effects of

Osteoprotegerin (OPG) against TNF α exposure, which is known to induce apoptosis (Saini et al., 2008).

Methods: WB Lean mass and fasting plasma concentrations of dickkopf-1 (DKK1), osteocalcin (OC), osteoprotegerin (OPG), osteopontin (OPN), sclerostin and tumour necrosis factor- α (TNF α) were measured in 272 older adults (aged 69 to 81 years; 52% were female) and 171 younger adults (18-30 years; 53% were female). Spearman's rho partial correlations and multivariate regression were used to examine the relationships between lean mass and the circulating factors. Human myoblast cells were cultured with TNF α alone, OPG alone and both molecules combined. Myoblast differentiation parameters including aspect ratio, fusion index (%) and myotube area (%) were calculated. OPG concentration was measured in cell supernatants and selected 'myokines' were measured from supernatants of myoblasts exposed to TNF α .

Results: WB Lean mass was 5% lower in old than young. Circulating osteocalcin was lower in old compared with young, while dickkopf-1, osteoprotegerin, sclerostin and TNF α were higher in old compared with young. These circulating factors were each significantly ($p < 0.03$) negatively associated with WB Lean mass in the older adults, (OC; $r = -0.175$, DKK1; $r = -0.312$, OPG; $r = -0.170$, Sclerostin; $r = -0.235$) apart from TNF α that was positively associated ($r = 0.140$) with WB Lean mass in older adults. In multivariate regression, height and OPG accounted for 45% of the WB Lean mass in the older adults. In young, the same combination of OPG and height accounted for 18% of variation in WBMD. Differentiation parameters (Figures 1 and 2) showed a significantly greater proportion of differentiation when OPG was added to the cultured myoblasts before TNF α ($p < 0.0001$). ELISA showed significant secretion of OPG released from human myoblast cells, particularly during the first 24h of incubation ($p = 0.001$) with an interaction effect found between TNF α and time $F(15,24) 3.340$, $p = 0.004$.

Conclusion: These results identify OPG as a circulating factor significantly associated with whole body lean mass in young and older adults. OPG may also protect myoblasts against TNF α exposure and may therefore be a therapeutic target for intervention against inflammation associated with ageing and, ultimately, reducing the ageing decline of muscle loss.

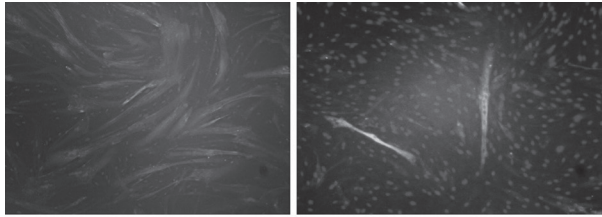


Figure 1: Myoblast tubes incubated with OPG on left, TNF α on right. OPG figure showing increased myotube differentiation compared to TNF α . Green; Myosin. Blue; nuclei

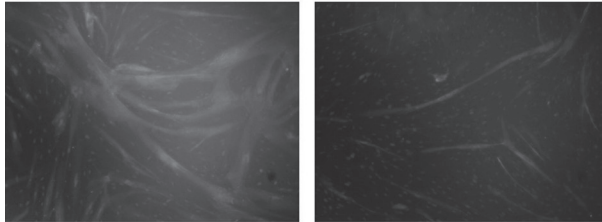


Figure 2: Myoblast tubes left, incubate with OPG adding TNF α after 24h, right; incubation with TNF α adding OPG at 24h. Green; Myosin. Blue; nuclei

COULSON, J., BAGLEY, L., BARNOUIN, Y., BRADBURN, S., BUTLER-BROWNE, G., GAPEYEVA, H., HOGREL, J. Y., MADEN-WILKINSON, T., MAIER, A. B., MESKERS, C., MURGATROYD, C., NARICI, M., PAASUKE, M., SASSANO, L., SIPILA, S., AL-SHANTI, N., STENROTH, L., JONES, D. A. & MCPHEE, J. S. 2017. Circulating levels of dickkopf-1, osteoprotegerin and sclerostin are higher in old compared with young men and women and positively associated with whole-body bone mineral density in older adults. *Osteoporos Int.* 28(9):2683-268

SAINI, A., AL-SHANTI, N., FAULKNER, S. H. & STEWART, C. E. 2008. Pro- and anti-apoptotic roles for IGF-I in TNF-alpha-induced apoptosis: a MAP kinase mediated mechanism. *Growth Factors*, 26, 239-53.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC094

Electric fields stimulate directional migration of synovial mesenchymal stem cells

S.G. Yayman¹, A. Molsberger^{2,3}, A.M. Rajnicek¹ and C. Mccaig¹

¹Institute of medical sciences, University of Aberdeen, Aberdeen, UK, ²Department of Orthopedics, Ruhr-University Bochum, Bochum, Germany and ³Clinic for Orthopedics and Pain Treatment, Düsseldorf, Germany

There is a need for novel clinical strategies to stimulate tissue regeneration after injury. Naturally occurring electric fields (EF) at injuries may promote wound healing and preliminary clinical studies have shown that electrical stimulation using acupuncture needles promoted tissue repair in tendon, muscle and joint

related injuries in elite athletes. We tested the hypothesis that EF stimulation of synovial mesenchymal stem cells (SMSCs) can induce tissue repair by promoting cell migration and mitosis.

SMSCs isolated from whole C57BC6 mouse knee joints were seeded in Ibidi μ -Slide I channels and an EF of 150 mV/mm was applied for 12 hours. Images were captured at 10-minute intervals for 10 visual fields and time-lapse videos were analysed with ImageJ (v. 1.47). Parameters quantified included the direction (Forward Migration Index; FMIx) and speed of cell migration, and the polarity of cell division. An FMIx value of 0 represents random migration, 1 is anodal migration and -1 is cathodal migration. The axis of cell division was scored between 0 degrees (parallel to EF) to 90 degrees (orthogonal to EF), so randomly directed division yields a mean angle of 45 degrees. Data are reported as mean $\pm$ standard error of mean (SEM) and compared with an unpaired 2-tailed Student's t test.

More unsorted (passages P4-P6) synovial cells migrated (44% of $n=837$; $p<0.001$) during EF exposure than controls (10% of $n=898$). Similarly, sorted SMSCs from later passages (P13-P15) migrated more frequently during EF stimulation (28% of $n=1465$; $p<0.001$) than controls (8% of $n=1546$). EF stimulation reduced the migration speed of unsorted (control $12 \pm 0.67 \mu\text{m/h}$; EF $8.4 \pm 0.23 \mu\text{m/h}$; $p<0.01$) but not sorted SMSCs (control $7.5 \pm 0.25 \mu\text{m/h}$; EF $8.3 \pm 0.20 \mu\text{m/h}$). Cell movement was directed strikingly to the anode of the EF for both cell types. The quantitative indices for directional migration (FMIx) of controls were -0.023 ± 0.045 and 0.001 ± 0.029 for unsorted and SMSCs, respectively but during EF exposure they were 0.651 ± 0.010 and 0.490 ± 0.013 for unsorted and SMSCs, respectively ($p<0.01$ for both). Unsorted cells displayed randomly oriented cell division relative to the EF, with the mitotic spindle oriented at mean angle 40 ± 7 degrees ($n=16$; $p=0.2$) compared to 50 ± 5 degrees for controls ($n=20$). In contrast, the mitotic spindle of sorted cells was more orthogonal to the EF, with a mean angle 52 ± 2 degrees ($n=138$) compared to 47 ± 2 degrees for controls ($n=173$; $p<0.05$).

The data support the idea that EF stimulation coupled with cell therapies may aid tissue regeneration.

We thank Columbus Health Products GmbH, Germany, for generous financial support.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC095

The plasticity of “exercise adaptability” in relation to resistance vs. endurance training

W.F. Farrash^{1,3}, E. Crombie¹, N. Gharahdaghi¹, P.J. Herrod¹, D. Wilkinson^{1,2}, K. Smith^{1,2}, B. Phillips^{1,2} and P. Atherton²

¹University of Nottingham, Derby, UK, ²NIHR BRC, Nottingham, UK and ³Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia

Introduction: Physical activity reduces the risk of diabetes, cardiovascular diseases, and chronic respiratory diseases and improves general health. Although physical activity per se, including both endurance exercise training (EET; prolonged aerobic activity) and resistance exercise training (RET; moving the resistive force of an external load), in the main lowers blood pressure and improves lipid profiles and insulin sensitivity¹, each of these distinct training modes triggers unique physiological adaptation. EET enhances aerobic capacity as a result of mitochondrial biogenesis and angiogenesis²; in contrast, RET increases muscle mass and associated strength/power³. However, in most cases, the magnitude of response varies among participants regardless of mode. For example, ~20% of participants showed limited improvement in aerobic fitness after several weeks-to-months of EET⁴. Similarly, hypertrophic responses ranged from 0-10% after supervised RET⁵. This response variation is not related to a pre-existing subject characteristic such as gender, age, muscle mass, or habitual physical activity, which make the underlying mechanisms of exercise resistance unclear.

Aim: To explore ‘within individual’ heterogeneity of adaptations to RET/EET using a cross-over design.

Methods: The study was conducted as a 14-week cross-over intervention (vis-à-vis: 4-weeks RET or EET; 6-weeks wash-out; 4-weeks EET or RET). Pre- and post-analyses were held for each training mode in relation to measures of aerobic capacity (VO₂ peak/anaerobic threshold (AT)) and muscle mass (by DXA). Paired and unpaired t-tests were used. Data are means±SD. *P* value <0.05 was considered significant. **Results:** 4-weeks of EET improved VO₂peak (42.8±10.4 vs. 45.9±9.4 ml/kg/min; *P*=0.004) and AT (24.9±8.1 vs. 28.7±8.9 ml/kg/min; *P*<0.0001), while RET enhanced appendicular lean body mass (ALBM) (26.8±4.3 vs. 27.4±4.1 kg; *P*=0.014). The 6-week washout was sufficient to reverse these adaptive values to baseline. i.e. VO₂peak pre-EET (44.1±7.7 ml/kg/min) was identical to pre-RET (44.6±5.7 ml/kg/min; *P*=0.89). The same was true for AT (*P*=0.88). Similarly, ALBM pre-RET (26.8±2.2 kg) was not different to ALBM pre-EET (27.2±2.6 kg; *P*=0.40). Finally, our data (Figure 1) illustrates a range of exercise-mode specific (i.e. only responding to one or the other mode) and non-specific (i.e. responding to neither or both modes) trainability traits.

Conclusion: Four-weeks of exercise training was sufficient to elicit anticipated exercise-mode specific adaptations i.e. aerobic capacity gains with EET and lean mass gains with RET. A 6-week washout was sufficient to reverse these gains. Finally, as

expected, responses to EET and RET within groups was variable; however, across exercise mode, responses were also unpredictable - traits for which biomarkers should be sought.

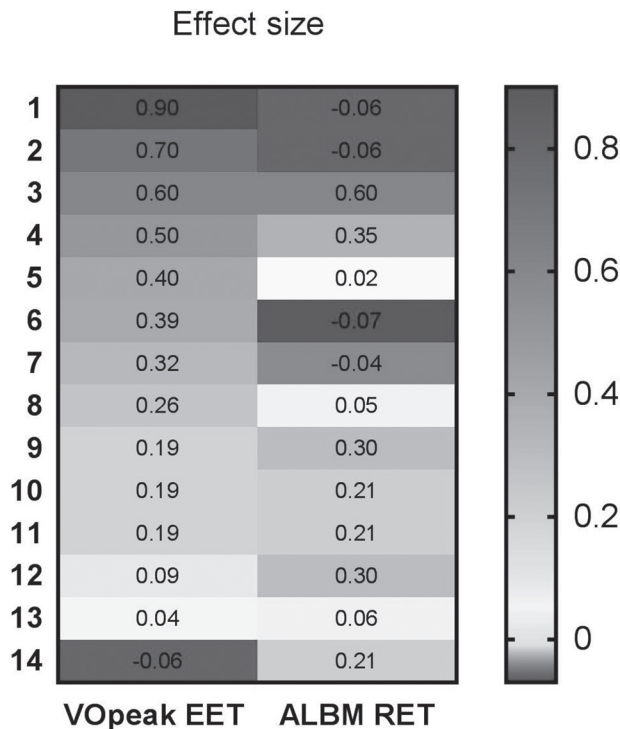


Figure 1: Effect size of two modes of exercise training on a primary endpoint expected to improve with that mode of exercise in 14 young individuals. The effect size was calculated by dividing difference (post-pre) by standard deviation of baseline (pre) values. VO₂ peak was used to represent the endurance exercise training (EET) response, from the highest to the lowest, with the corresponding appendicular lean body mass (ALBM) response for resistance exercise training (RET) presented.

Green, D. J. *et al.* Exercise training and artery function in humans: nonresponse and its relationship to cardiovascular risk factors. *J. Appl. Physiol.* 117, 345–352 (2014).

Hoier, B., Passos, M., Bangsbo, J. & Hellsten, Y. Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle. *Exp. Physiol.* 98, 585–597 (2013).

Phillips, B. *et al.* Resistance exercise training improves age-related declines in leg vascular conductance and rejuvenates acute leg blood flow responses to feeding and exercise. *J Appl Physiol* 112, 347–353 (2012).

Phillips, S. M. *et al.* High responders to resistance exercise training demonstrate differential regulation of skeletal muscle microRNA expression. *J. Appl. Physiol.* (2010). doi:10.1152/jappphysiol.00901.2010

Phillips, B. E. et al. Molecular Networks of Human Muscle Adaptation to Exercise and Age. *PLoS Genet.* (2013). doi:10.1371/journal.pgen.1003389

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC096

Maximum inspiratory pressure vs functional capacity in Portuguese active elderly undergoing Multicomponent Training

C. Blasco-Lafarga², A. Roldán¹, N. Blasco-Lafarga³, M. Gómez-Cabrera⁴, J.R. Duarte⁵ and J. Carvalho⁵

¹Physical Education and Sport Department, University of Valencia, Valencia, Spain,

²Physical Education and Sport Department, University of Valencia, Valencia, Spain,

³Primary Health Centre in Peset Hospital area, Valencia, Spain, ⁴Physiology Department, University of Valencia, Valencia, Spain and ⁵Research Centre in Physical Activity Leisure and Health, Faculty of Sports, University of Porto, Porto, Portugal

Lack or little relationship between the strength of the inspiratory muscles and the functional capacity in a group of healthy active elderly undergoing a multicomponent training program (MCTP), suggest the assessment of the Maximum Inspiratory Pressure (MIP) and its specific training to ensure cardiorespiratory health in neuromuscular interventions (1). In order to deepen in this rationale, the present study reproduces the experimental procedure and testing methodology in a group of Portuguese elderly undergoing a different MCTP. Training dose was larger (three days/week vs two days), and cognitive demands were reduced compared to EFAM-UV[®] (1).

12 healthy, active, non-smokers elderly (73.08±6.08 years; 54.58±16.14 cmH₂O; 5 women: 73.40±7.67 years, 53.60±21.90 cmH₂O; 7 men: 72.86±5.37 years, 55.29±12.50 cmH₂O) volunteered to participated. MIP was assessed by means of the Powerbreathe[®] K5 (2), following Neder, Andreoni (3) protocol. Functional capacity included: cardiorespiratory fitness (6MWT), and upper-limb and lower-limb strength tests (Handgrip & 30STS). The association between MIP and functional capacity was analysed, and age and gender were introduced as covariates. The Portuguese sample showed MIP values closed to those expected for the Powerbreath[®] K5 in similar samples (1, 4), but only gender showed a significant a priori association with the handgrip ($r=0.690$; $p<0.05$). MIP, 6MWT and 30STS failed to show it. In disagreement with previous studies in elderly undergoing EFAM-UV[®] (1), there was lack of association between MIP and the functional capacity outcomes in the Portuguese group.

This study underspin the small association between MIP and functional capacity in elderly following neuromuscular training when considering the influence of age and gender. Larger dose and reduced cognitive demands do not improve this relationship, so specific inspiratory training should be added to MCTP, although

the small sample size is an important limitation in our study. In fact, Giua, Pedone (5) found a relationship between MIP and 6MWT in a group of elderly (men and women), without considering our covariates, and predicted the 6MWT including MIP in the formula, what increased the explained variability from 22% to 34%. On the other hand, differences in testing devices (traditional vs. electronic) is also a limitation and suggests the need of further studies with better normative references, and bigger samples of healthy active individuals.

Table 1. *Performance characteristics*

	Total (n=12) Mean $\pm$ SD	CV (%)	Women (n=5) Mean $\pm$ SD	CV (%)	Men (n=7) Mean $\pm$ SD	CV (%)
MIP (cmH ₂ O)	54.58 $\pm$ 16.14	31.00	53.60 $\pm$ 21.90	41.00	55.29 $\pm$ 12.50	23.00
6MWT (m)	528.04 $\pm$ 55.51	25.00	524.80 $\pm$ 44.59	8.00	530.36 $\pm$ 65.64	12.00
HG¹ (Kg)	32.21 $\pm$ 5.50	36.00	27.43 $\pm$ 5.07	34.30	34.94 $\pm$ 3.68	11.00
30STS (rep)	18.00 $\pm$ 3.88	23.00	18.80 $\pm$ 3.96	21.00	17.43 $\pm$ 4.04	23.00

¹ Only 11 participants for this variable

BMI: Body Mass Index; MIP: Maximum Inspiratory Pressure; 6MWT: 6 minutes walking test; HG: Handgrip Strength; 30STS: 30 seconds seat to stand test.

Table 1. *Performance and functional capacities*

¹ Only 11 participants for this variable

BMI: Body Mass Index; MIP: Maximum Inspiratory Pressure; 6MWT: 6 minutes walking test; HG: Handgrip Strength; 30STS: 30 seconds seat to stand test

1. Roldán A, Cordellat A, Monteagudo P, Blasco-Lafarga NM, Gómez-Cabrera MC, Blasco-Lafarga C. Age and gender mediate the small association of MIP and functional capacity in healthy active elderly. In: Society TP, editor. Physiology 2019; Poster communication submitted to The Pshyological congress 2019, Aberdeen2019.

2. Lee KB, Kim MK, Jeong JR, Lee WH. Reliability of an Electronic Inspiratory Loading Device for Assessing Pulmonary Function in Post-Stroke Patients. Medical Science Monitor. 2016;22:191-6.

3. Neder JA, Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Brazilian Journal of Medical and Biological Research. 1999;32:719-27.

4. Alvarenga GM, Charkovski SA, dos Santos LK, da Silva MAB, Tomaz GO, Gamba HR. The influence of inspiratory muscle training combined with the Pilates method on lung function in elderly women. Clinics in chest medicine. 2018;73:e356-e.

5. Giua R, Pedone C, Scarlata S, Carrozzo I, Rossi FF, Valiani V, et al. Relationship Between Respiratory Muscle Strength an Physical Performance in Elderly Hospitalized Patients. Rejuvenation Research. 2014;17(4):366-71.

Special thanks to all the elderly, the student and the acedemic staff in the CIAFEL (University of Porto).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC097

Steady state of respiratory gases is not necessary to achieve reliable resting metabolic rate measurements: a reliability study using the Vyntus CPX system.

J.L. Areta¹, J. Iraki², I. Garthe³, G. Paulsen³ and G. Slater⁴

¹*School of Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK,* ²*Iraki Nutrition, Oslo, Norway,* ³*Norwegian Olympic Centre, Oslo, Norway and* ⁴*University of the Sunshine Coast, Sunshine Coast, QLD, Australia*

Reliable measurements of resting metabolic rate (RMR) in humans are crucial to detect meaningful changes in its status. Past research suggests that low intra-measurement variation of respiratory gases (*steady state*) is necessary to achieve valid measurements on critically ill patients (1). However, it is unclear how stability of continuously assessed gases may affect RMR reliability in healthy individuals (2,3). Therefore, this study aimed at defining the effect of data analysis methods determining lowest coefficient of variation within indirect calorimetry measurements on the reliability of between and within-day RMR.

To assess this, healthy participants (n=26) completed three measurements of RMR (two on the same day, and two on consecutive days) using a ventilated hood linked to the Vyntus CPX system.

Each measurement consisted of 30 min of individuals resting in supine position in standardised conditions, with the first 10 min used for adaptation, providing the subsequent 20 min of net collected data per measurement. Within this 20 min period we determined RMRs for the intervals of lowest coefficient of variation (CV) for VO₂, VCO₂, and respiratory exchange ratio of 5 and 10 min duration, using data sampling rate (smoothing) of 10, 20 and 30 sec as well the whole 20 min of data collected and the first and second 10 min intervals, providing a total of 21 methods of interval determination for RMR.

Between-day RMRs showed no systematic bias, but intervals ≥ 10 min showed better typical error of measurement (TEM; 3.7-4.6%) and intraclass correlation (ICC; 0.92-0.95) than intervals of 5 min (TEM, 4.8-6.1%; ICC, 0.86-0.92). There was no relationship between average within-measurement CV and between day CV (Pearson $R^2 \leq 0.009$, $p \geq 0.2$). Whole period and the first 10 min interval showed no systematic bias, a nearly perfect correlation of 0.92 (0.84-0.97; 95%CL) and 0.93 (0.85-0.97) respectively and a small TEM of 4.5 (3.5-6.2) and 4.3% (3.3-5.9%) respectively. There was a nearly perfect correlation between the 1st 10 min interval and the whole 20 min period ($R^2 = 0.991$, $p < 0.0001$).

Within-day RMR showed a lower reliability, evidenced by lower correlations between measurements (ICC, 0.85-0.89) and higher typical error (TEM, 6.8-8%). Systematic bias seemed negligible, but in some cases the second measurement was lower than the first one (effect sizes ~ 0.20 ; 95%CL ~ 0.45 -0.05).

In conclusion, criteria for determining lowest variation (CV) during measurements seemed to have no meaningful effects on the reliability of measurements, but longer periods provided more reliable between day RMR data. Additionally, there

was no relationship between within-measurement CV and RMR reliability. Ten and 20' of data collected under standardised conditions provide reliable RMR measurements without need of considering the CV of data analysed.

McClave S et al. (2003) *JPEN* 27(1):16-20.

Fullmer S et al. (2015) *J. Acad. Nutr. Diet.* 115(9):1417-46.

Sanchez-Delgado et al. (2018) *Clin. Nutr.* 37(5):1618-24.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC098

The influences of skeletal muscle temperature on muscle deoxygenation and metabolism at fixed exercise workload

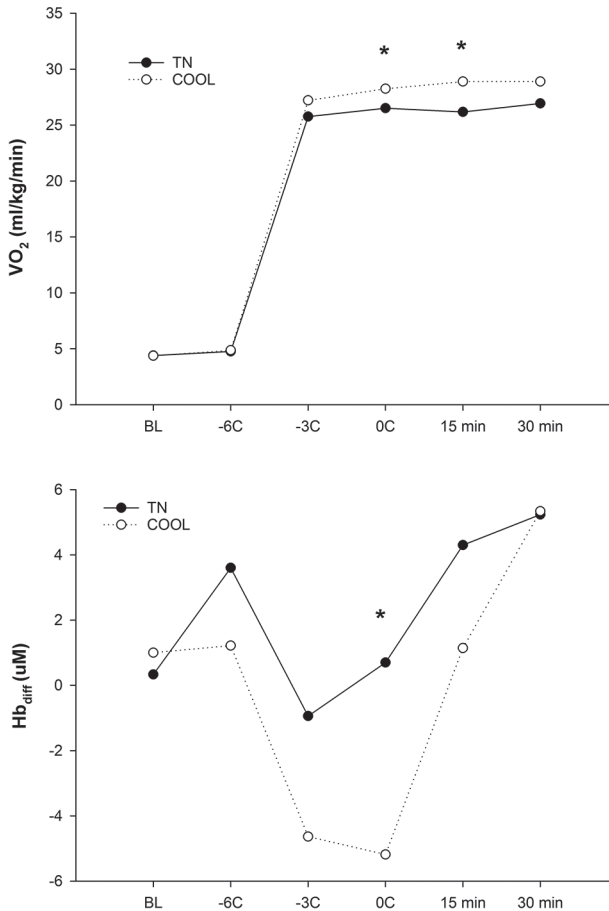
D. Gagnon^{1,2} and S. Munten¹

¹Human Kinetics, Laurentian University, Sudbury, ON, Canada and ²Center for Research in Occupational Safety and Health, Laurentian University, Sudbury, ON, Canada

Muscle cooling has been associated with reduced physical performance due to increased O₂ consumption requirements, reduced mechanical efficiency, altered neuromuscular functions, blood volume, and O₂ extraction (Oksa et al., 2002; Gagnon et al., 2017). Whether these factors influence the skeletal muscle deoxygenation response, and consequently metabolism, during physical performance, is unclear. This study investigated skeletal muscle oxidative responses, metabolism, and cardiovascular hemodynamics during cycling at a fixed workload following muscle cooling. Seven healthy participants (26 ± 5.7 years, 174 ± 8.8 cm, 75 ± 13.1 kg, 21 ± 3.4%BF, 49 ± 8.7 VO₂peak) dressed in shorts and a t-shirt, performed two steady-state exercise sessions at 70% of ventilatory threshold (VT), on a cycle ergometer, in a fasted state. Sessions were conducted in a thermoneutral environment (22.0°C), with (LCO) and without (CON) a pre-exercise leg-cooling protocol in 10°C water until muscle temperature (T_m) decreased by 6°C. Exercise was then initiated at 70%VT until T_m returned to baseline. Cycling continued for another 30 min. Cardiorespiratory and metabolic variables were assessed via indirect calorimetry, whereas skeletal muscle tissue oxygenation (%TSI) and the difference in oxygenated and deoxygenated hemoglobin content (diffHb) were assessed with near-infrared spectroscopy (NIRS). Cardiovascular hemodynamic responses were collected via intrathoracic bioimpedance. Venous blood samples were collected to assess changes in lactate, pH, pO₂ and pCO₂. Two-way ANOVA were used (conditions and times) to assess differences with a level of significance set at p<0.05. HR, SV, and CO all increased across time but were not different between conditions. VO₂ was greater in LCO vs. CON when muscle temperature was restored (28 ± 2 vs. 26 ± 3 mL O₂/kg/min; p<0.05), and at 15 min of exercise (29 ± 4 vs. 26 ± 3 mL O₂/kg/min; p<0.05). %TSI was higher at the onset of exercise (58 ± 5%) compared when muscle temperature was restored (49 ± 5%; p<0.001) and at 15 min of exercise (53 ± 7 %; p<0.001) but no differences were

observed between conditions. While diffHb followed the same trend, it was also lower in LCO vs. TN when muscle temperature was restored (-5.2 ± 5 vs. 1 ± 2 μM ; $p < 0.05$). Blood pH, pO_2 were lower while pCO_2 was higher when muscle temperature was restored in the LCO condition but at no other point in time ($p < 0.05$).

The present study demonstrates an increase in oxygen consumption from pre-exercise muscle cooling, associated with lower tissue oxygenation despite the return of muscle temperature to baseline values. A delayed metabolic response could explain the present results. Nonetheless, whether the increase in oxygen demand drives additional O_2 uptake, or, the lower O_2 availability drives a greater energy demand, remains to be determined.



Oksa J et al. (2002). *J. Appl. Physiol.* 35(20), 1995-2004

Gagnon DD et al. (2017). *J. Sports Sci.* 35(20), 1995-2004

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC099

Diving-related flow-mediated dilation responses following imposed oscillatory shear stress

O.F. Barak³, R.L. Hoiland¹, T. Mijackica², N. Janjic³, D. Vlahovic³, P. Ainslie¹ and Z. Dujic²

¹Centre for Heart, Lung and Vascular Health, University of British Columbia, Okanagan Campus, Kelowna, BC, Canada, ²University of Split School of Medicine, Split, Croatia and ³University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

Diving with self-contained underwater breathing apparatus (SCUBA) induces endothelial dysfunction that seems to be an important factor in development of decompression sickness (1). Flow-mediated dilation (FMD) is a measure of endothelial function and is reported to be decreased following single and repetitive dives (2,3). An impaired endothelial function has also been observed following acute periods of oscillatory shear stress (OSI) in the conduit arteries, which induces the expression of pro-atherogenic factors (4). The aim of this study was to assess whether post-dive endothelial dysfunction could further be worsened with a short period of OSI. Twelve male divers (age 39.3 ± 9.2 years, height 186 ± 6 cm, weight 88.0 ± 11.6 kg) completed a single dive to a depth of 18m sea water with a 47-min bottom time. Endothelium-dependent vasodilator function of the brachial artery was assessed pre- and post-intervention of imposed oscillatory shear stress by a 20-min cuff inflation to 75 mmHg over the forearm (5), using the FMD approach. All FMD measures were analysed by two-way repeated measure analysis of variance (RM-ANOVA; factors: time – pre-/post-intervention, condition – pre-/post-dive). There was an interaction effect for absolute and relative FMD ($p=0.017$ and $p=0.009$, respectively). Absolute FMD was reduced by $30.7 \pm 18.2\%$ following the intervention ($p<0.05$), but only in the pre-dive condition. Diving reduced FMD by $24.1 \pm 11.9\%$ ($p<0.05$) but there was no further reduction induced by the intervention. The interaction effect persisted following allometric scaling for baseline diameter and including SRAUC and baseline diameters as covariates ($p=0.006$). Endothelial function measured through FMD seems to be susceptible to oscillatory shear stress pre-dive, but the effect OSI is mitigated post-dive, as the values of FMD are already decreased by diving induced stress factors.

Thom SR, Bennett M, Banham ND, Chin W, Blake DF, Rosen A, Pollock NW, Madden D, Barak OF, Marroni A, Balestra C, Germonpre P, Pieri M, Cialoni D, Le P-NJ, Logue C, Lambert D, Hardy KR, Sward D, Yang M, Bhopale VB, Dujic Z. Association of microparticles and neutrophil activation with decompression sickness. *J Appl Physiol.* 2015;119:427–434.

Lambrechts K, Pontier J-M, Balestra C, Mazur A, Wang Q, Buzzacott P, Theron M, Mansourati J, Guerrero F. Effect of a single, open-sea, air scuba dive on human micro- and macrovascular function. *Eur J Appl Physiol.* 2013;113:2637–2645.

Barak OF, Caljkusic K, Hoiland RL, Ainslie PN, Thom S, Yang M, Jovanov P, Dujic Z. Differential influence of vitamin C on the peripheral and cerebral circulation following diving and exposure to hyperoxia. *Am J Physiol Regul Integr Comp Physiol*. 2018;315(4):R759-R767.

Barak OF, Mladinov S, Hoiland RL, Tremblay JC, Thom SR, Yang M, Mijacka T, Dujic Z. Disturbed blood flow worsens endothelial dysfunction in moderate-severe chronic obstructive pulmonary disease. *Sci Rep*. 2017;7(1):16929.

Jenkins NT, Padilla J, Boyle LJ, Credeur DP, Laughlin MH, Fadel PJ. Disturbed blood flow acutely induces activation and apoptosis of the human vascular endothelium. *Hypertension* 2013;61:615–621.

This study was partly funded by a grant of the Croatian Science Foundation (IP-2014-09-1937) and of the Autonomous Province of Vojvodina (142-451-2455/2018).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC100

Impaired flow-mediated dilation stimulated by sustained increases in shear stress in high-altitude excessive erythrocytosis

J.C. Tremblay¹, G.B. Coombs², C.A. Howe², G.A. Vizcardo-Galindo³, R.J. Figueroa-Mujica³, D. Bermudez³, M.M. Tymko², F. Villafuerte³, P. Ainslie² and K.E. Pyke¹

¹*School of Kinesiology and Health Studies, Queen's University, Kingston, ON, Canada,*

²*School of Health and Exercise Science, University of British Columbia - Okanagan, Kelowna, BC, Canada and* ³*Departamento de Ciencias Biológicas y Fisiológicas, Universidad Peruana Cayetano Heredia, Lima, Peru*

Excessive erythrocytosis (EE; hemoglobin concentration [Hb] ≥ 21 g dl⁻¹ in adult males) is a maladaptive pathology associated with increased cardiovascular risk in Andean highlanders (Corante et al. (2018)). It has been demonstrated that flow-mediated dilation (FMD) in response to a transient increase in shear stress is reduced in Andeans with EE (Tremblay et al. (2019)); however, whether a similar impairment in response to more commonly encountered sustained increases in shear stress occurs is unknown. We sought to characterize sustained shear stress stimulus FMD (SS-FMD) in response to progressive handgrip exercise in Andeans with and without EE in Cerro de Pasco, Peru (4330 m). Male Andean highlanders with (n=17, [Hb] = 23.2 ± 1.2 g dl⁻¹ (mean $\pm$ SD)) and without EE (n=23, [Hb] = 18.7 ± 1.9 g dl⁻¹) performed three-minutes of rhythmic handgrip exercise at intensities of 20%, 35%, and 50% of their maximum voluntary contraction (MVC). Duplex ultrasound was used to record brachial artery blood velocity and diameter in the last minute of each handgrip intensity. Venous whole blood viscosity was assessed to calculate shear stress. Although baseline shear stress and shear stress at 20% and 35% MVC did not differ between cohorts, Andeans with EE had a 22% lower shear stress at 50% MVC compared to Andeans without EE (P=0.004). At

20% MVC, SS-FMD did not differ between groups, however at 35 % and 50 % MVC, SS-FMD was $2.1 \pm 2.0\%$ and $2.8 \pm 2.7\%$ in Andeans with EE compared to $4.1 \pm 3.4\%$ and $7.5 \pm 4.5\%$ in those without EE ($P = 0.048$ and $P < 0.001$). The stimulus-response slope (change in shear stress *versus* change in diameter) was lower in Andeans with EE compared to Andeans without EE ($P = 0.028$). When participants were pooled, this stimulus-response slope was negatively related to Hb ($r^2 = 0.203$, $P = 0.0035$) and blood viscosity ($r^2 = 0.148$, $P = 0.014$), and positively related to oxyhemoglobin saturation ($r^2 = 0.136$, $P = 0.019$). These findings report, for the first time, an impaired SS-FMD in response to small muscle mass exercise in Andeans with EE. Exercise results in a physiologically-relevant and commonly encountered shear stress stimulus, and in combination with results indicating impaired FMD in response to transient increases in shear stress, these findings suggest a generalized reduction in endothelial sensitivity to shear stress in this population.

Corante N *et al.* (2018). *High Alt Med Biol* 19, 221-231

Tremblay JC *et al.* (2019). *Hypertension* In Press

This work was funded by an American College of Sports Medicine Doctoral Student Research Grant (JCT), the Natural Sciences and Engineering Research Council of Canada (PNA), and a Canada Research Chair (PNA) with travel support provided by The Physiological Society (JCT). This study received funding from the Wilderness Medical Society Research-in-Training Grant, supported by the Academy of Wilderness Medicine®.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC101

Genotyping analyses of lean body mass and hand grip strength-associated single nucleotide polymorphisms in master athletes

H. Crossland¹, D. McCormick¹, J. Piasecki², D. Wilkinson¹, K. Smith¹, J. McPhee³, M. Piasecki¹ and P. Atherton¹

¹MRC-ARUK Centre for Musculoskeletal Ageing Research & NIHR Nottingham BRC, University of Nottingham, Derby, UK, ²Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK and ³Department of Sport and Exercise Sciences, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester, UK

Recent genome-wide association studies (GWAS) have identified a number of candidate genetic loci associated with lean body mass (LBM) (Zillikens *et al.*, 2017) and hand grip strength (HGS) (Willems *et al.*, 2017). We aimed to determine whether these associations could be observed in a unique population of master athletes (both sprint and endurance), by assessing allele distributions of selected SNPs

linked to LBM and HGS and evaluating potential links to physiological parameters. Genomic DNA was isolated from buffy coat samples of 48 master athletes (MA; 70.6 ± 5.9 yrs; age-graded performance $83.4 \pm 8.6\%$) and 48 older controls (75.3 ± 6.0 yrs). The tetra-primer amplification refractory mutation system (ARMS) PCR technique (Ye et al., 2001), combined with agarose gel electrophoresis, was used to validate SNPs and genotype samples. Out of the 3 SNPs analysed that were previously associated with LBM (*IRS1*, *FTO* and *ADAMTSL3*), neither *IRS1* nor *FTO* showed any differences in allelic distribution between MA and control or correlation with LBM, however for *ADAMTSL3*, there was a significant enrichment in the effect allele in the MA group ($P < 0.01$ with Fisher's exact test). Irrespective of groupings, there was also a significant association of genotype with %LBM ($P < 0.01$ with one-way ANOVA). For the 3 HGS-linked SNPs analysed (*GBF1*, *GLIS1* and *TGFA*), neither *GBF1* nor *GLIS1* showed any significant association with HGS in the samples analysed, or any differences in allelic distribution between MA and controls. While allele frequencies for the *TGFA* SNP were not significantly different between MA and controls, there was a significant association with HGS and genotype ($P < 0.05$ with one-way ANOVA), and there was a significant enrichment in the effect allele in those individuals with the highest 25% versus the lowest 25% HGS (irrespective of groupings; $P < 0.05$ with Fisher's exact test). Out of the 6 SNPs analysed, 4 showed no difference in allele frequencies or associations with LBM or HGS (*IRS1*, *FTO*, *GLIS1* or *GBF1*), however the SNPs linked to *ADAMTSL3* and *TGFA* showed significant associations with LBM and HGS, respectively, as predicted by the GWAS analyses. The potential functional relevance of the *ADAMTSL3* gene in lean mass regulation, and of *TGFA* in muscular strength, remains to be evaluated.

Willems SM et al. (2017). *Nat Commun* 8, 16015.

Ye S et al. (2001). *Nucleic Acids Res* 29, E88-8.

Zillikens MC et al. (2017). *Nat Commun* 8, 1414.

This work was supported by the Medical Research Council [grant number MR/P021220/1] as part of the MRC-ARUK Centre for Musculoskeletal Ageing Research awarded to the Universities of Nottingham and Birmingham, and the National Institute for Health Research, Nottingham Biomedical Research Centre.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Post-prandial hyperlipidaemia impairs dynamic cerebral autoregulation

C. Marley¹, H. Tsukamoto^{1,4}, D. Davis^{1,2}, J. Brugniaux^{1,3}, J. Smirl⁵ and D. Bailey¹

¹Neurovascular Research Laboratory, University of South Wales, Pontypridd, UK, ²Leeds Trinity University, Leeds, UK, ³University Grenoble Alpes, Grenoble, France, ⁴Ritsumeikan University, Kyoto, Japan and ⁵University of British Columbia, Okanagan, BC, Canada

Background: It is well established that post-prandial hyperlipidaemia (PPH) impairs systemic vascular endothelium function¹. However, we have recently demonstrated that this is not simply confined to the systemic circulation and equally extends to the cerebral vasculature in the form of impaired cerebrovascular reactivity to carbon dioxide². To what extent PPH may influence other markers of cerebrovascular health such as dynamic cerebral autoregulation (dCA) are yet to be investigated.

Methods: Forty-four males were recruited for the study of which 20 were young (age: 24 ± 5 yrs; body mass: 76 ± 13 kg) and 24 were aged (age: 67 ± 5 yrs; body mass: 82 ± 11 kg). dCA was assessed using transfer function analysis in response to forced oscillations in mean arterial pressure (MAP; finger photoplethysmography) via squat-stand manoeuvres at a frequency of 0.05Hz for 3 minutes as described previously³. During these manoeuvres, continuous measurements of middle cerebral artery velocity (MCAv; transcranial Doppler) were made. Cerebrovascular resistance and conductance indices were calculated as MAP/MCAv and MCAv/MAP, respectively. Venous blood samples were also obtained from an indwelling cannula to assess triglycerides (photometry). All measurements were made prior to and 4 hours following consumption of a standardised high-fat meal (1362 kcal; 130g fat)⁴. Data were analysed using a 2-way repeated measures analysis of variance. Significance was established at $P < 0.05$ and data are expressed as mean $\pm$ SD.

Results: By design, the high-fat meal increased circulating triglycerides by 1.38 ± 1.03 mmol/L in the young and 1.49 ± 0.90 mmol/L in the aged ($P < 0.05$). PPH had no effect on resting MCAv, MAP, CVRi or CVCi (Table 1; $P > 0.05$), but was associated with impaired dCA in the form of reduced phase (Table 1; $P < 0.05$).

Conclusion: These findings show for the first time that PPH has the capacity to impair dCA across the lifespan. These findings have important clinical implications given that impaired dCA is associated with increased risk of stroke and neurodegenerative disease⁵.

Table 1. Changes in cerebrovascular function following a high-fat meal

Meal	Young		Aged		Significance (P Values)		
	Pre-meal	Post-meal	Pre-meal	Post-meal	Age	Meal	Age x Meal
MCAv (cm.s ⁻¹)	62 ± 13	61 ± 10	48 ± 11	46 ± 10	0.000	0.435	0.911
MAP (mmHg)	88 ± 9	85 ± 7	90 ± 11	88 ± 10	0.316	0.102	0.451
CVRI (mmHg.cm.s ⁻¹)	1.49 ± 0.31	1.44 ± 0.28	1.96 ± 0.42	1.97 ± 0.44	0.000	0.681	0.435
CVCI (cm.s ⁻¹ .mmHg)	0.70 ± 0.14	0.72 ± 0.15	0.54 ± 0.13	0.53 ± 0.13	0.000	0.528	0.375
MAP Power (mmHg) ²	19960 ± 10271	23377 ± 11916	20355 ± 15556	26971 ± 22199	0.666	0.003	0.324
MCAv Power (cm.s ⁻¹) ²	10590 ± 8956	10754 ± 8193	6894 ± 5160	8689 ± 6469	0.177	0.119	0.192
Coherence	0.97 ± 0.03	0.98 ± 0.01	0.97 ± 0.03	0.98 ± 0.02	0.951	0.278	0.965
Gain (cm.s ⁻¹ .mmHg ⁻¹)	0.71 ± 0.31	0.66 ± 0.19	0.57 ± 0.19	0.61 ± 0.20	0.124	0.772	0.083
Normalised Gain (%.mmHg ⁻¹)	1.05 ± 0.34	1.08 ± 0.33	1.02 ± 0.28	1.19 ± 0.43	0.612	0.062	0.188
Phase (radians)	0.61 ± 0.30	0.54 ± 0.20	0.61 ± 0.29	0.55 ± 0.25	0.962	0.044	0.949

Values are mean + SD; MCAv indicates middle cerebral artery velocity; MAP, mean arterial pressure; CVRI/CVCI, cerebrovascular resistance/conductance indices.

Vogel *et al.* (1997) *Am J Cardiol*; **79**, 350-354.

Marley *et al.* (2017) *Clin Sci*; **131**, 2807-2812.

Bailey *et al.* (2019) *J Physiol*; **597**, 611-629.

Patsch *et al.* (1983) *PNAS*; **80**, 1449-1453.

Panerai (2008) *Cardiovasc Eng*; **8**, 42-59.

DMB is supported by a Royal Society Wolfson Research Fellowship (#WM170007).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC103

The Effects of a Short-Term Heat Acclimation Protocol in Elite Amateur Boxers

B. Stone^{1,2}, J. Ashley¹, R. Skinner², J. Polanco², M. Walters² and J. Kellawan¹

¹Health and Exercise Science, University of Oklahoma, Colorado Springs, CO, USA and

²United States Olympic Committee, Colorado Springs, CO, USA

Background: The 2020 Summer Olympics will be held in Tokyo, Japan where the heat and humid often reach 40°C and 70% relative humidity (RH) (1). Heat and humidity (H&H) have a substantial negative effect on exercise performance, however, exposure to H&H can lead to physiological adaptations (e.g. expanded plasma volume and sweat rate) can enhance exercise performance in both H&H and in 'normal' environmental conditions (2, 3). In highly trained endurance athletes, exercise induced acclimation can occur in as little as 5-days (known as short term heat acclimation, STHA) (4, 5). However, it is unknown if STHA can be achieved in different athletic populations such as elite amateur boxers. Aims: To examine the effects of a 5-day STHA protocol on cardiovascular performance, sweat loss, and hydration status in elite amateur boxers. Methods: 9 (3 female) elite level amateur boxers (mean ± SD: 22.5 ± 5.1 years, 179.1 ± 9.8 cm, 73.3 ± 20.5kg)

underwent a 5-day STHA protocol. All sessions were conducted in an environmental chamber set at 32°C and 70% RH, during the same time of day, with a minimum of 3 hours post-prandial. Each session was 60 minutes in length which consisted of dynamic warm up, exercise (eight, 1-min intervals at 85% of heart rate max (HRmax) followed by 20-min steady-state at 75% HRmax)) conducted on a treadmill and cycle ergometer (alternated daily), cool down and stretch. Each session consisted of a pre-weight and urine specific gravity (USG) measurement with a post-session weigh out. Pre and post STHA protocol cardiovascular performance testing: 24 hours pre and post STHA, cardiovascular performance was assessed via repeat sprint assessment test in normal environmental conditions (22°C). Data were analyzed using a One-way ANOVA followed by post hoc t tests when appropriate with a priori value set at ($p \leq 0.05$). Results: Data are absolute and change (Δ) pre-to-post STHA $\pm$ SD. Repeat sprint assessment significantly improved pre-to post-STHA $\sim 44\%$ ($+\Delta 6 \pm 6.9$ more sprints completed, $p = 0.02$, $d = 0.9$). Daily sweat loss did not change significantly from Day 1-5 ($p = 0.3$), however, intra-day loss was significant ($1.5 \pm 1.5\text{kg}$, $\Delta 2\%$, $p < 0.05$). USG was significantly increased from Days 1-5 (1.0188 ± 0.0101 to 1.01453 ± 0.006 , $\Delta -0.42\%$, $p = 0.05$, $d = 0.54$). Conclusion: 5-Day STHA in elite boxers significantly improved cardiovascular performance, hydration status, and increased intra-day sweat loss. These observations are indicative of positive physiological adaptations that improve exercise performance in H&H. Therefore, we conclude that a 5-Day STHA protocol can enhance physiological exercise responses in elite amateur boxers.

Nakamura S, Wada K, Yanagisawa N, Smith DR. Health risks and precautions for visitors to the Tokyo 2020 Olympic and Paralympic Games. Travel medicine and infectious disease. 2018;22:3-7.

Baker LB. Sweating Rate and Sweat Sodium Concentration in Athletes: A Review of Methodology and Intra/Interindividual Variability. Sports medicine (Auckland, NZ). 2017;47(Suppl 1):111-28.

Guy JH, Deakin GB, Edwards AM, Miller CM, Pyne DB. Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. Sports medicine (Auckland, NZ). 2015;45(3):303-11.

Garrett A, Creasy R, Rehner N, Patterson M, Cotter J. Effectiveness of short-term heat acclimation for highly trained athletes. European Journal of Applied Physiology. 2012;112(5):1827-37.

Periard JD, Racinais S, Sawka MN. Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports. Scand J Med Sci Sports. 2015;25 Suppl 1:20-38.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Comparative assessment of serum and urine indices of renal function amongst athletes and non-athletes in Port Harcourt, Nigeria.

D.V. Dapper¹, K.I. Davies¹, P. Emem-Chioma² and A.N. CHUEMERE¹

¹Department of Human Physiology, University of Port Harcourt, Port Harcourt, Nigeria, Port Harcourt, Nigeria and ²Department of Medicine, University of Port Harcourt, Port Harcourt, Nigeria

The prevalence of hypertension and its associated complications including renal impairment is on the increase amongst Nigerians. The present study aims to evaluate the value of serum and urine electrolytes and indices in the assessment of renal function amongst athletic and non-athletic subjects. Further, the study aims to estimate 24hour sodium intake using spot-urine sodium concentration.

A total of 277 apparently healthy subjects (131 males and 146 females) aged between 18 and 40 years were recruited into the study. Subjects consisted of 133 athletes (94 males and 39 females) and 144 non-athletes (37 males and 107 females). All subjects were apparently healthy. Anthropometric parameters and blood pressure indices were determined. 5ml of venous blood and spot urine samples were subsequently collected for determination of venous and urinary concentration of various electrolytes and indices of renal function: sodium, potassium, chloride, urea, creatinine and uric acid using ion selective electrode, kinetic and end point spectrometry as appropriate. Glomerular filtration rate was calculated using the CKD-EPI equation. Athletic male subjects were found to have higher values of age, height, weight, body mass index, diastolic and mean arterial pressure compared to non-athletic male subjects ($p<0.05$): However, athletic female subjects were observed to have higher values of height and lower values of diastolic blood pressure and mean arterial blood pressure only compared to non-athletic female subjects ($p<0.05$). Athletic male subjects were found to have significantly higher values of both serum sodium and urine sodium concentrations compared to non-athletic male subjects ($p<0.05$); while, non-athletic male subjects were found to have significantly higher values of urine potassium and urine chloride concentrations compared to athletic male subjects. For female subjects, non-athletic female subjects were found to have significantly higher serum sodium concentration compared to athletic female subjects ($p<0.05$); while athletic female subjects were found to have significantly higher urine creatinine concentration compared to non-athletic female subjects ($p<0.05$). No significant differences were observed in the values of other indices assessed in either male or female athletes ($p>0.05$).

We attempt to establish normative values of these electrolytes and indices of renal function amongst apparently healthy athletic and non-athletic Nigerian subjects and estimated values of daily sodium intake from spot-urine sodium concentration. Our study would be of immense public health importance since previous reports have been relatively scanty.

Ordinioha, B (2013) *Niger J Clin Pract* 16, 1-4

Ighorjoe AD & Dapper DV (2005) *Niger J Physiol Sci* 20, 33-38

Levey AS, Stevens LA, Schmid CH, et al. (2009) *Ann Intern Med.* 150, 604-612

He FJ & MacGregor GA. (2009) *J Hum Hypertens* 23, 363-384

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC105

Recurrent concussion in retired rugby union players is associated with cerebral hypoperfusion and cognitive impairment

T.S. Owens, T.A. Calverley, B.S. Stacey, C. Marley, H. Tsukamoto, M. Steggall, G. Jones, L. Fall, L. Venables, B. Davies, P. Williams and D. Bailey

Faculty of Life Science and Education, University of South Wales, Llanelli, United Arab Emirates

Sports-related concussion (SRC) represents a significant and growing public health concern yet remain one of the least understood injuries facing the sports medicine community today. There is increasing concern that prior recurrent concussion may contribute to long-term neurologic sequelae in later-life as retired contact sport athletes with three or more SRC's present with a fivefold prevalence of mild cognitive impairment (MCI, 1). To what extent this relates to an accelerated decline in cerebral perfusion, a major risk factor for cognitive decline and dementia, remains to be explored. We recruited 40 aged participants who were divided into two distinct groups; 20 retired rugby union players aged 65 (mean) $\pm$ 7 (SD) years with a self-report history of 3 (mean) concussions $\pm$ 3 (SD) incurred over the course of their playing careers and 20 non-concussed, non-contact, age, education and fitness-matched controls aged 63 $\pm$ 6 years. Middle cerebral artery blood flow velocity (MCAv) was measured via Doppler ultrasonography at rest and in response to hypercapnia/hypocapnia ($\pm$ 5 mmHg change in end-tidal PCO₂) with cerebral oxygen content (CDO₂) calculated retrospectively(2). Ongoing symptoms of concussion were reporting using the Sports Concussion Assessment Tool 5 (SCAT5). Cognitive function was assessed via neuropsychometric testing and screened for MCI using the Montreal Cognitive Assessment (MoCA). Rugby players had played for 23 $\pm$ 8 years and reported between 3 - 10 previous concussions with ongoing, persistent symptoms related to concussion (7 $\pm$ 6 vs. 3 $\pm$ 4, P = 0.01) with greater severity (16 $\pm$ 13 vs. 4 $\pm$ 8, P = 0.00). Resting cerebral perfusion was lower in rugby players (45 $\pm$ 9 cm.s⁻¹ vs. 51 $\pm$ 7 cm.s⁻¹, P = 0.01) and in response to hypercapnia (57 $\pm$ 10 cm.s⁻¹ vs. 69 $\pm$ 15 cm.s⁻¹, P = 0.01). Subsequently, CDO₂ was lower in rugby players at rest (1048 $\pm$ 170 ml/cm/svs. 878 $\pm$ 184 ml/cm/s, P = 0.00), throughout hypercapnia (1406 $\pm$ 289 ml/cm/s vs. 1118 $\pm$ 242 ml/cm/s P = 0.00) and hypocapnia (683 $\pm$ 136 ml/cm/s vs. 577 $\pm$ 139 ml/cm/s P = 0.02). Executive motor function was slower in rugby players using the non-dominant hand on the Grooved Pegboard (92 $\pm$ 18 vs. 79 $\pm$ 15 seconds, P = 0.02) in addition to lower MoCA scores (24 $\pm$ 3 vs. 26 $\pm$ 2, P = 0.02).

These findings indicate that neurological complications associated with SRC persist after retirement from rugby union. Cerebral hypoperfusion is identified as a haemodynamic risk factor that may precede the observed cognitive impairment that in later-life, may accelerate a (former) player's trajectory towards neurodegeneration(3, 4). Our prior research has identified that the cerebral hypoperfusion characteristic of SRC, albeit in the younger demographic, is associated with a free radical-mediated reduction in nitric oxide bioavailability(5).

Manley G, Gardner AJ, Schneider KJ, Guskiewicz KM, Bailes J, Cantu RC, et al. A systematic review of potential long-term effects of sport-related concussion. *British Journal of Sports Medicine*. 2017;51(12):969-77.

Bailey DM, Brugniaux JV, Filipponi T, Marley CJ, Stacey B, Soria R, et al. Exaggerated systemic oxidative inflammatory nitrosative stress in chronic mountain sickness is associated with cognitive decline and depression. *The Journal of physiology*. 2019;597(2):611-29.

Shively S, Scher AI, Perl DP, Diaz-Arrastia R. Dementia resulting from traumatic brain injury: what is the pathology? *Archives of neurology*. 2012;69(10):1245-51.

Wolters FJ, Zonneveld HI, Hofman A, van der Lugt A, Koudstaal PJ, Vernooij MW, et al. Cerebral perfusion and the risk of dementia: a population-based study. *Circulation*. 2017;136(8):719-28.

Owens TS, Calverley TA, Stacey BS, Marley CJ, Rose G, Fall LA, et al., editors. Elevated Systemic Oxidative Nitrosative Stress and Cerebrovascular Function in Professional Rugby Union Players: The Link to Impaired Cognition. *Europhysiology* 2018; 2018 15/09/2018; London.

The Authors would like to thank the JPR Williams Research Fellowships. D.M. Bailey is a Royal Society Wolfson Research Fellow (#WM170007).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC106

The effects of work rate and pedalling cadence on skeletal muscle oxygenation during cycling

C. Dockerill^{1,2}, L. Shastri², M. Alkhalil², C. Forbes², G. Rafferty², T. Takaishi⁴, L. Zhang³, K. Ishida^{3,5} and F. Formenti^{2,6,7}

¹Cardiovascular Clinical Research Facility, The University of Oxford, Oxford, UK, ²Centre for Human and Applied Physiological Sciences, King's College London, London, UK, ³Graduate School of Medicine, Nagoya University, Nagoya, Japan, ⁴Graduate School of Natural Sciences, Nagoya City University, Nagoya, Japan, ⁵Research Centre of Health, Physical Fitness and Sport, Nagoya University, Nagoya, Japan, ⁶Nuffield Division of Anaesthetics, The University of Oxford, Oxford, UK and ⁷Department of Biomechanics, University of Nebraska at Omaha, Omaha, United States Minor Outlying Islands

The selection of pedalling cadence during cycling may be determined by a number of factors, including the degree of oxygenation in the exercising skeletal muscle. It is generally accepted that muscle oxygenation decreases while cycling at increasing work rates, when metabolic rate increases (1). In contrast, muscle oxygenation

response to increased cadence at a fixed work rate is less clear (2, 3). The purpose of the two studies presented here was to determine muscle oxygenation at different cycling cadences and work rates.

Sub- T_{vent} study: Cardiopulmonary and metabolic responses to cycling were recorded in 12 participants at work rates of 70% and 90% of the ventilatory threshold (T_{vent}). Participants cycled at cadences of 30, 50, 70, 90, and 110 revolutions per minute (rpm), in a randomised sequence with 110 rpm performed last. Each cadence was sustained for 4 min, interspersed with active recovery periods (4). Skeletal muscle (*vastus lateralis*) oxygenation was measured using near-infrared spectroscopy and tissue saturation index (TSI) determined.

T_{vent} study: A similar protocol was used to measure TSI in 9 participants at cadences of 40, 50, 60, 70, 80 and 90 rpm. Work rate was equal to 100% T_{vent} (5), with cadences tested as above for 4 min each, in a randomised sequence with 90 rpm undertaken last.

Data were analysed with two-way repeated measures ANOVA corrected for multiple comparisons with Bonferroni's post-hoc test.

Cardiopulmonary and metabolic responses were greater at 90% than at 70% T_{vent} , and at 110 rpm compared with lower cadences (Figure 1), but *vastus lateralis* oxygenation changes from baseline were not different between the two work rates or between the five cadences tested (Figure 2). At a work rate equal to 100% T_{vent} , the cardiopulmonary and metabolic responses were again greater at the higher pedalling cadences (Figure 3). At this higher work rate, the TSI reduction from baseline was significantly greater at 90 rpm ($-14 \pm 4\%$) than at 40 rpm ($-12 \pm 3\%$) and 50 rpm ($-12 \pm 3\%$) ($P=0.027$ and 0.017 , respectively) (Figure 4).

Our results demonstrate that elevated pedalling cadence at a work rate equal to 100% T_{vent} can significantly reduce *vastus lateralis* TSI and confirm previous findings (1) for skeletal muscle oxygenation being unaffected by pedalling cadence at workloads below T_{vent} . These findings suggest that skeletal muscle oxygenation is affected by high cadence when cycling at a work rate equal to T_{vent} and may only be a determinant of self-selected cadence during cycling at this exercise intensity.

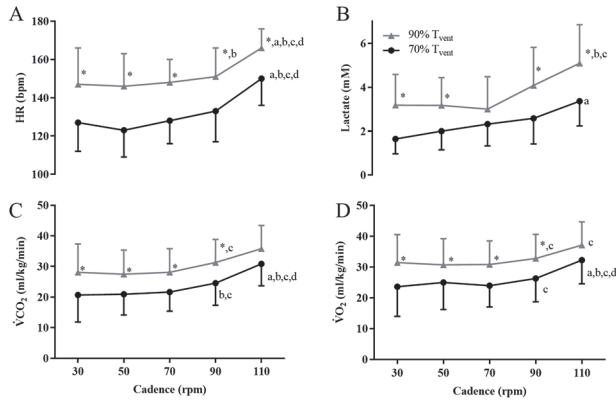


Figure 1. Physiological responses to cycling exercise at different work rates and pedalling cadences.

Mean and standard deviation values: (A) HR (bpm), (B) Lactate (mM), (C) $\dot{V}CO_2$ (ml/kg/min) and (D) $\dot{V}O_2$ (ml/kg/min) for each cadence (30, 50, 70, 90, and 110 rpm) during 70% T_{vent} (black symbols) and 90% T_{vent} (grey symbols) tests. * $P < 0.05$ when compared to the same cadence at 70% T_{vent} ; a, b, c, d: $P < 0.05$ when compared to 30, 50, 70, and 90 rpm respectively, at the same T_{vent} .

HR, heart rate; bpm, beats per minute; rpm: revolutions per minute; T_{vent} , Ventilatory threshold; $\dot{V}CO_2$, carbon dioxide output; $\dot{V}O_2$: oxygen uptake.

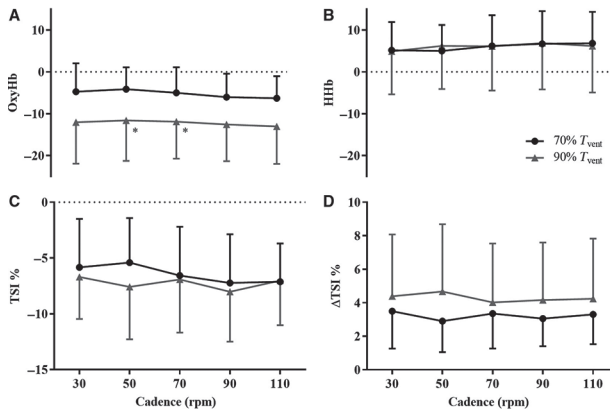


Figure 2. Indices of skeletal muscle oxygenation at different work rates and pedalling cadences.

Mean and standard deviation values: (A) OxyHb, (B) HHb, (C) TSI, (D) ΔTSI for each cadence (30, 50, 70, 90, and 110 rpm) during the 70% T_{vent} (black symbols) and 90% T_{vent} (grey symbols) tests. TSI changes from baseline and ΔTSI are presented as percentage values. * $P < 0.05$ when compared to the same cadence at 70% T_{vent} .

TSI, Tissue Saturation Index; ΔTSI , amplitude of TSI cyclical oscillation within pedal revolution; Hb, Haemoglobin; OxyHb, Oxygenated Hb; HHb, Deoxygenated Hb; T_{vent} , Ventilatory threshold; rpm, revolutions per min.

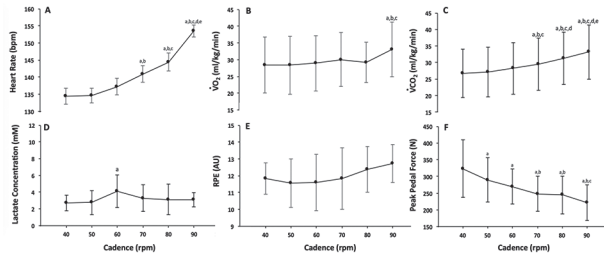


Figure 3. Physiological responses to cycling exercise at different pedalling cadences at 100% T_{vent} . Mean and standard deviation values: (A) Heart Rate (bpm), (B) VO_2 (ml/kg/min), (C) VCO_2 (ml/kg/min), (D) Lactate concentration (mM), (E) RPE and (F) Peak pedal force (N) for each cadence (40, 50, 60, 70, 80 and 90 rpm) performed at 100% T_{vent} . a, b, c, d, e: $P < 0.05$ when compared to 40, 50, 60, 70 and 80 rpm respectively.

RPE, Rate of perceived exertion; N, Newtons. Other abbreviations as in Fig. 1.

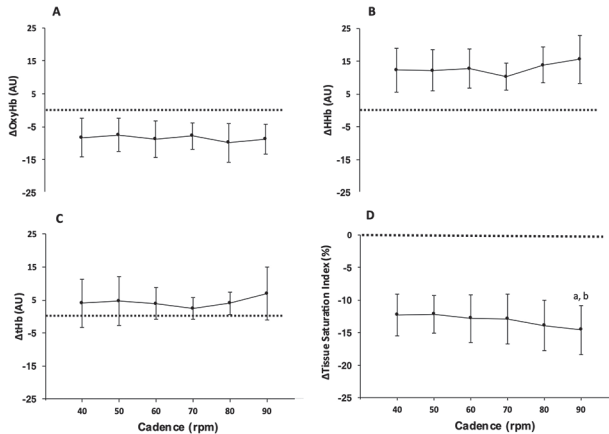


Figure 4. Indices of skeletal muscle oxygenation at different pedalling cadences at 100% T_{vent} .

Mean and standard deviation values: (A) OxyHb, (B) HHb, (C) tHb and (D) TSI for each cadence (40, 50, 60, 70, 80 and 90 rpm) performed at 100% T_{vent} . All data are presented as changes from resting values. TSI is presented as percentage values; OxyHb, HHb and tHb are presented in arbitrary units. a, b: $P < 0.05$ when compared to 40 and 50 rpm respectively, at the same T_{vent} .

AU, Arbitrary units; Δ , change from baseline; tHb, Total haemoglobin. Other abbreviations as in Fig. 2.

Takaishi T *et al.* (2002). *Med Sci Sports Exerc* 34, 520–8

Skovereng K *et al.* (2017). *PLoS ONE* 12, e0169573

Kounalakis SN and Geladas ND. (2012). *Appl Physiol Nutr Metab* 37, 407-417

Shastri L *et al.* (2019). *Physiol Rep*, 7(3), e13963

Formenti F *et al.* (2019). *Intl J Sports Med* doi.org/10.1055/a-0835-6286

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC107

Healthy pregnancy does not exaggerate the cardiac response to submaximal exercise

V.L. Meah^{2,1}, R.E. Shave^{2,3}, K. Backx², J.R. Cockcroft⁴ and E.J. Stöhr^{2,4}

¹Faculty of Kinesiology, Sport and Recreation, University of Alberta, Edmonton, AB, Canada, ²School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, UK, ³Health and Exercise Sciences, University of British Columbia, Kelowna, BC, Canada and ⁴Department of Medicine, Division of Cardiology, Columbia University Irving Medical Center, New York, NY, USA

Pregnancy results in profound adaptation to maternal cardiovascular demand as reflected by greater left ventricular (LV) size and cardiac output (Q) at rest. However, it is not known whether structural cardiac adaptations alter the LV twist mechanics that underpin normal function, and if the changes in resting structure and function influence the maternal cardiac responses to exercise. The aim of this study was to examine the interplay between structural adaptation and functional responses to submaximal exercise in healthy pregnancy. We hypothesised that pregnant women would have an increased LV twist at rest and, exaggerated cardiac responses to exercise due to the elevated demands of pregnancy. Ethical approval was obtained from Cardiff Metropolitan University Ethics Committee. Healthy non-pregnant women ($n=18$), pregnant women ($n=14$, 22-26 weeks of singleton pregnancy) and postpartum women ($n=13$, 12-16 weeks after birth) provided informed consent to participate in the study. Women underwent standardised assessments of global cardiovascular function (including Q [allometrically scaled], LV mass and end-systolic wall stress [ESWS]) and LV twist (speckle tracking echocardiography) at rest and during cycling exercise at 25 and 50% peak power output. Significant differences ($P<0.05$) were determined for between-subjects (group), within-subjects (exercise) and group*exercise interaction using a general linear model. The relationship between Q and LV twist at rest and during exercise was identified using a second order polynomial equation of the mean group data per condition. At rest, pregnant women had significantly larger Q (data presented as mean $\pm$ SD; non-pregnant: 1.2 ± 0.2 , postpartum: 1.3 ± 0.3 , pregnant: 1.9 ± 0.3 L.min⁻¹/m^{1.83}, $P=0.01$) but similar LV mass (non-pregnant: 33 ± 6 , postpartum: 33 ± 6 , pregnant: 35 ± 5 g/m^{2.7}, $P=0.56$), ESWS (non-pregnant: 73 ± 14 , postpartum: 75 ± 14 , pregnant: 67 ± 15 kilodynes.cm², $P=0.23$) and LV twist (non-pregnant: 13.5 ± 4.0 , postpartum: 15.6 ± 4.6 , pregnant: $16.5\pm6.0^\circ$, $P=0.21$) compared to non-pregnant and postpartum women. In contrast to our hypothesis, pregnant women did not have exaggerated cardiac responses to cycling exercise at 25 and 50% peak power output (LV twist: pregnant: 22 ± 7 and 25 ± 12 , non-pregnant: 20 ± 6 and 24 ± 7 , postpartum: 19 ± 8 and $24\pm8^\circ$, $P=0.50$; Q: pregnant: 2.8 ± 0.4 and 3.4 ± 0.7 , non-pregnant: 2.4 ± 0.3 and 3.0 ± 0.3 , postpartum: 2.3 ± 0.4

and $3.0 \pm 0.5 \text{ L} \cdot \text{min}^{-1} / \text{m}^{1.83}$, $P=0.96$, see Figure 1). These data support that healthy pregnancy-induced structural remodelling does not result in increased cardiac stress, as evidenced by no differences in ESWS or LV twist (a parameter related to intra-myocardial stress distribution). Additionally, despite higher Q at rest, the cardiac output response to exercise during healthy pregnancy is proportionate to the level of exertion, similar to non-pregnant women. These findings support that submaximal exercise does not place undue stress on a healthy pregnant woman's heart.

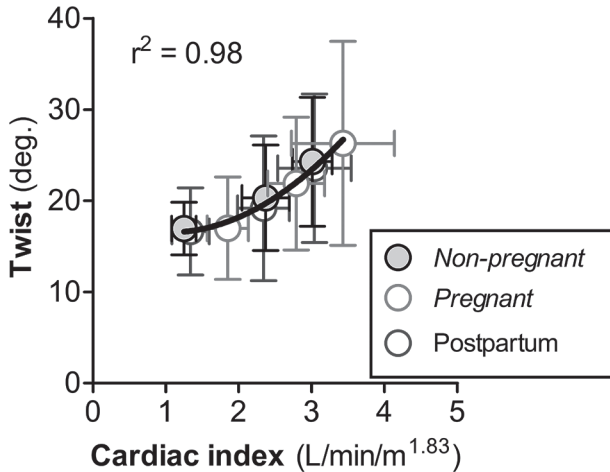


Figure 1. At rest and during submaximal exercise, cardiac output (allometrically scaled to height) was consistently higher in pregnant women compared to non-pregnant and postpartum women, but the relationship with left ventricular twist (a parameter related to intra-myocardial stress distribution) was maintained across all groups.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC108

Synchronization of blood pressure and heart rate during cycling with various intensities

E. Orlova, O. Tarasova, O. Vinogradova and A. Borovik

Exercise physiology, Institute of Biomedical Problems of the Russian Academy of Sciences, Moscow, Russian Federation

OBJECTIVES: It is long established that baroreflex control of cardiovascular system is modified during physical exercise. However, the effect of exercise intensity on baroreflex activity and time-dependence of the transient processes in baroreflex control are poorly understood. The aim of this study was to study the intensity-dependence and dynamic characteristics of baroreflex activity changes during exercise.

For evaluation of baroreflex activity we used phase synchronization of blood pressure and heart rate.

METHODS: 10 young healthy men performed a 15-minute bicycle exercise twice: with an intensity of 60% and 80% of anaerobic threshold (AT). The exercises were preceded by a 15-min rest period. To determine AT the volunteers performed bicycle ramp test before the experiment. Blood pressure and ECG were continuously recorded during the experiment, then phase synchronization index (PSI) was calculated off-line as described elsewhere (Borovik et al, 2014). The dynamics of baroreflex activity changes was studied for 8 subjects who performed the cycling exercise with sine-like intensity modulation (mean value 50% AT, amplitude 30% AT, frequency changed from 1 to 10 mHz), PSI were then calculated in successive 4-min episodes.

RESULTS: Mean value of PSI in the frequency range of baroreflex waves (near 0.1 Hz) tended to be lower during light exercise (0.15 ± 0.04) and was significantly reduced during heavy exercise (0.11 ± 0.04) compared to PSI value at rest (0.19 ± 0.07). The experiments with sine-like intensity pattern showed that PSI changes with alternating power are rather slow – PSI modulation disappeared at 2 mHz, whereas blood pressure and heart rate modulations were clearly seen at this frequency and higher.

CONCLUSION: The phase synchronization of blood pressure and heart rate during exercise might be masked by modulating effect of the intensity-dependent muscle pressor reflex upon baroreflex activity. Exercise intensity-induced PSI changes develop slowly, during several tens of seconds.

The work is performed according to the Plan for Fundamental research of SRC RF Institute of Biomedical Problems RAS and with partial financial support of the RFBR (N17-04-01943).

Borovik A.S., Kuznetsov, S.Y., Vinogradova, O.L. Phase synchronization of arterial pressure and heart rate as a measure of baroreflex activity. IEEE Xplore Digital Library, 2014, p. 217-218.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC109

A Combined Approach of Vitamin D Supplementation and a Physical Activity Intervention in a CVD and Diabetes ‘at risk’ UAE cohort

S. Dalibalta¹ and G. Davison²

¹American University of Sharjah, Sharjah, United Arab Emirates and ²university of ulster, Jordanstown, UK

In the United Arab Emirates (UAE), low nutritional intake of vitamin D and inadequate exposure to sunlight have resulted in notable deficiencies in blood vitamin D concentrations, which have been implicated in diabetes, cardiovascular disease (CVD), and cancers. There are also notable associations between vitamin D

deficiency and reduced exercise tolerance. The aim of this study was to determine how vitamin D status may be correlated with physical inactivity and other disease risk factors in a young UAE population.

Upon ethical approval, 35 male and female students, with an average age of 20 years, participated in this study. Primary data was obtained on vitamin D status, cardiorespiratory fitness, body composition, and blood profiles of participants. A cohort of the vitamin D deficient individuals were supplemented with vitamin D3 (1000IU). All students participated in an 8-week exercise intervention program, where they engaged in moderate-high intensity exercise at least 3 times a week for 60 minutes. Physiological and biochemical profiling was obtained post-intervention as well. Accordingly, students were grouped based on whether they were vitamin D deficient/insufficient and not supplemented (Group A, n=15), vitamin D deficient/insufficient and supplemented (Group B, n=18), or vitamin D sufficient and not supplemented (Group C, n=2).

Results show that this cohort exhibited clear risk factors for CVD as well as distinct deficiencies/insufficiencies in blood vitamin D3 levels. The cardiorespiratory fitness of all groups was generally poor, as determined using a beep test, with average predicted VO_2 max values of 28.3 ± 5 ml/kg/min. Nearly 38% of the overall cohort were overweight/obese ($\text{BMI} \geq 25$), and 41% had systolic blood pressure values over 120 mmHg. The average vitamin D3 levels of Group A, B and C were 19, 13.1, and 34.8 ng/mL respectively. Post intervention, there was an increase in the VO_2 max of all groups to 31 ± 5.8 ml/kg/min showing a change from poor to average cardiorespiratory fitness, although there were no statistical differences between groups, pre-and post-intervention. High blood pressure also dropped from 41% of participants to 27%. There was a significant increase in vitamin D3 levels in group B upon supplementation ($p=0.001$). Although a larger sample set is needed, preliminary data demonstrates that an 8-week intervention can have a positive change on the cardiorespiratory health and fitness in this young population although increasing vitamin D levels does not appear to play a role in markedly enhancing their cardiorespiratory fitness.

In conclusion, a combined approach of vitamin D supplementation and a physical activity intervention was used to investigate changes on CVD and diabetes risk factors after an 8-week intervention. Although improvements in cardiorespiratory fitness and health were notable, vitamin D supplementation did not correlate with greater cardiorespiratory and physiologic changes in this subpopulation.

The authors would like to acknowledge the American University of Sharjah for funding this project (Grant #: FRG17-R-10) and all the students who volunteered to take part in this study.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

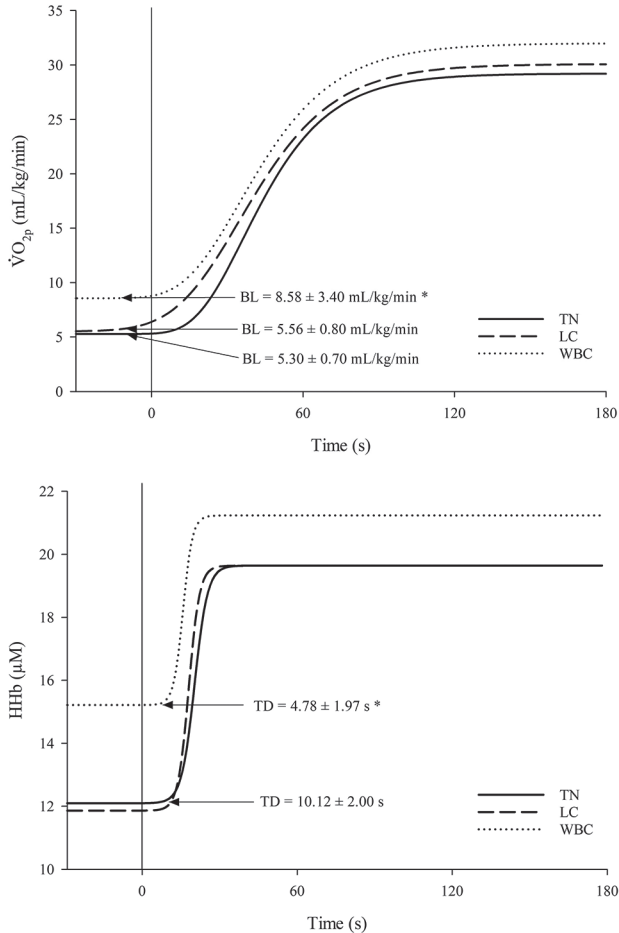
Whole-body cooling results in reduced muscle blood flow but faster muscle oxygen uptake kinetics

N. Beckett-Brown^{1,2}, O. Seresse¹, A. McCue^{1,2}, J. Peltonen³, L. Vilén⁵, D. Marsh⁴, D. MacLean⁴ and D. Gagnon^{1,2}

¹Laboratory of Environmental Exercise Physiology, School of Human Kinetics, Faculty of Health, Laurentian University, Sudbury, ON, Canada, ²Center for Research in Occupational Safety and Health, Laurentian University, Sudbury, ON, Canada, ³Department of Sport and Exercise Medicine, Clinicum, University of Helsinki, Helsinki, Finland, ⁴Northern Ontario School of Medicine, Sudbury, ON, Canada and ⁵University of Jyväskylä, Jyväskylä, Finland

Temperature changes peripheral and core tissue dynamics, resulting in profound effects on blood flow and subsequent oxygen transport and uptake (Yanagisawa et al., 2007). Increases in muscle temperature (T_m) suggest limited influences on pulmonary oxygen uptake kinetics (VO_{2p}), while decreases in T_m , instead, have indicated a slower VO_{2p} kinetic response due to reduced oxidative reactions, during low exercise intensity following lower-body cooling (Koga et al., 1997; Shiojiri et al., 1997). We are unaware if these changes occur locally or via systemic cooling, and if this is parallel in muscle oxygen uptake responses (VO_{2m}). This study investigated VO_{2p} and VO_{2m} kinetics in response to core (T_c) and T_m cooling of the vastus lateralis muscle during exercise. Eight males (mean $\pm$ SD; 25 ± 5.5 years, 175 ± 6.9 cm, 76 ± 8.0 kg, 17 ± 3.0 %BF, 53 ± 6.1 mL/kg/min VO_{2max}) performed two rest-to-exercise sessions at 80% of the first ventilatory threshold for three minutes on a cycle ergometer, in the following three conditions: thermoneutral (TN: no change in T_c and T_m), local leg cooling (LC: no change in T_c and T_m decreased by 6°C), and whole-body cooling (WBC: T_c decreased by 1°C and T_m decreased by 6°C). T_m was measured with an intramuscular temperature probe. Cardiorespiratory variables were assessed via metabolic cart. Cardiac output was assessed via impedance cardiography. Near-infrared spectroscopy was used to evaluate concentration changes in oxy/deoxygenated hemoglobin and was combined with venous and arterial occlusions to calculate VO_{2m} and muscle blood flow (Q_m). One-way RM ANOVA were used to assess differences in VO_{2p} , deoxygenated hemoglobin (HHb) and Q kinetics (baseline, amplitude, time delay; TD, and time constant; τ), relative VO_{2p} , VO_{2m} , Q_m , T_c and T_m were assessed at baseline, pre and post exercise with two-way RM ANOVA. Statistical significance was set at $p < 0.05$. Despite lower T_c and T_m , higher baseline values of VO_{2p} in WBC vs. TN ($+3.3 \pm 2.67$ mL O_2 /kg/min) and LC ($+3.0 \pm 2.98$ mL O_2 /kg/min) were observed. VO_{2p} kinetics TD and τ remained similar across all conditions. Relative VO_{2p} in WBC was higher than TN ($+2.7 \pm 1.78$ mL O_2 /kg/min). There were no changes in cardiac output kinetics. A shorter HHb kinetics TD was observed in WBC vs. TN (-5.3 ± 3.32 s), and a decrease in Q_m in WBC vs. TN (-1.7 ± 1.73 mL/min/100mL) and LC (-0.6 ± 1.06 mL/min/100mL). HHb kinetics τ did not change and relative changes in VO_{2m} were not different. These

results indicate faster $\dot{V}O_{2m}$ kinetics, seen by a shorter TD in WBC during moderate intensity exercise. The higher $\dot{V}O_{2p}$ demand in WBC suggests a greater recruitment of muscle mass to compensate for the limited rate of oxidative reactions following cooling, and could explain the lack of differences in kinetics values (Ferretti, 1992). Future investigations should examine the complex role that exercise intensity has on oxidative reactions under systemic and local cooling.



Yanagisawa, O., Homma, T., Okuwaki, T., Shimao, D., & Takahashi, H. (2007). Effects of cooling on human skin and skeletal muscle. *European Journal of Applied Physiology*, 100(6), 737–745.

Koga, S., Shiojiri, T., Kondo, N., & Barstow, T. J. (1997). Effect of increased muscle temperature on oxygen uptake kinetics during exercise. *Journal of Applied Physiology*, 83(4), 1333–1338.

Shiojiri, T., Shibasaki, M., Aoki, K., Kondo, N., & Koga, S. (1997). Effects of reduced muscle temperature on the oxygen uptake kinetics at the start of exercise. *Acta Physiologica Scandinavica*, 159(4), 327–33.

Ferretti, G. (1992). Cold and Muscle Performance. *International Journal of Sports Medicine*, 13, 185–187.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC111

SPECTRA ANALYSIS OF THE INTERACTIONS BETWEEN N-ACETYLCYSTEINE AND SPERM DNA FOLLOWING CYCLOPHOSPHAMIDE EXPOSURE *IN-VITRO*

S.A. Shittu, O.B. Owofeye and Y. Raji

Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Cyclophosphamide used in chemotherapy of cancerous cells was reported to threaten DNA integrity of normal cells like spermatozoa via its ability to generate free radicals. Pre-treatment with antioxidants like N-acetylcysteine was therefore found useful as an adjunct drug against cyclophosphamide-induced cardiac failure and sperm toxicity without interfering with its chemotherapeutic efficacy. In an earlier report from our laboratory, N-acetylcysteine enhanced sperm Histone-Protamine replacement and protected sperm DNA integrity in cyclophosphamide treated male Wistar rats. This suggests a possible interaction between sperm DNA and N-acetylcysteine, however, the nature of the interaction is yet to be elucidated. This study was therefore designed to investigate the molecular interaction between N-acetylcysteine and DNA following cyclophosphamide exposure *in-vitro*. Herring sperm DNA (1.38×10^{-3} mol/L), cyclophosphamide (0.6×10^{-3} mol/L) and N-acetylcysteine (3.58×10^{-3} mol/L) were prepared freshly and their absorbances were recorded as A_{DNA} , A_{CYCLO} , and A_{NAC} respectively at 260nm. The DNA was then incubated with either cyclophosphamide or N-acetylcysteine and the absorbances were recorded as A_{DCYCLO} or A_{DNAC} respectively. Chromicity change estimated by comparing the sum of A_{DNA} and A_{CYCLO} or A_{NAC} with A_{DCYCLO} or A_{DNAC} respectively were used as indices of molecular interaction. $A_{DNA+CYCLO \text{ OR } NAC}$ greater than or less than $A_{DNA} + A_{CYCLO \text{ OR } NAC}$ were considered hyperchromic due to DNA stabilization or hypochromic effects due to DNA fragmentation respectively while unchanged chromicity was considered no molecular interaction. $DCYCLO$ and $DNAC$ were further incubated with NAC and $CYCLO$ respectively and recorded as $A_{DCYCLO+NAC}$ and $A_{DNAC+CYCLO}$. The sum of $A_{DCYCLO} + A_{NAC}$ or $A_{DNAC} + A_{CYCLO}$ was compared with $A_{DCYCLO+NAC}$ or $A_{DNAC+CYCLO}$ respectively to assess competitive interaction between cyclophosphamide and N-acetylcysteine on DNA. All procedures were repeated 7 times. Data were presented as mean $\pm$ SEM and compared by ANOVA at $p \leq 0.05$. Hypochromic effect was observed when cyclophosphamide was incubated with DNA alone ($A_{CYCLO} = 32.44 \times 10^{-3} \pm 0.09 \times 10^{-3}$; $A_{DNA} = 374.03 \times 10^{-3} \pm 3.10 \times 10^{-3}$;

$A_{\text{DCYCLO}} = 384.07 \times 10^{-3} \pm 19.02 \times 10^{-3}$) but there was no effect when DNA was pre-incubated with N-acetylcysteine ($A_{\text{DNAC+CYCLO}} = 412.37 \times 10^{-3} \pm 18.07 \times 10^{-3}$). On the other hand, there was hyperchromic effect when N-acetylcysteine was incubated with DNA alone and when the DNA was pre-incubated with cyclophosphamide ($A_{\text{NAC}} = -117.14 \times 10^{-3}$; $A_{\text{DNA}} = 374.03 \times 10^{-3} \pm 3.10 \times 10^{-3}$; $A_{\text{DNAC}} = 457.93 \times 10^{-3} \pm 19.65 \times 10^{-3}$ and $A_{\text{DCYCLO+NAC}} = 457.77 \times 10^{-3} \pm 19.02 \times 10^{-3}$).

It was therefore concluded that N-acetylcysteine may proffer a DNA structural stability before cyclophosphamide administration while it could ameliorate DNA damage after cyclophosphamide administration.

Codrington A *et al.* (2004). *J Androl.* 25:354-62. PMID: 15064312 DOI: 10.1002/j.1939-4640.2004.tb02800.x

Shittu SA *et al.* (2019). *JBRA Assisted Reproduction.* PMID:30633472 DOI:10.5935/1518-0557.20180079

Sirajuddin M *et al.* (2013). *Journal of Photochemistry and Photobiology B: Biology* 124, 1-19.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC112

The impact of acute exercise on microRNAs associated with inflammation in healthy subjects: a pilot study

C. Balchin¹, R. Peters¹, N. Wood¹, D.L. Johnson², O. Wilson¹ and A. Stavropoulos-Kalinoglou¹

¹*Carnegie School of Sport, Leeds Beckett University, Leeds, UK and* ²*School of Clinical and Applied Sciences, Leeds Beckett University, Leeds, UK*

Introduction: MicroRNA's (miRNAs) are single stranded RNAs, which negatively regulate gene expression by repressing mRNA translation (Churov, Oleinik and Knip, 2015). They are essential intracellular mediators of inflammation in exercise adaptation (Davidson-Moncada, Papavasiliou and Tam, 2010) and their expression is inherent in the inflammatory post-acute exercise response. However, the effects of exercise stimuli of variable type and intensity on circulating miRNA expression has not yet been established. Consequently, this study will assess the impact of acute aerobic and resistance exercise on miRNAs associated with inflammation (miRNA-146a, miRNA-155 and miRNA-222). **Methods:** Following institutional ethical approval, 10 healthy recreationally active males (Age=24±3 years; BMI =25.5±2.8kg/m², mean±sd) volunteered for the study. They were assessed for maximal aerobic capacity (VO₂max) and maximal strength at leg press (6-10 repetition maximum (RM) sub-maximal protocol). Thereafter they completed 1 aerobic exercise trial: 30 minutes of sub-maximal cycling at a workload equivalent to 70% VO₂max, plus 2 lower body resistance exercise trials: 3 sets of repetitions to failure at 30%1RM and 3 sets of 10 repetitions at 70%1RM. Exercise trials were randomised and separated by a washout period of 3-7 days. Venous blood samples were collected pre- and

immediately post- exercise. Real-time quantitative polymerase chain reaction (RT-qPCR) analyses were performed to investigate acute changes in circulating miRNAs associated with inflammation, log2fold expression was then calculated from the RT-qPCR data. Results: Following aerobic and 30%1RM resistance exercise, miRNA-146a increased (1.52 ± 3.64 and 0.07 ± 0.56 respectively) whilst levels decreased post 70%1RM resistance exercise (-0.46 ± 0.69). Both MiRNA-155 and miRNA-222 levels increased in all trials (aerobic= 0.55 ± 0.46 , 30%1RM= 0.41 ± 0.75 , 70%1RM= 0.52 ± 0.85 ; aerobic= 1.41 ± 3.84 , 30%1RM= 0.41 ± 0.62 , 70%1RM= 0.05 ± 0.44 respectively). One way repeated measures ANOVA revealed no significant differences between the trials (miRNA-146a= $F(1.07, 9.65)=2.289$, $p=0.162$; $n^2=0.203$; miRNA-155= $F(2, 18)=0.111$, $p=0.896$; $n^2=0.012$; miRNA-222= $F(1.07, 9.62)=0.981$, $p=0.353$; $n^2=0.098$), whilst a large effect size was only identified between 30%1RM and 70%1RM in miRNA-146a ($d=0.85$). Conclusion: Our findings suggest that acute aerobic and resistance exercise induces small changes in miRNA expression. Both aerobic and 30%1RM resistance exercise resulted in increased miRNA expression; however, the magnitude of change was minimal. The major limitation in this study was the small sample size, which may have impacted our findings. Nevertheless, the impact of changes in miRNA expression is not known and longitudinal studies are required to examine the role of miRNA in mediating inflammation in response to varying exercise modes and intensities.

Churov, A., Oleinik, E., and Knip, M. (2015) MicroRNAs in rheumatoid arthritis: Altered expression and diagnostic potential. *Autoimmunity Reviews*, 14 (11) July, pp.1029-37.

Davidson-Moncada, J., Papavasiliou, F., and Tam, W. (2010) MicroRNAs of the immune system: roles in inflammation and cancer. *Ann N Y Acad Sci*, 1183, pp.183-94.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC113

A 'Combined Oral Stable Isotope Assessment of Muscle' (COSIAM) approach to simultaneously quantify muscle mass and protein turnover: implications for ageing

J. Cegielski, D. Wilkinson, M.S. Brook, B. Catherine, B. Phillips, J. Gladman, K. Smith and P.J. Atherton

MRC/ARUK Centre for Musculoskeletal Ageing Research & NIHR Nottingham BRC, School of Medicine, University of Nottingham, Derby, UK

Introduction: With increased lifespan associating with age-related muscle loss (sarcopenia), optimising approaches for quantifying skeletal muscle 'health biomarkers' (i.e. muscle mass, muscle protein synthesis (MPS) and breakdown (MPB)) remains a major need. Further, any such methods must be broadly applicable, minimally-invasive, robust and cost-effective if they are to be used for comprehending, and developing treatments relating to mitigating age-related

loss of muscle mass. Currently, technical approaches for measurement of MPS/MPB are time-limited, require the infusion of expensive sterile isotopically labelled tracers, and involve blood and tissue sampling under sterile conditions. Similarly, muscle mass imaging methods (e.g. MRI, CT, DXA), while minimally-invasive, are costly and not readily accessible. Aim: First, to develop a combinatorial approach to concurrently quantify muscle mass and muscle protein turnover (i.e. MPS and MPB) using the stable-isotope tracers D₃-creatine (D₃-Cr)¹, deuterium oxide (D₂O)², and D₃-3-methylhistidine (D₃-3MH)³, respectively; second, to uncover novel relationships between skeletal muscle mass/strength and MPS/MPB. Methods: We recruited 10 older men (~70y, BMI: ~25kg.m⁻²) into a 4-day study, with a DXA and D₃-Cr being orally consumed (dissolved in D₂O) on day 1. D₃-3MH was provided (single tube, dissolved in water) to be taken home and consumed on day 3. From timed urine, saliva, blood samples and one muscle biopsy (m. vastus lateralis, VL) on day 4, we determined muscle mass, MPS and MPB via mass spectrometry. Additionally, we assessed leg power (Nottingham Rig), strength (1-RM) and handgrip strength (dynamometry). Results: D₃-Cr derived muscle mass was positively correlated to appendicular fat-free mass (AFFM) estimated by DXA (r=0.69, P=0.013), leg strength (D₃-Cr: r=0.64, P=0.0234) and leg power (D₃-Cr: r=0.67, P=0.017). There were no correlations between whole-body muscle mass and cumulative myofibrillar protein synthesis (FSR; representative of VL MPS) measured over the 4-days, or whole-body MPB. However, FSR had a positive trend with muscle mass (D₃-Cr: r=0.52, P=0.12, AFFM: r=0.50, P=0.072). Furthermore, FSR positively correlated with leg power (r=0.56, P=0.0455); and had a significantly negative correlation with handgrip strength (r=-0.74, P=0.007). Conclusions: We demonstrate the efficacy of COSIAM for concurrent measurement of MPS, MPB and muscle mass in humans. The COSIAM approach is minimally-invasive and has broad applicability to wider clinical, frailty and care home studies. Moreover, COSIAM uncovered novel relationships linking cumulative deficits in MPS to muscle function and potentially age-related muscle loss. A larger cohort will be required to investigate these relationships further.

Clark R V, Walker AC, O'Connor-Semmes RL, et al. Total body skeletal muscle mass: estimation by creatine (methyl-d3) dilution in humans. *J Appl Physiol*. 2014;116(12):1605-1613. doi:10.1152/japplphysiol.00045.2014

Wilkinson DJ, Franchi M V, Brook MS, et al. A validation of the application of D2O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *Am J Physiol Endocrinol Metab*. 2014;306(5):E571-9. doi:10.1152/ajpendo.00650.2013.

Sheffield-Moore M, Dillon EL, Randolph KM, et al. Isotopic decay of urinary or plasma 3-methylhistidine as a potential biomarker of pathologic skeletal muscle loss. *J Cachexia Sarcopenia Muscle*. 2014;5(1):19-25. doi:10.1007/s13539-013-0117-7

We would like to thank Abbeyfield Foundation, charity number 1167685 (RGS117347) for funding this work and supporting the PhD studentship for J Cegielski. Additionally, the MRC/ARUK (MRC: MR/K00414X/1 and ARUK: 19891) and NIHR Nottingham Biomedical Research Centre for providing centre funding and supporting our work.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC114

Comparative assessment of some cardiovascular risk factors amongst HIV seronegative subjects, HIV seropositive ART naïve subjects and HIV seropositive subjects on ART in Port Harcourt, Nigeria

S.D. Lawson, C.O. Iyama-Boma, I. Tamuno, S.O. Ojeka, A.N. CHUEMERE and D.V. Dapper

Department of Human Physiology, University of Port Harcourt, Port Harcourt, Nigeria, Port Harcourt, Nigeria

Background: On account of reported possible cardiovascular complications of antiretroviral therapy (ART), the present study attempted a comparative assessment of some cardiovascular risk factors amongst a cohort of HIV seronegative subjects, HIV seropositive ART naïve subjects and HIV seropositive subjects on ART attending a tertiary health facility in Port Harcourt, Nigeria.

Materials and Methods: Total of 400 subjects (143 males and 257 females) were recruited into this cross-sectional study. Subjects were separated according to sex and divided into three groups consisting of 100 apparently healthy HIV seronegative subjects who served as control; 100 recently diagnosed HIV seropositive ART naïve subjects and; 200 HIV seropositive subjects on ART. Age, anthropometric parameters and blood pressure indices were determined and venous blood obtained for determination of serum lipid profile, fasting blood sugar and CD₄ cell count using standard procedures and methods. The Atherogenic index of plasma (AIP) and Castelli's risk indices I and II were also determined using appropriate formulae. Statistical significance was determined using analysis of variance; $p < 0.05$ was considered significant.

Results: Amongst the three groups of subjects, significant variations were found in weight and body mass index for both male and female subjects; however, significant variations were observed for blood pressure indices only amongst male subjects. Further, significant variations were observed in the values of total cholesterol and high density lipoprotein concentrations: For male and female subjects, both parameters were highest amongst HIV seropositive ART naïve males and apparently healthy HIV seronegative females respectively. No significant variations were observed in fasting blood glucose among the three group for both male and female subjects. Predictably, the CD₄ cell count were significantly higher amongst apparently healthy HIV seronegative subjects compared to other groups in both males and females. Although AIP showed significant variations amongst male subjects being highest amongst HIV seropositive ART naïve male subjects; no significant variations were observed amongst female subjects. Castelli's risk index (CRI) I and Castelli's risk index (CRI) II were found to show significant variations

amongst both sexes: highest amongst HIV seronegative apparently healthy male subjects and amongst HIV seropositive female Subjects on ART.

Conclusion: Female HIV seropositive subjects on ART apparently are at higher cardiovascular risk due to a significantly increased Castelli's risk indices. The significance of our findings are described in the light of the cardiovascular risk to our subjects. We recommend continuous evaluation of the cardiovascular status of HIV seropositive subjects on antiretroviral therapy (ART) for early intervention and treatment and lipid work up for all newly diagnosed HIV seropositive subjects prior to commencement of ART.

Dapper V, Emem-Chioma P, & Didia B (2008). *Turk J Haematol* 25, 181-186.

Niroumand S, Khajedaluae M, Khadem-Rezaian M, Abrishami M, Juya M, Khodae G, & Dadgarmoghaddam (2015) M. *Med J Islam Repub Iran* 29, 1-9.

Adedokun AK, Olisekodiaka MJ, Adeyeye DA, Muhibi AM, Ojokuku OH, Adepeju AA, et al., *Int J Clin Trials Case Stud.* (2017) 2, 8-15.

Yusuf R, Sambo AI, Mohammed MH & Abdulaziz H. (2014). *Sub-Saharan Afr J Med* 1, 31-35

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC115

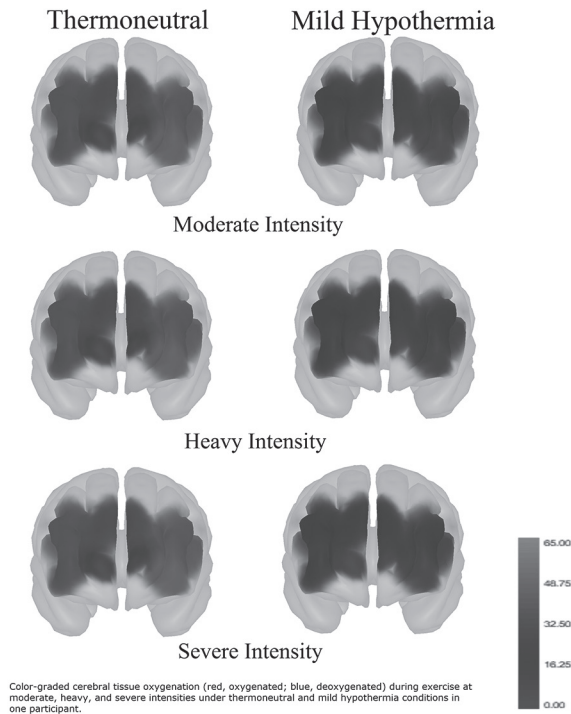
Effects of mild whole-body hypothermia on prefrontal cortex cerebral oxygenation during cycling at moderate, heavy, and severe intensities

D. Gagnon^{1,2}, N. Barclay^{1,2} and S. Munten¹

¹Human Kinetics, Laurentian University, Sudbury, ON, Canada and ²Center for Research in Occupational Safety and Health, Laurentian University, Sudbury, ON, Canada

Mild hypothermia lowers cerebral oxygenation, via lower cerebral blood flow or oxygen consumption, and leads to impaired cognitive functions (Minett et al. 2014; Ferguson et al. 2018). Exercise, however, is known to increase cerebral oxygenation up to heavy intensities before it drops at higher workload (Rooks et al. 2010). Whether the increase in systemic blood flow during exercise, at higher intensities, could be sufficient to re-establish adequate cerebral oxygenation during exercise is unclear. This study examined the combined influences of mild hypothermia and exercise from moderate, to heavy and severe intensities on cerebral oxygenation in healthy adults. Six participants (21 ± 1 yrs, 75 ± 13 kg, 177 ± 10 cm, 21 ± 5 %BF) completed six randomized 5-min cycling sessions as follows: 1) moderate intensity (70% of VT), 2) heavy intensity (40% of the difference between VT and VO₂max), and 3) severe intensity (80% of the difference between VT and VO₂max), each performed in thermoneutral (TN) (22°C) and cold (CO) (0°C) environmental conditions. The CO condition was initiated following a 0.5°C drop in core temperature to induce mild hypothermia. Each exercise sessions were conducted with a minimum of 20 min or rest. Measures of prefrontal cerebral oxygenation, via changes in oxy-deoxygenated haemoglobin difference (Δ Hbdiff), was assessed with near-

infrared spectroscopy from 6 emitter-detector pairs spread evenly across the right and left prefrontal areas of the head. Heart rate (HR), cardiac output (Q), stroke volume (SV) responses were collected via intrathoracic bioimpedance. Two-way RM ANOVA were used (exercise intensities and environments) to determine differences with a level of significance set at $p < 0.05$. SV and Q were not influenced by temperature while HR was lower in CO (151 ± 3 beats/min) compared to TN (157 ± 4) across exercise conditions ($p < 0.05$). Hbdiff was consistently lower during CO vs. TN in moderate (-3.9 ± 4.9 vs. 3.6 ± 6.0 μM), heavy (-7.0 ± 5.6 vs. 3.5 ± 5.3 μM), and severe (-4.9 ± 7.0 vs. 4.1 ± 6.1 μM) ($p < 0.05$). In conclusion, while mild hypothermia reduces cerebral oxygenation, exercising, at any intensities, did not re-establish tissue oxygenation. The implications of the results suggest that the systemic increase in blood flow, expected at higher exercise intensities, was inconsequential to the lower hypothermia-induced cerebral metabolic demand and oxygenation.



Minett et al. (2014). *Scand. J. Med. & Sci. Sports* 24(4), 656–666

Ferguson et al. (2018). *J. Appl. Physiol.* 125(2), 479–485

Rooks et al. (2010). *Progress in Neurobiol.* 92(2), 134–150

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC116

The acute effect of ischemic preconditioning on cycling performance

Z. Smite^{1,2} and L. Plakane^{1,3}

¹Faculty of Biology, Department of Human and Animal Physiology, University of Latvia, Riga, Latvia, ²Department of Anatomy, Latvian Academy of Sport Education, Riga, Latvia and ³Research Institute of Cardiology and Regenerative Medicine, University of Latvia, Riga, Latvia

Ischemic preconditioning (IPC) is an intervention where brief, repeated periods of limb ischemia are followed by reperfusion. It has been shown that IPC can protect the heart, skeletal muscle and endothelial cells from ischemia-reperfusion injury and improve their functions during stress situation [1, 2, 3]. As during high-intensity exercise local tissue hypoxia and metabolite accumulation are induced, high-intensity exercise partially can be considered as a form of ischemic stress [4]. There are studies that have shown improvements in exercise performance after acute IPC but still the results are controversial [5]. The aim of this study was to examine the impact of IPC on cycling performance during an incremental exercise test.

Ten healthy individuals (5♂, 5♀; 29 ± 5 years, 175 ± 8 cm, 76 ± 11 kg; values are means ± SD) on separate occasions did incremental cycling tests. Cycling test started at the power output of 50W and after every third minute power was increased by 30W until exhaustion. One of the tests was perceived by IPC that consisted of 3 cycles of 5 min ischemia followed by 5 min of reperfusion. Ischemia was induced 5 min before an exercise on both thighs with cuffs inflated at 200 mmHg pressure. Arterial blood pressure was measured before and directly after IPC. Cardiorespiratory parameters continuously were measured through the incremental exercise stress test. For aerobic and anaerobic threshold determination three methods were used (V-Slope, ventilation vs workload, EqO₂ and EqCO₂ vs workload). All values in results are represented as mean ± SE. To evaluate the difference between conditions paired samples t-test was used.

IPC did not affect resting arterial blood pressure ($p > 0,05$). Before and after IPC systolic blood pressure was 121,2 ± 4,1 mmHg and 121,6 ± 4,6 mmHg; diastolic blood pressure 74,5 ± 3,0 mmHg and 74,6 ± 3,3 mmHg, respectively. There was no difference between conditions at which intensity aerobic and anaerobic threshold was reached ($p > 0,05$). Aerobic and anaerobic threshold in control condition (CON) was reached at VO₂/kg 27,1 ± 1,4 ml/kg/min and 37,6 ± 1,9 ml/kg/min but in IPC condition at 26,8 ± 1,6 ml/kg/min and 37,9 ± 2,0 ml/kg/min, respectively. IPC increased time till exhaustion ($p < 0,05$) (CON 1324,8 ± 61,6 s; IPC 1358,4 ± 62,6 s) that was accompanied by 4,6 ± 1,5 % improvements in VO₂peak (CON 39,8 ± 2,0 ml/kg/min, IPC 41,6 ± 2,0 ml/kg/min, $p < 0,01$). Arterial blood pressure before, during and after exercise did not differ between condition ($p > 0,05$) except

at maximal exercise intensity where systolic blood pressure was higher in IPC condition ($204,7 \pm 9,5$ mmHg) than in CON ($194,6 \pm 9,1$ mmHg) ($p < 0,01$).

IPC acutely does not affect exercise performance at submaximal intensity but improves aerobic capacity and endurance by modulating cardiovascular response at maximal intensity exercise.

Hausenloy DJ et al. (2007). Lancet 370(9587), 575 – 579.

Andreas M et al. (2011). J Cardiovasc Magn Reson 13, 32.

Loukogeorgakis SP et al. (2005). J Am Coll Cardiol 46(3), 450 – 456.

Marongiu E & Crisafulli A (2014). Curr Cardiol Rev 10(4), 336 – 348.

Caru M et al. (2019). J Sport Health Sci In press, <https://doi.org/10.1016/j.jshs.2019.01.008>

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC117

Overexpression of major histocompatibility complex I induces mitochondrial dysfunction in human skeletal muscle myoblasts

A. Thoma², T. Akter-Miah², H.L. Bond², G.A. Nye¹, R. Cooper³ and A. Lightfoot²

¹Chester Medical School, University of Chester, Chester, UK, ²Musculoskeletal Science & Sports Medicine Research Centre, Manchester Metropolitan University, Manchester, UK and ³University of Liverpool, Liverpool, UK

Myositis is a rare acquired autoimmune disorder which results in patients experiencing profound muscle weakness, dysfunction and disability. Myositis is characterised by inflammatory cell infiltration (CD4+/CD8+ T-cells) into muscle and the presence of an array of circulating myositis-specific and -associated autoantibodies (1). A key diagnostic feature of myositis is the overexpression of major histocompatibility complex (MHC) I upon the surface of muscle fibres. MHC I overexpression persists in the absence of inflammatory cells or overt disease, where patients remain profoundly weak (2). Moreover, genome wide single nucleotide polymorphism association studies have identified the MHC region of chromosome 6 to have the strongest disease association (3). Thus, MHC has been identified as an important pathogenic factor in myositis; however, the mechanisms associated with this are poorly understood. In this study, we examine the impact of overexpressing the MHC I isoform (HLA-A2/K^b) on mitochondrial function and dynamics, as described by mitochondrial structure, in human skeletal muscle cells. Human skeletal muscle myoblasts were transfected with a mammalian HLA-A2/K^b overexpression vector using transit-X2 transfection reagent for 18 hours; transfection efficiency was determined by immunofluorescent staining with an anti-HLA class I antibody. Mitochondrial function was assessed using seahorse extracellular flux analysis. Mitochondrial structure and morphology were observed with the MitoTracker Red probe using fluorescent microscopy and quantified using a NIH

ImageJ-based macro (4). Analysis for statistical significance was determined by ANOVA with Tukey's *post hoc* test, data are expressed as mean $\pm$ SEM, $n=4$. Overexpression of MHC I results in a decline in maximal (Ctrl, 368.4 ± 43.28 vs. MHC I, 209.1 ± 39.64 pmol/min/mg; $p<0.05$), basal (Ctrl, 208.6 ± 39.77 vs. MHC I, 108.6 ± 38.64 pmol/min/mg), and ATP-linked respiration (Ctrl, 93.81 ± 35.7 vs. MHC I, 47.36 ± 35.57 pmol/min/mg), alongside a decline in overall reserve capacity of cells (Ctrl, 231.4 ± 27.03 vs. MHC I, 128.9 ± 58.14 pmol/min/mg). Morphological analysis and quantitation of mitochondria show significantly increased mitochondrial perimeter ($p<0.05$) and decreased mitochondrial solidity ($p<0.05$) in MHC I overexpressing cells, two predictive features of mitochondrial fission (5). Even though elongated mitochondria are present following MHC I overexpression ($p<0.05$), no significant changes are found in mitochondrial interconnectivity, a vital characteristic of mitochondrial fusion events (4). These findings suggest that overexpression of MHC I induces mitochondrial dysfunction by altering mitochondrial bioenergetics and biogenesis. Overall, this study provides an insight into the mechanisms by which MHC I may induce dysfunction in myositic muscle.

Miller FW, Lamb JA, Schmidt J & Nagaraju K (2018). Risk factors and disease mechanisms in myositis. *Nature Reviews Rheumatology* 14(5), 255.

Nagaraju K, Raben N, Loeffler L, Parker T, Rochon PJ, Lee E, *et al.* (2000). Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. *Proceedings of the National Academy of Sciences* 97(16), 9209-9214.

Rothwell S, Cooper RG, Lundberg IE, Miller FW, Gregersen PK, Bowes J, *et al.* (2016). Dense genotyping of immune-related loci in idiopathic inflammatory myopathies confirms HLA alleles as the strongest genetic risk factor and suggests different genetic background for major clinical subgroups. *Annals of the rheumatic diseases* 75(8), 1558-1566.

Dagda RK, Cherra SJ, Kulich SM, Tandon A, Park D. & Chu CT (2009). Loss of PINK1 function promotes mitophagy through effects on oxidative stress and mitochondrial fission. *Journal of Biological Chemistry* 284(20), 13843-13855.

Westrate LM, Drocco JA, Martin KR, Hlavacek WS & MacKeigan JP (2014). Mitochondrial morphological features are associated with fission and fusion events. *PLoS One* 9(4), e95265.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC118

Characterisation of the decline in maximal voluntary isokinetic power during ramp-incremental cycle ergometry exercise

M.M. Baldwin, M.R. Chadwick, B.J. Taylor, K.M. Birch and C. Ferguson

School of Biomedical Sciences, University of Leeds, Leeds, UK

Rapid switching from hyperbolic to isokinetic cycling allows for periodic assessment of exercise-induced fatigue through measurement of the reduction in maximal voluntary isokinetic power (MVIP). During constant-power exercise MVIP is reduced during tests above, but not below, the lactate threshold (LT) (1). During supra-LT

constant-power tests MVIP decreases to a greater extent during very-heavy vs. heavy intensity exercise (1). At the limit of ramp-incremental (RI) cycle ergometry MVIP is reduced; however, the time-course is unknown. RI exercise spans all intensity domains (2); therefore, we hypothesised a progressive decrease in MVIP above LT. Characterising this time-course is likely to provide insight into the mechanisms of intolerance. Therefore, the aim of this study was to characterise the decrease in MVIP throughout a maximal RI test.

Eight healthy participants (mean $\pm$ SD: age 23 ± 2 yr, height 180 ± 8 cm, mass 76 ± 8.5 kg) performed RI exercise ($25 \text{ W} \cdot \text{min}^{-1}$) to the limit of tolerance to determine LT and peak oxygen uptake ($\text{VO}_{2\text{peak}}$). On a separate visit (randomised), participants repeated this maximal RI test and MVIP was measured every 90 s during 6 s of isokinetic cycling at 80rpm (RI-MVIP). MVIP was also measured at baseline and instantaneously at the limit of tolerance, and breath-by-breath pulmonary gas exchange was measured throughout both tests.

Lactate threshold was $1.8 \pm 0.3 \text{ L} \cdot \text{min}^{-1}$. $\text{VO}_{2\text{peak}}$ was not different between RI protocols (3.8 ± 0.4 vs. $3.7 \pm 0.5 \text{ L} \cdot \text{min}^{-1}$; $p > 0.05$). During RI-MVIP, below LT there was no decrease in MVIP compared to baseline ($p > 0.05$). In the subsequent three measures of MVIP made above LT (Post-LT 1, Post-LT 2, Post-LT 3), there was a trend for Post-LT 1 MVIP to decrease compared to baseline (Baseline vs. Post-LT 1: 836 ± 117 vs. $781 \pm 136 \text{ W}$; $p = 0.07$). There was then a progressive decline in MVIP until the limit of tolerance was attained (Post-LT 1 vs. Post-LT 2 vs. Post-LT 3: 781 ± 136 vs. 712 ± 147 vs. $550 \pm 151 \text{ W}$; all $p < 0.05$). At the limit of tolerance, MVIP was greater than the RI peak power (410 ± 116 vs. $285 \pm 24 \text{ W}$; $p = 0.05$).

The absence of a decrease in MVIP below LT is consistent with known MVIP and metabolic responses to moderate-intensity constant-power exercise (1, 3). The progressive decrease in MVIP during RI exercise above LT is consistent with the greater decline in MVIP during very-heavy vs. heavy intensity constant-power exercise (1). The time course of the decline in MVIP above LT resembles that of fatigue-related metabolite accumulation during RI exercise (4). However, the presence of a power reserve (MVIP at intolerance $>$ RI work rate peak) suggests central fatigue is also contributory, although the specific mechanism through which peripheral and central fatigue integrate to induce task intolerance remains unknown.

Cannon DT, White AC, Andriano MF, Kolkhorst FW & Rossiter HB (2011). Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans. *J Physiol* 589, 727–739.

Whipp BJ, Davis JA, Torres F, Wasserman K (1981). A test to determine parameters of aerobic function during exercise. *J Appl Physiol* 50, 217–221.

Cannon DT, Bimson WE, Hampson SA, Bowen TS, Murgatroyd SR, Marwood S, Kemp GJ, Rossiter HB (2014). Skeletal muscle ATP turnover by ^{31}P magnetic resonance spectroscopy during moderate and heavy bilateral knee extension. *J Physiol* 592, 5287–5300.

Cannon DT, Howe FA, Whipp BJ, Ward SA, McIntyre DJ, Ladroue C, Griffiths JR, Kemp GJ, Rossiter HB (2013). Muscle metabolism and activation heterogeneity by combined ^{31}P chemical shift and T2 imaging, and pulmonary O_2 uptake during incremental knee-extensor exercise. *J Appl Physiol* 115, 839–849.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC119

Pharmaceutical endogenous testosterone depletion blunts resistance exercise-induced hypertrophy in younger men via impairing muscle growth programming

N. Gharahdaghi, S. Rudrappa, B. Phillips, M.S. Brook, W.F. Farrash, M. Aziz, I. Idris, P.J. Herrod, T. Sian, D.J. Wilkinson, N.J. Szewczyk, K. Smith and P.J. Atherton

Medical Sciences & Graduate Entry Medicine, University of Nottingham, Derby, UK

The role of endogenous testosterone(T) in mediating hypertrophic responses to resistance exercise training(RET) remains contentious with correlative studies suggesting a limited role¹, and others a more central role². We thus investigated the physiological and mechanistic effects of chemical endogenous T depletion adjuvant to 6-wk of RET on muscle mass, function, myogenic regulatory factors and muscle anabolic signalling, protein synthesis and glycaemic control in younger men. Sixteen non-hypogonadal healthy young men (18-30y, BMI $\leq$ 30kg.m⁻², serum T>230 ng.dl⁻¹) were randomised in a double-blinded fashion to receive either: placebo (P, saline n=8) or the GnRH analogue, Goserelin (Zoladex (Z) (3.6-mg, n=8)) injections before undertaking 6-wks of RET(6-exercises,3-sets,8-10-reps,80% 1-RM). Participants underwent Dual-energy X-ray Absorptiometry, ultrasound of m.vastus lateralis (VL) and Oral Glucose Tolerance Testing; finally, m.vastus biopsies were taken to quantify myogenic gene expression, anabolic pathways and muscle protein synthesis (MPS) and break down rates (MPB).

Provision of Z during RET suppressed endogenous T (Z:45.2 $\pm$ 4, vs. P:323 $\pm$ 30 ng.dl⁻¹, P<0.001). The Z group exhibited blunted whole-body (Z:55 $\pm$ 3 to 56 $\pm$ 3, P=0.6 vs. P:56 $\pm$ 2 to 58 $\pm$ 2kg, P=0.006), appendicular (Z:8.2 $\pm$ 0.3 to 8.4 $\pm$ 0.4, P>0.9 vs. P:7.6 $\pm$ 0.3 to 8.3 $\pm$ 0.3kg.m⁻², P=0.07), and VL (Z:0.01 $\pm$ 0.01 vs. P:6.2 $\pm$ 1.1%, P=0.007) lean mass gains, and composite strength gains (Z:40 $\pm$ 2 vs. P:50 $\pm$ 3%, P=0.03). Reductions in body fat percentage were only evident in the P group (Z:23 $\pm$ 2 to 24 $\pm$ 2%, P=0.2 vs. P:24 $\pm$ 3 to 23 $\pm$ 3%, P=0.04). The P group exhibited augmented gene expression related to T metabolism (e.g. *Androgen Receptor*: 1.8-fold; *HSD17B3*: 2.8-fold, P<0.05); anabolism/myogenesis (e.g. *IGF-1EA* (3.3-fold), *Myogenin* (2.6-fold), *Myf5* (2.6-fold), *c-Met* (2.7-fold), P<0.05) in addition to RNA/DNA (P:0.5 $\pm$ 0.1 to 0.6 $\pm$ 0.1, P=0.0003 vs. Z:0.5 $\pm$ 0.1 to 0.5 $\pm$ 0.1, P=0.3), and RNA/ASP ratio (P:6.5 $\pm$ 0.2 to 8.9 $\pm$ 1.1, P=0.008 vs. Z:5.8 $\pm$ 0.4 to 6.8 $\pm$ 0.5, P>0.9). Also, acute RET-induced phosphorylation of AKT^{ser473} (Z:4.9 $\pm$ 1.6-fold, P=0.7 vs. P:10.1 $\pm$ 5.3-fold, P=0.003) and mTORC1^{ser2448} (Z:2.5 $\pm$ 1.3-fold, P>0.9 vs. P:4.8 $\pm$ 2.1-fold, P=0.03) were attenuated in the Z group. Both MPS (P:2.01 $\pm$ 0.1 vs. Z:1.54 $\pm$ 0.1%.day⁻¹, P=0.01) and MPB rates (P:129.1 $\pm$ 13 vs. Z:93.1 $\pm$ 7.8 g.day⁻¹, P=0.04) were higher in P group, which led to higher net turnover and protein accretion (P:3.9 $\pm$ 1.1 vs. Z:1.2 $\pm$ 1.1 g.day⁻¹, P=0.04). Only the P group enhanced insulin sensitivity (Cederholm: P:14.5 $\pm$ 4.9 vs. Z:2.1 $\pm$ 0.4 mg.L².mmol.L⁻¹.mU⁻¹.min⁻¹, P=0.04).

Chemical T depletion during RET blunted muscle insulin/anabolic signalling, T processing enzyme and pro-myogenic gene expression, perhaps explaining attenuated muscle growth and insulin sensitivity in this group. Thus, decreasing endogenous levels of T coupled to RET attenuates numerous positive adaptations to RET - thereby, demonstrating the, at least, permissive importance of endogenous T in regulating muscle hypertrophy.

West DW, et al. (2009). *Journal of Applied Physiology*, 108(1), 60-67.

Brook MS, et al. (2016). *The Journal of physiology*, 594(24), 7399-7417.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC120

The influence of temperature on localized muscle oxygen uptake, oxygenation, and blood flow during exercise

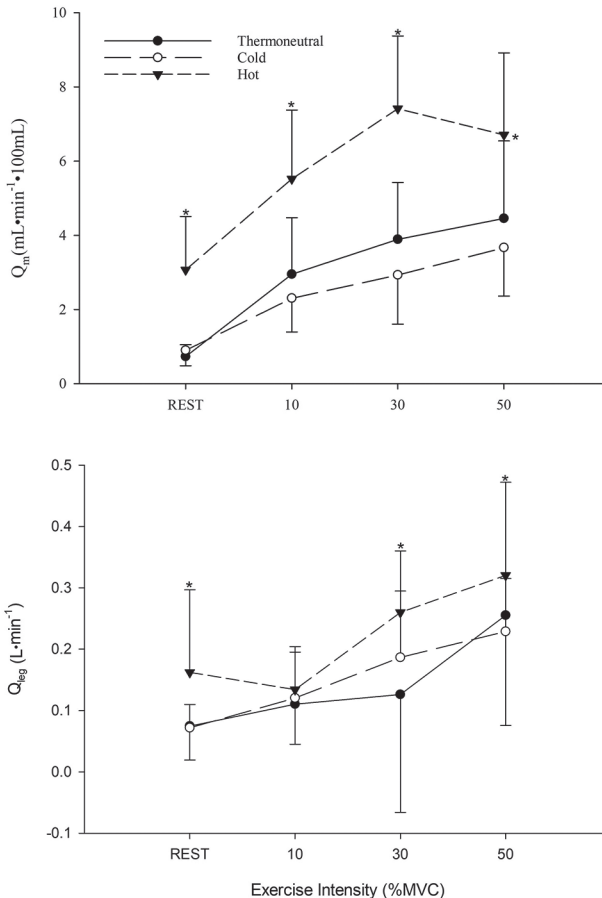
A. McCue¹, J. Peltonen³, N. Beckett-Brown¹, O. Seresse¹, D. Marsh², D. MacLean² and D. Gagnon¹

¹Human Kinetics, Laurentian University, Sudbury, ON, Canada, ²Northern Ontario School of Medicine, Sudbury, ON, Canada and ³Foundation of Sports and Exercise Medicine, University of Helsinki, Helsinki, UK

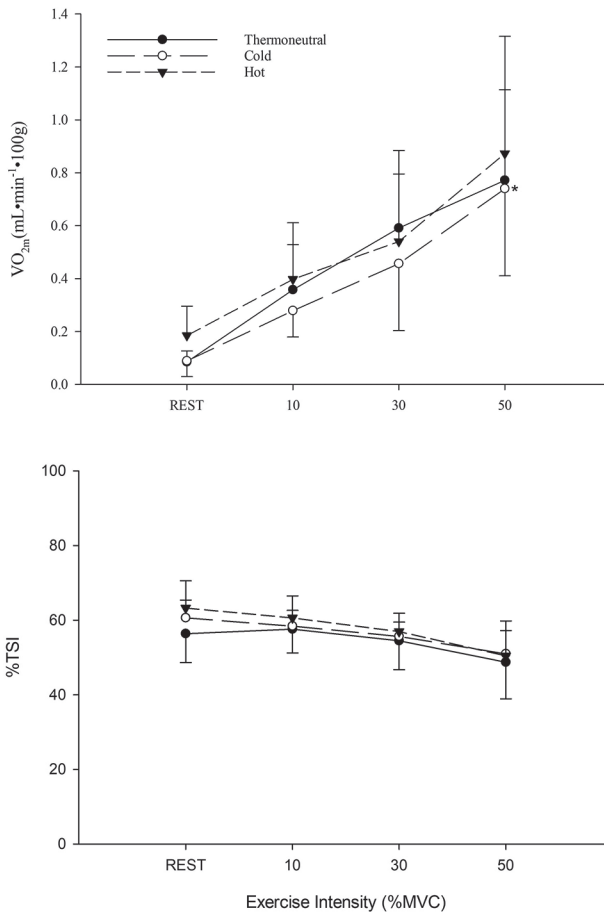
Thermal changes in muscle tissue are known to influence its perfusive and diffusive O₂ capacity (Hom et al, 2004; Yanagisawa et al, 2007; Wakabayashi et al, 2017). Nonetheless, whether a shift in oxygen transport and extraction in skeletal muscle during exercise modulates oxidative pathways, and is reflected in oxygen consumption (VO_{2m}) and tissue oxygenation (%TSI), is unclear. The purpose of this study was to assess the effects of skeletal muscle cooling and heating on femoral artery blood flow (Q_{leg}), muscle blood flow (Q_m), VO_{2m}, and %TSI during single-leg exercises. Eleven healthy males (23 ± 3.1 yrs, 77.6 ± 8.65 kg, 176.2 ± 6.99 cm) participated in a total of nine three-minute single-leg extension bouts on an isokinetic dynamometer set at 60° • sec⁻¹. They each exercised at 10, 30, and 50% of their maximal voluntary contraction (MVC) under the following three thermal conditions: thermoneutral (TN: no change in muscle temperature (T_m)), cold (CO: -3°C in T_m), and hot (HT: +3°C in T_m). T_m was measured using an intramuscular flexible probe. Near-infrared spectroscopy was used to assess %TSI, as well as changes in Q_m and VO_{2m} through venous and arterial occlusion techniques. Q_{leg} was assessed via Doppler ultrasound. Two-way RM ANOVA was used to assess differences in muscle oxidative and hemodynamic responses. Statistical significance was set at p<0.05. Results are reported as mean ± standard error.

Q_{leg} was greater in HT vs. TN and CO at rest only (p>0.05). Q_{leg} was further influenced by exercise intensity (p<0.05) as rest was lower compared to 30%, and 50% MVC (0.103 ± 0.021 vs. 0.191 ± 0.024, and 0.268 ± 0.042 L • min⁻¹; p<0.05). At rest Q_m and VO_{2m} were influenced by exercise intensity (p<0.001) and thermal

conditions ($p < 0.05$). Q_m was higher in HT vs. TN, and CO (5.680 ± 0.488 vs. 3.008 ± 0.35 , and 2.453 ± 0.241 $\text{mL} \cdot \text{min}^{-1} \cdot 100\text{mL}$; $p < 0.001$). Q_m increased with exercise intensity but no differences were observed between 30% and 50% (4.747 ± 0.406 vs. 4.946 ± 0.473 $\text{mL} \cdot \text{min}^{-1} \cdot 100\text{mL}$; $p > 0.05$). VO_{2m} was lower in CO vs. TN, and HT (0.391 ± 0.050 vs. 0.451 ± 0.056 , and 4.99 ± 0.066 $\text{mL} \cdot \text{min}^{-1} \cdot 100\text{g}$; $p < 0.05$). VO_{2m} steadily increased with exercise intensity and was different between all intensities ($p < 0.05$). %TSI was significantly lower at 50% MVC vs. rest, 10%MVC, and 30%MVC (50.068 ± 2.461 vs. 60.104 ± 1.751 , 58.893 ± 1.441 , and 55.715 ± 1.405 %; $p < 0.05$). These results indicate that localized thigh cooling and heating modulate the well established link between Q_{leg} and Q_m at rest and during single-leg exercise. Despite clear influences of cooling and heating on perfusive and diffusive O_2 capacity via changes in Q_m and VO_{2m} , tissue oxygenation was not significantly affected, suggesting intrinsic factors involved in maintaining tissue oxygenation under homeostatic challenges.



Poster Communications



Hom et al. 2004. *J. Thermal Biol.* 29(7-8), 785-789)

Yanagisawa et al. 2007. *Eur. J. Appl. Physiol.* 100(6), 737-745

Wakabayashi et al. 2017. *Int. J. Biometeorol.* 61(7), 1261-1267

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC121

Comparative assessment of some indices of renal function amongst HIV seronegative subjects, HIV seropositive ART naïve subjects and HIV seropositive subjects on ART in Port Harcourt, Nigeria.

C.O. Iyama-Boma, S.D. Lawson, F. Saronee, A.N. CHUEMERE and D.V. Dapper

Department of Human Physiology, University of Port Harcourt, Port Harcourt, Nigeria, Port Harcourt, Nigeria

Study attempts a comparative assessment of some indices of renal function amongst a cohort of apparently healthy HIV seronegative HIV seropositive (antiretroviral) ART naïve and HIV seropositive subjects on ART in Port Harcourt, Nigeria. This is to determine appropriate biomarkers of renal impairment in HIV seropositive subjects in our environment.

400 subjects (143 males and 257 females) were recruited into a cross-sectional prospective study. Subjects were separated according to sex and divided into three groups: 100 apparently healthy HIV seronegative subjects who served as controls; 100 recently diagnosed HIV seropositive ART naïve subjects and; 200 HIV seropositive subjects on ART. Venous blood and spot-urine samples were collected and the following parameters determined using appropriate methods: serum and urine uric acid; serum and urine creatinine; serum and urine phosphate; fasting blood sugar and urine glucose concentration. Total serum protein, C-reactive protein and albumin concentration were also determined in serum while microalbumin, microprotein and macroglobulin were determined in urine. Albumin-Creatinine ratio and Protein-Creatinine ratio were calculated and estimated Glomerular filtration rate determined using the MDRD equation. Statistical significance was determined using analysis of variance; $p < 0.05$ was considered significant.

Urine creatinine, urine micro albumin, urine macroglobulin, urine phosphate, urine uric acid and serum C- reactive protein showed significant variations amongst the various groups being consistently highest amongst HIV seropositive subjects on ART in both sexes; both serum uric acid and urine micro protein were also found to show significant variations amongst the various groups but were however, highest amongst apparently healthy HIV seronegative subjects in both sexes. Serum albumin also showed significant variations in the various groups in both sexes; however, male HIV seronegative subjects and female HIV seropositive ART naïve subjects were found to have the highest values. Serum albumin creatinine ratio, serum protein creatinine ratio and serum phosphate concentration were found to show significant variations only amongst the female groups: serum albumin creatinine ratio was highest amongst female HIV seropositive subjects on ART; serum protein creatinine ratio was highest amongst apparently healthy female HIV seronegative subjects; while serum phosphate was highest amongst female HIV seropositive ART naïve subjects.

Apparently, sex variations exists in the pattern of changes of renal function indices assessed amongst our subjects; with female subjects at a probable higher risk.

Attempt was made to identify possible biomarkers of renal impairment amongst HIV seropositive subjects in our environment. Our results are of import since previous studies from our environment have been relatively scanty.

Foy MC, Estrella MM, Lucas GM, Tahir F, Fine DM, Moore RD, & Atta MG. (2013). *Clin J Am Soc Nephrol*. 8, 1524-1532.

Malipattu SK, Wyatt CM, He JC. (2012). *J AIDS Clin Res. Suppl* 4:001.

Cristelli MP, Trullàs JC, Cofán F, Rico N, Manzardo C, Ambrosioni J, Bedini JL, Moreno A, Diekmann F, Miro JM; CKD-H. Clinical Investigators. (2018). *Braz J Infect Dis*. 22, 193-201.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC122

Central and peripheral contributions to submaximal exercise performance in older adults

S. Jones¹, M.G. Schultz², T. Tillin¹, S. Williams¹, N. Chaturvedi¹ and A.D. Hughes¹

¹University College London, London, UK and ²Menzies Institute for Medical Research, Hobart, TAS, Australia

Background

Functional capacity declines with age leading to disability and increased cost of health and social care.(1) In older adults, factors that influence the ability to sustain sub-maximal exercise (a marker of functional capacity) are not well defined. We aimed to determine the contribution of cardiac function, peripheral desaturation, oxygen extraction and skeletal muscle oxidative capacity to sub-maximal exercise performance in a population-based sample of older adults.

Methods

Participants were older adults (>60 years old) enrolled in the Southall And Brent REvisited (SABRE) study.(2) Sub-maximal exercise performance was assessed as the highest achieved whole-body oxygen uptake (VO_2) during a 6-minute stepper test (6MST). Near Infrared Spectroscopy (NIRS) was used to assess local skeletal muscle oxygen extraction and desaturation at the start and end of exercise and maximum oxidative capacity. Echocardiography was used to measure resting cardiac output (CO) and CO during exercise was estimated by multiplying resting stroke volume by exercise heart rate. Analysis was by multiple linear regression adjusted for confounders: age, sex, ethnicity, diabetes and obesity.

Results

363 participants (71.3 $\pm$ 6.4 years old, male; n=212) undertook the 6MST, skeletal muscle desaturation measurements and echocardiography. Skeletal muscle desaturation and cardiac output were independently associated with whole-body VO_2 (standardized β adjusted for confounders: 0.27, $p<0.001$ & 0.30, $p<0.001$, respectively). Local skeletal muscle oxygen extraction was independently associated with whole-body VO_2 (standardized β adjusted for confounders & cardiac

output: 0.37, $p < 0.001$). Maximum oxidative capacity was not associated with VO_2 (β : 0.01, $p = 0.4$).

Conclusion

Local oxygen extraction and desaturation in skeletal muscle are independent predictors of self-selected exercise performance in older adults. These associations persist with adjustment for cardiac output suggesting local capacity to extract oxygen could be a major limiting factor for self-paced exercise in older adults.

Ferrucci L *et al.* (2016). *J Gerontol. A Biol. Sci. Med. Sci.* 71, 1184–1194.

Tillin T *et al.* (2012). *Int. J. Epi.* 41, 33–42

We acknowledge the participants of the SABRE study who voluntarily gave up their time to undertake this study.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC123

Physical activity confers neuroprotective benefits in young females; focus on improved cerebrovascular reactivity.

T.A. Calverley¹, C. Marley¹, T.S. Owens¹, B.S. Stacey¹, M. Steggall¹, L. Fall¹, L. Venables², H.J. Tsukamoto¹ and D. Bailey¹

¹University of South Wales, Cardiff, UK and ²Cardiff University, Cardiff, UK

The aged population are living longer and more sedentary lives, which in part contributes towards an increased prevalence of stroke and neurodegenerative disease. This is especially apparent in females (1, 2), however the fundamental mechanisms remain unclear. It has previously been demonstrated that lifelong physical activity in males can attenuate the inexorable age-related decline in cerebral perfusion and vasoreactivity (3), though to what extent comparable neuroprotection is conferred among females remains to be established. To force the “adaptive” signal out of the background “biological” noise, the current study examined the potential relationship between cardiorespiratory fitness and cerebral perfusion both at rest and in response to an acute vascular stressor (high-fat meal) known to incur cerebrovascular impairment subsequent to elevated oxidative-nitrosative stress (4). Eight active (21 ± 2 years of age; maximal oxygen consumption ($\text{VO}_{2\text{max}}$) = 40 ± 3 ml/kg/min) and eight inactive (21 ± 2 years of age; $\text{VO}_{2\text{max}}$ = 31 ± 3 ml/kg/min) females participated in the study. On the first visit, $\text{VO}_{2\text{max}}$ was assessed via a standardised incremental exercise test. During the second visit, transcranial Doppler ultrasound and capnography were employed to establish middle cerebral artery blood flow velocity (MCAv) and cerebrovascular reactivity to hypercapnia ($\text{CVR}_{\text{CO}_2\text{HYPER}}$) and hypocapnia ($\text{CVR}_{\text{CO}_2\text{HYPO}}$) (4), which subsequently determined cerebrovasomotor range ($\text{CVR}_{\text{CO}_2\text{RANGE}}$). Measurements were obtained prior to and four hours following consumption of a standardised high-fat meal (1362 kcal; 130 g

of fat) to induce post-prandial lipaemia (PPL) and elicit peak systemic oxidative-nitrosative stress. Comparative data were analysed via a two-way repeated measures analysis of variance and relationships via a Pearson product-moment correlation. The active group exhibited elevated $\text{CVR}_{\text{CO}_2\text{HYPO}}$ and $\text{CVR}_{\text{CO}_2\text{RANGE}}$ ($P < 0.05$ vs. inactive group). Furthermore, a positive relationship between $\text{VO}_{2\text{max}}$ and $\text{CVR}_{\text{CO}_2\text{HYPER}}$ was observed ($r = 0.6$; $P < 0.05$). No between group differences were observed in resting cerebral perfusion and PPL-induced hypoperfusion was also comparable ($P > 0.05$ between groups). Collectively, our findings highlight a selective improvement in $\text{CVR}_{\text{CO}_2\text{HYPO}}$ but not $\text{CVR}_{\text{CO}_2\text{HYPER}}$ implying that the neuro-protection afforded by physical activity appears to be more related to changes in calcium sensitivity and not purely ascribed to vascular NO bioavailability. Given that the ageing female brain is more vulnerable to stroke and neurodegenerative disease in later life (1, 2), it would appear that they may likely suffer more pronounced cerebrovascular impairment during the critical phase of middle-age reinforcing the importance of lifelong physical activity.

1. Compton J, Van Amelsvoort T, Murphy D. HRT and its effect on normal ageing of the brain and dementia. *British journal of clinical pharmacology*. 2001;52(6):647-53.
2. Bushnell CD. Stroke and the female brain. *Nature Reviews Neurology*. 2008;4(1):22.
3. Bailey DM, Marley CJ, Brugniaux JV, Hodson D, New KJ, Ogoh S, et al. Elevated aerobic fitness sustained throughout the adult lifespan is associated with improved cerebral hemodynamics. *Stroke*. 2013;44(11):3235-8.
4. Marley CJ, Hodson D, Brugniaux JV, Fall L, Bailey DM. Post-prandial hyperlipidaemia results in systemic nitrosative stress and impaired cerebrovascular function in the aged. *Clinical Science*. 2017;131(23):2807-12.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC124

Age and gender mediate the small association of MIP and functional capacity in healthy active elderly

A. Roldán¹, A. Cordellat¹, P. Monteagudo¹, N. Blasco-Lafarga², M. Gomez-Cabrera³ and C. Blasco-Lafarga¹

¹Physical Education and Sports Department, University of Valencia, Valencia, Spain,

²Primary Health Centre Peset-Hospital area, Valencia, Spain and ³Physiology, University of Valencia, Valencia, Spain

Maximum inspiratory pressure (MIP) is a measure of inspiratory muscle strength which decreases with age and is higher in men compared to women (1). Physical Activity on regular basis helps to maintain it (2), so some authors have analysed its relationship with regard to functional capacity in aging and pathology (3). However, up to our knowledge this is the first study to analyse any mediating influence of age and gender in this association, considering healthy active elderly.

67 healthy, active, non-smokers elderly (73.02 ± 4.54 years; 48.96 ± 16.93 cmH₂O, 55 women) volunteered to participated. Twice a week, they underwent 60 min of EFAM-UV[®], a multicomponent training program (MCTP) focused on gait training, postural control and strength training under dual-task constraints (4). Body composition and functional capacity were assessed, followed by correlation analyses between MIP and functional capacity (6MWT, Hand Grip [HG] and 30 seconds seat to stand test [30STS]), with age and gender as covariates. Age and gender influence on MIP and functional capacity was previously tested.

Despite being trained (Table 1), our sample showed similar MIP compared to recent studies assessing it with the same electronic device (Powerbreathe[®]K5) (5). As expected, age (a) and gender (g) confirmed their influence on MIP ($r^a = -0.297$; $p < 0.05$, $r^g = 0.444$; $p < 0.005$) and functional capacity (6MWT: $r^a = -0.348$; $p < 0.005$, $r^g = 0.348$; $p < 0.005$, HG: $r^a = -0.417$; $p < 0.005$, $r^g = 0.637$; $p < 0.005$). In addition, the association between MIP and functional capacity was clearly reduced by both covariates, with gender as the main mediator (6MWT: $r = 0.468$; $p < 0.005$ vs $r^a = 0.397$, $p < 0.005$, $r^g = 0.360$, $p < 0.005$, HG: $r = 0.493$; $p < 0.005$ vs $r^a = 0.404$; $p < 0.005$, $r^g = 0.270$; $p < 0.05$, 30STS: $r = 0.305$; $p < 0.05$ vs $r^a = 0.276$; $p < 0.05$ vs $r^g = 0.260$; $p < 0.05$). Whereas 6MWT and 30STS displayed an almost small association with MIP when accounting for age and gender together, this association disappeared in HG (6MWT: $r = 0.468$; $p < 0.005$ vs $r^{a+g} = 0.286$; $p < 0.05$, 30STS: $r = 0.305$; $p < 0.05$ vs $r^{a+g} = 0.234$; $p = 0.061$). Current disparity in the normative scores for MIP prevent to conclude that general neuromuscular health does not ensure respiratory functioning (i.e. optimal inspiratory strength), although small values or absence of correlation with MIP (HG), when accounting for age and gender, suggest it. Higher cardiorespiratory demands in 6MWT and 30STS might explain their slightly larger association. Specific respiratory training and MIP measurement might be introduced to ensure respiratory health in active elderly undergoing neuromuscular MCTPs.

Table 1. Performance characteristics

	Total (n=67) Mean $\pm$ SD	CV (%)	Women (n=55) Mean $\pm$ SD	CV (%)	Men (n=12) Mean $\pm$ SD	CV (%)
MIP (cmH ₂ O)	48.96 $\pm$ 16.93	34.00	45.57 $\pm$ 14.46	32.00	64.92 $\pm$ 18.81	31.00
6MWT (m)	558.04 $\pm$ 63.07	11.00	547.67 $\pm$ 56.54	10.00	609.92 $\pm$ 68.56	11.00
HG (Kg)	28.63 $\pm$ 6.93	24.00	26.20 $\pm$ 4.48	17.00	39.72 $\pm$ 5.12	15.00
30STS (rep)	18.42 $\pm$ 4.60	24.00	18.05 $\pm$ 4.42	25.00	20.08 $\pm$ 5.21	26.00

MIP: Maximum Inspiratory Pressure; 6MWT: 6 minutes walking test; HG: Handgrip Strength; 30STS: 30 seconds Seat to Stand Test.

Harik-khan R, Wise R, Fozard J. Determinants of Maximal Inspiratory Pressure. American Journal of Respiratory and Critical Care Medicine. 1998;158:1459-64.

McConnell AK, Copestake AJ. Maximum Static Respiratory Pressures in Healthy Elderly Men and Women: Issues of Reproducibility and Interpretation. Respiration. 1999;66:251-8.

Efstathiou ID, Mavrou IP, Grigoriadis KE. Correlation Between Maximum Inspiratory Pressure and Hand-Grip Force in Healthy Young and Middle-Age individuals. Respiratory Care. 2016;61(7):925-9.

Blasco-Lafarga C, Martínez-Navarro I, Cordellat A, Roldán A, Monteagudo P, Sanchis-Soler G, et al. Método de Entrenamiento Funcional Cognitivo Neuromuscular. Propiedad Intelectual num.: 156069. 2016;España (2016).

Alvarenga GM, Charkovski SA, dos Santos LK, da Silva MAB, Tomaz GO, Gamba HR. The influence of inspiratory muscle training combined with the Pilates method on lung function in elderly women. *Clinics*. 2018;73:e356.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC125

Exercise-onset-induced hypotension activates cerebrovascular response to exercise

J.D. Ashley, J.H. Shelley, J. Sun, B. Stone, L. Ambrosio and J. Kellawan

Health and Exercise Science, University of Oklahoma, Norman, OK, USA

Current models of cerebral blood flow (CBF) regulation at the onset of exercise suggests more than a ~50 second delay in the CBF response (1). When compared to other autoregulatory challenges [thigh cuff release and sit-to-stand test], CBF is restored within 20 seconds (2,3). Rest-to-exercise transition induces a brief hypotensive response due to rapid vasodilation of the periphery. Within this context, an investigation into the early blood flow adaptation during the onset of exercise is warranted as previous monoexponential modeling may have omitted these potentially important responses. **PURPOSE:** The purpose of this study was to identify if exercise-onset-induced hypotension activates a cerebrovascular response prior to monoexponential increases in CBF. **METHODS:** 7 healthy adults (24 ± 4.1 yrs) completed 2 minutes of quiet rest (baseline, BLN) then cycled for 3-min at 50W on a recumbent cycle ergometer. Middle cerebral artery velocity, a surrogate for CBF (MCAv; transcranial Doppler ultrasound) and mean arterial pressure (MAP; finger photoplethysmography), were measured on a beat-by-beat basis to calculate $CVCi = (MCAv / MAP) * 100 \text{ mmHg}$. End-tidal CO_2 was measured using breath-by-breath capnography (ET_{CO_2}). Results were analyzed using a paired samples t-test, significance was set at $p \leq 0.05$. **RESULTS:** Data are mean $\pm$ S.D. Both MCAv and MAP significantly decreased from BLN ($MCAv_{\text{NADIR}} -4.4 \pm 4.4 \text{ cm/s}$ Δ from $MCAv_{\text{BLN}}$, $p=0.01$; $MAP_{\text{NADIR}} -10.7 \pm 6.3 \text{ mmHg}$ Δ from MAP_{BLN} , $p=0.001$). The MAP_{NADIR} occurred at $10.3 \pm 2.6 \text{ sec}$. The $CVCi$ at $MCAv_{\text{NADIR}}$ was not different than BLN (73.4 ± 7.6 vs. 72.4 ± 12 , $p=0.7$). However, $CVCi$ at MAP_{NADIR} was elevated compared to BLN (84.7 ± 8.5 VS. 73.4 ± 7.6 , $p=0.02$). ET_{CO_2} did not change within the same timeframe. **DISCUSSION:** These results indicate the drop in CBF at the onset of exercise is primarily due to exercise-onset-induced hypotension, as opposed to changes in end-tidal CO_2 . Further, there is activation of vasoregulatory mechanisms that blunt cerebral hypoperfusion at exercise onset. Therefore, monoexponential models overlook potentially significant cerebrovascular countermeasures occurring prior to model predicted blood flow increase.

Billinger SA, Craig JC, Kwapiszeski SJ, Sisante JF, Vidoni ED, Maletsky R, Poole DC. Dynamics of middle cerebral artery blood flow velocity during moderate-intensity exercise. *J Appl Physiol*. 2017 Mar 9;122(5):1125-33.

Aaslid R, Lindegaard KF, Sorteberg W, Nornes H. Cerebral autoregulation dynamics in humans. *Stroke*. 1989 Jan;20(1):45-52.

Favre ME, Serrador JM. Sex Differences in Cerebral Autoregulation is Unaffected by Menstrual Cycle Phase in Young, Healthy Women. *Am J Physiol Heart Circ Physiol*. 2019 Feb 1.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC126

Cerebrovascular Response To Graded Exercise In Young Men and Women: A Pilot Study

J.H. Shelley, J. Ashley, B. Stone, J. Sun, L. Ambrosio and J. Kellawan

Department of Health and Exercise Science, University of Oklahoma, Norman, OK, USA

Reductions in cerebral blood flow (CBF) and poor vascular control are associated with increased risk of stroke and neurodegenerative disease (e.g. vascular dementia, Alzheimer's)(1). Recently, research initiatives have been initiated to examine sex-specific cerebrovascular regulation due to observations of differing expressions and treatment responses to cognitive and vascular diseases between Women (WN) and Men (MN)(2). Within this context, exercise is a promising non-pharmacological intervention to help combat cerebrovascular dysfunction(3). Previous research has shown differing vasodilator responses to both handgrip and knee extensor exercise in healthy WN and MN (4-5). Despite, these observations, the vast amount of research investigating CBF responses to exercise in humans have been conducted in men at lower exercise intensities. It is unknown if the sex differences in vascular response to exercise observed in the periphery exist in the brain. **PURPOSE:** Therefore, the aim of our pilot study was to compare CBF and cerebrovascular conductance index (CVC_i) – Exercise Intensity relationships between MN and WN. **METHODS:** 10 healthy adults (5 WN, 24 ± 4.1 yrs) completed a graded-exercise-test (GXT, stage length 3-min, 50W, 75W, 100W; after which MN increases by 40W, WN increased by 30W) on a recumbent cycle ergometer to volitional exhaustion (unable to maintain RPM of 60-80). Maximal Wattage (W_{max}) was determined to be the wattage of the highest completed stage. Middle cerebral artery velocity a proxy of CBF (MCA_v; transcranial Doppler ultrasound) and mean arterial pressure (MAP; finger photoplethysmography), were measured on a beat-by-beat basis to calculate CVC_i = MCA_v/MAP. **RESULTS:** mean ± S.E.M. Peak MCA_v were similar between WN & MN (82.3 ± 4.6 cm/s vs. 81.9.5 ± 4.8, p = 0.94). However, WN reached their peak MCA_v at a lower relative exercise intensity (41.9 ± 3.1 vs. 61.6 ± 6.5 %W_{max}, p ≤ 0.05). WN & MN did not differ in CVC_i (0.69 ± 0.1 vs. 0.73 ± 0.09 cm/s/mmHg, p = 0.5) at the %W_{max} where peak MCA_v was achieved. **CONCLUSION:** WN achieve peak MCA_v at a lower relative exercise intensity than MN. However, there appears to be no sex difference in vasodilatory responses to graded exercise as there was no difference in CVC_i at peak MCA_v. Thus, while the CBF – Exercise Intensity relationship differs between the sexes, evidence for a greater exercise vasodilatory response to exercise in brain of WN, as observed in other circulations, is not apparent.

Leijenaar JF, van Maurik IS, Kuijter JPA, van der Flier WM, Scheltens P, Barkhof F, et al. Lower cerebral blood flow in subjects with Alzheimer's dementia, mild cognitive impairment, and subjective cognitive decline using two-dimensional phase-contrast magnetic resonance imaging. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2017;9:76-83.

Miller VM, Garovic VD, Kantarci K, Barnes JN, Jayachandran M, Mielke MM, et al. Sex-specific risk of cardiovascular disease and cognitive decline: pregnancy and menopause. *Biology of sex differences*. 2013;4(1):6

DeSouza CA, Shapiro LF, Clevenger CM, Dinunno FA, Monahan KD, Tanaka H, et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation*. 2000;102(12):1351-7.

Green DJ, Hopkins ND, Jones H, Thijssen DH, Eijssvogels TM, Yeap BB. Sex differences in vascular endothelial function and health in humans: impacts of exercise. *Experimental physiology*. 2016;101(2):230-42.

Hicks A, Kent-Braun J, Ditor D. Sex Differences in Human Skeletal Muscle Fatigue 2001. 109-12 p.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC127

Electrical stimulation via the motor nerve may recruit from a more variable motor unit pool than direct muscle stimulation

T.B. Inns, D. McCormick, B. Phillips and M. Piasecki

School of Medicine, MRC-ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Derby, UK

Electromuscular stimulation (EMS) has been employed as an interventional strategy to mitigate the deleterious effects of age on neuromuscular function (Kern et al., 2014). However, studies using EMS use variable protocols and to date, show inconsistent results (Trethowey et al., 2019). One previous study compared EMS over the quadriceps muscle belly versus over the femoral nerve and reported greater central pathway recruitment with nerve stimulation, compared to greater peripheral recruitment with muscle stimulation (Bergquist, Wiest and Collins, 2012). However, the effects of each method on inducing muscle fatigue are unclear, and there is a need to develop consistent EMS intervention protocols that target the large, functionally important lower limb muscle groups, such as the quadriceps. Seven young (27 ± 3 y), healthy participants were recruited to this study. In session 1, EMS was applied via the femoral nerve (nStim), followed by application via the muscle belly (mStim) >48h later. For nStim, stimulating electrodes were placed on the lower back and over the femoral nerve. mStim involved the electrodes being placed over the quadriceps muscle belly. For each modality, a single 30Hz stimulus was applied, with sufficient current to achieve involuntary contractile force equivalent to 30% maximal voluntary isometric contraction (MVC). The electrically stimulated intermittent contraction fatigue test (McPhee et al, 2014) was then applied to the quadriceps (60 stimuli at 30Hz with a predetermined current

(Figure 1A/B). MVC was assessed pre and post fatigue test, with vastus lateralis compound motor action potentials (CMAP) measured throughout to provide an estimate of electrophysiological activity. Isometric force generated by voluntary and involuntary contractions were recorded using Spike2 software (Cambridge Electronic Design) while the participant was seated in a chair fixing the knee at 90°. Tolerance of each protocol was assessed using a visual analogue scale.

nStim and mStim induced muscle fatigue to a similar extent with similar reductions in both MVC ($14 \pm 9\%$ and $16 \pm 10\%$, both $P < 0.01$) and involuntary force ($46 \pm 20\%$, $P < 0.01$ and $41 \pm 8\%$, $P < 0.001$, respectively). The coefficient of variation for mean involuntary peak contraction force was higher for nStim ($28 \pm 11\%$) compared to mStim ($19 \pm 4\%$). Correlation of mean CMAP area versus peak contraction force was $r^2 = 0.49$ ($P < 0.001$) for nStim, and $r^2 = 0.097$ ($P < 0.001$) for mStim (Figure 1C/D). There was no significant difference between self-reported discomfort in mStim versus nStim (6.3 ± 1.9 vs. 3.8 ± 2.1 , $P = 0.09$).

Both forms of EMS are able to induce muscle fatigue to a similar extent. The greater variability in force production observed with nStim may reflect a more variable pool of recruited motor units. Force produced via nStim was highly correlated with electrophysiological activity, and as such may be a useful indicator of muscle activity where force recording is not possible.

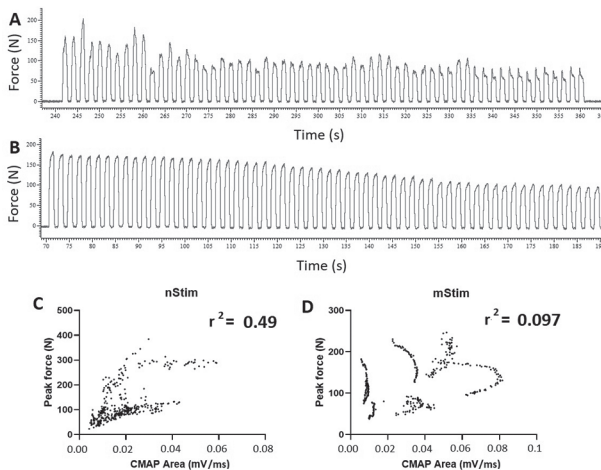


Figure 1. The effects of nerve trunk (A) and quadriceps muscle belly (B) electromuscular stimulation on knee extension contractile force, and the correlation between compound motor action potentials (CMAP) area and peak knee extension contractile force with nerve (nStim) and muscle (mStim) stimulation.

Bergquist, A. J., Wiest, M. J. and Collins, D. F. (2012) *Journal of Applied Physiology*, 113(1), pp. 78–89.

Kern, H. *et al.* (2014) *Frontiers in Aging Neuroscience*, 6(JUL) pp. 1–11.

Mcphee, J. S. *et al.* (2014) *Muscle and Nerve*, 50(3), pp. 393–400.

Trethewey, S. P. *et al.* (2019) *Journal of Critical Care*, 50, pp. 287–295.

We would like to acknowledge funding from the MRC/ARUK Centre for Musculoskeletal Ageing Research and the Nottingham Biomedical Research Centre.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC128

Mouse chromosome X locus flanking *Pou3f4* gene affects muscle mass by affecting the number of muscle fibres

A.M. Albloshi¹, P.M. Brooks², T.M. Coate² and A. Lionikas¹

¹University of Aberdeen, Aberdeen, UK and ²Georgetown University, Washington, District of Columbia, USA

Muscle mass differs by more than two-fold between healthy adult individuals of the same sex, age and height. Genetic factors account for 40 – 80% of these differences (1), however, specific genes responsible for the effects remain largely unknown. It also remains unclear to what extent these differences are due to the variation in the fibre number, determined prenatally, or fibre size, which can change over the course of a lifespan. The loss of muscle due to aging, known as sarcopenia, increases the risk of disability in the elderly 1.5 to 4.6 times (2). Understanding the mechanisms determining muscle mass and fibre numbers would help early assessment of the risk of sarcopenia when preventive interventions are more effective. The aim of this study was to shed light on genetic mechanisms determining muscle mass differences in a population of CFW outbred mice.

The study was motivated by the findings of a genome-wide association analysis that a locus on chromosome X, encompassing *Pou3f4*, *Cycl1* and *Rps6ka6* genes, affected the weight of the glycolytic muscle (tibialis anterior, extensor digitorum longus (EDL), gastrocnemius and plantaris) in CFW mice (3). To follow that up, we carried out histological analyses in a subset of CFW EDL samples to explore the cellular mechanisms of the effect of that locus on muscle weight in male mice. We found that carriers of the weight-increasing allele had 10% more fibres ($P < 0.03$) in EDL muscle, $1,091 \pm 92$ ($n=10$), than the carriers of the decreasing allele, 988 ± 95 ($n=10$), whereas we did not find differences in the cross-sectional area of muscle fibres. We then tested the hypothesis that *Pou3f4* gene might be responsible for the effect on muscle fibre number. We carried out histochemical staining to compare the fibre numbers between *Pou3f4*^{-/-} mice ($n=7$) and their wildtype littermates ($n=5-7$). The knockout mice had 21% reduction in EDL fibres, 847 ± 85 vs $1,068 \pm 114$ ($P < 0.01$), and 22% reduction in fibres of oxidative soleus muscle, 591 ± 144 vs 759 ± 117 ($P < 0.04$), compared to their wildtype littermates.

We conclude that chromosome X locus affects muscle mass by influencing the number of muscle fibres. The *Pou3f4* gene appears an underlying cause of the effect. Hence, the study identified a novel myogenic modifier that might be contributing to variability in muscle mass by determining fibre number.

Livshits G, et al. Contribution of Heritability and Epigenetic Factors to Skeletal Muscle Mass Variation in United Kingdom Twins. *J Clin Endocrinol Metab* 101, 2450-2459 (2016).

Janssen I, Shepard DS, Katzmarzyk PT, Roubenoff R. The healthcare costs of sarcopenia in the United States. *Journal of the American Geriatrics Society* 52, 80-85 (2004).

Nicod J, et al. Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. *Nature Genetics* 48, 912-918 (2016).

The authors would like to acknowledge Mr James Archibald for carrying out histological analyses of CFW EDL samples.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC129

Isolated fast-twitch extensor digitorum longus muscles from old mdx dystrophic mice show little force recovery 120 minutes after eccentric damage

L. Kiriaev¹, S. Kueh¹, J. Morley¹, K. North², P. Houweling² and S.I. Head¹

¹Medicine, Western Sydney University, Sydney, NSW, Australia and ²Murdoch Children's Research Institute, Melbourne, VIC, Australia

Duchenne muscular dystrophy (DMD) is characterized by progressive wasting and cycles of regeneration in skeletal muscle. Work from our laboratory (1.), suggests that branched fibres, that form as a consequence of repeated bouts of regeneration, could be responsible for the terminal phase of mechanical muscle damage in old (58-112 weeks) dystrophic mice. A recent study (2.) in adult 12 week old *mdx* dystrophic mice reported that the majority of force loss produced by a series of eccentric contractions (EC) in extensor digitorum longus (EDL) muscles recovers (65%) within 120 minutes. The authors conclude this is incompatible with the assumption that EC force loss is due to mechanical damage. The majority of studies assess force recovery 10 minutes post EC. Our aim was to assess the recovery post EC damage at up to 120 minutes in old and adult dystrophic muscles. Male *mdx* mice (C57BL/10) at either 88 weeks or 16 weeks of age were killed with an overdose of isoflurane and fast-twitch EDL muscles dissected from the hind limbs. Each muscle was maintained in Krebs solution with carbogen at room temperature. The muscle was maximally stimulated at 125 Hz and a series of EC were given at 10% (three) and 20% (three) stretch from optimal length (L_0), the muscles were then rested for up to 120 minutes before measuring the recovery. Single muscle fibres were isolated enzymatically using collagenase to assess the degree of fibre branching in 16 week *mdx* EDL. Table1 shows a small recovery in the old 88 week group and a statistically significant larger recovery in the adult 16 week group. These findings of minimal recovery at 120 minutes post EC force loss in EDL muscles from 88 week *mdx* dystrophic mice support our "tipping point" hypothesis (1.) that there is a distinct pathophysiology, EC force loss due to acute fibre rupture at branch nodes, which occurs in "aged" (58-112 weeks) dystrophic EDL muscles (>70% complex branched fibres). Our branched fibre "tipping point" hypothesis predicts the reduced membrane

damage in the younger dystrophic age group is a consequence of the fact that the number and complexity of regenerated branched fibres has not passed a “tipping point” where branching will mechanically compromise the strength of fibres. These findings have important implications for pre-clinical drug studies which have used protection from EC damage as a marker for drug efficacy, but have only recorded force recovery after 10-20 minutes in young/adult *mdx* mice.

Table 1

	% Force lost after 1st 10% EC $\pm$ SD	%Force lost after all 6 EC $\pm$ SD	%Recovery 120 minutes $\pm$ SD	% fibres with >3 branches
88 weeks <i>mdx</i> EDL	73 $\pm$ 11	95 $\pm$ 2	23 $\pm$ 10	77%
16 weeks <i>mdx</i> EDL	42 $\pm$ 10	86 $\pm$ 2	55 $\pm$ 20	20%
T test (2 tail)	** P=0.0059 n=4	*** P=0.0007 n=4	*P=0.0296 n=4	NA

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

1. Kiriaev L, Kueh S, Morley JW, North KN, Houweling PJ, Head SI. Branched fibers from old fast-twitch dystrophic muscles are the sites of terminal damage in muscular dystrophy. *American Journal of Physiology-Cell Physiology*. 2018;314(6):C662-C74.
2. Olthoff JT, Lindsay A, Abo-Zahrah R, Baltgalvis KA, Patrino X, Belanto JJ, et al. Loss of peroxiredoxin-2 exacerbates eccentric contraction-induced force loss in dystrophin-deficient muscle. *Nature communications*. 2018;9(1):5104.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC130

Motor Unit Characteristics of the Human Vastus Lateralis following Eccentric/ Concentric induced Functional Decline

D. McCormick, J. Gladman, P.L. Greenhaff, P.J. Atherton and M. Piasecki

MRC-ARUK Centre for Musculoskeletal Ageing Research & NIHR Nottingham Biomedical Research Centre, University of Nottingham, Royal Derby Hospital Centre, UK

Generation of muscle force relies on a combination of altered motor unit firing rates, and recruitment through a progressively increasing range of sizes. Alterations in these mechanisms may influence muscle functional decline in the fatigued and damaged state. Although eccentric exercise elicits a more significant loss of function, muscle damage and soreness than concentric, it is unclear how these forms of exercise may influence the size of MU recruited to achieve a given force (Semmler et al., 2014). We hypothesized that any functional deficits observed in muscle force-generating capacity of the quadriceps would be also observed in the size of recruited vastus lateralis motor units.

The ‘Step Up, Step Down’ exercise technique (Kostek et al., 2007) was applied in 8 untrained healthy young adults (2 Male; 23 $\pm$ 2yrs; BMI 27 $\pm$ 6), designed to induce eccentric and concentric exercise in opposing quadriceps. Maximal voluntary

isometric contraction (MVC) and serum creatine kinase were assessed as an indicator of functional decline and muscle damage, respectively. Intramuscular electromyography was used to sample individual motor units during isometric contractions held at 25% MVC, and the area of the motor unit potential was used as an indicator of motor unit size (Piasecki et al., 2018). All measures were performed at baseline, post-exercise & 48hrs post-exercise.

Creatine kinase increased $26 \pm 10\%$ from baseline (121 ± 49 IU/L) to post-exercise (149 ± 59 IU/L, $P = 0.0471$), and the mean value remained elevated at 48 hours post-exercise (165 ± 69 IU/L, $P = 0.2122$) when compared to baseline. In the concentric limb, MVC was reduced immediately post-exercise ($18 \pm 10\%$ $P = 0.0071$), but did not differ significantly at 48 hours ($13 \pm 8\%$ $P = 0.139$) compared to baseline (Figure 1A). A similar pattern was observed for MUP area in the concentric limb which reduced by $40 \pm 14\%$ ($P = 0.051$) immediately post-exercise, and $12 \pm 3\%$ ($P = 0.658$) at 48 hours post-exercise. In the eccentric limb MVC was reduced by $24 \pm 8\%$ immediately post-exercise ($P = 0.0019$), and remained significantly reduced by $26 \pm 18\%$ ($P = 0.0016$) at 48 hours. However, unlike the concentric limb, the change in MUP area in the eccentric limb did not correspond with the prolonged reduction in MVC, decreasing by $41 \pm 25\%$ ($P = 0.0062$) immediately post, and by $9 \pm 18\%$ ($P = 0.212$) at 48 hours post-exercise, thus reverting to near baseline values (Figure 1B). The restoration of the MU size suggests that in healthy young adults the recruitment strategy can recover after a significant eccentric-exercise induced disturbance, though the recovery is not equalled by force producing ability. Changes in MUP area did not differ between the two exercise conditions, at either time points tested, whereas the expected differences in muscle function were observed. These data are highly suggestive of a minimal relevance of motor unit size recruitment strategies in the observed functional decline following eccentric exercise.

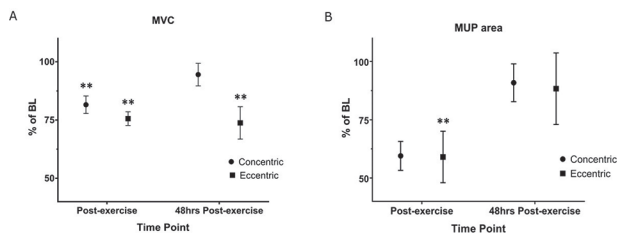


Figure 1. Maximum voluntary isometric contraction (A) and motor unit potential area (B) immediately post and 48 hours post eccentric and concentric exercise

Kostek, M., Chen, Y., Cuthbertson, D., Shi, R., Fedele, M., Esser, K. and Rennie, M. (2007). Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: major changes in CSR3P, MUSTN1, SIX1, and FBXO32. *Physiological Genomics*, 31(1), pp.42-52.

Piasecki, M., Ireland, A., Piasecki, J., Stashuk, D., Swiecicka, A., Rutter, M., Jones, D. and McPhee, J. (2018). Failure to expand the motor unit size to compensate for declining motor unit numbers distinguishes sarcopenic from non-sarcopenic older men. *The Journal of Physiology*, 596(9), pp.1627-1637.

Semmler, J. (2014). Motor unit activity after eccentric exercise and muscle damage in humans. *Acta Physiologica*, 210(4), pp.754-767.

This research was supported by grants from the MRC-ARUK Centre for Musculoskeletal Ageing Research & NIHR Nottingham Biomedical Research Centre

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC131

Imposed expiratory resistance and pulmonary function in young healthy volunteers

M. Bari, J. Erram and D.T. Cannon

School of Exercise & Nutritional Sciences, San Diego State University, San Diego, CA, USA

Expiratory flow limitation is a primary characteristic in chronic obstructive pulmonary disease (COPD) and asthma. Increased airway resistance occurs due to bronchoconstriction, destruction of elastic tissue in the airways, and mucus hypersecretion from goblet cells caused by irritation of the epithelium. As a result, the airways become narrowed. Obstruction can lead to dynamic hyperinflation, dyspnea, and exercise intolerance. However, increased airway resistance is just one of many abnormalities in COPD and asthma - COPD in particular is an exceedingly heterogeneous disease. Isolating the effects of expiratory airflow limitation, alone, is therefore challenging. In order to study abnormal lung mechanics in isolation, we aimed to test whether adjustable imposed expiratory resistance in young healthy volunteers produced deficits in the flow-volume relationship similar to that of patients with COPD or asthma. **PURPOSE** Measure pulmonary function in young healthy volunteers with and without imposed expiratory resistance. **METHODS** Twenty-seven participants (27 ± 5 years, 170 ± 11 cm, 71.4 ± 13.0 kg) completed standard pulmonary function testing according to the ATS/ERS standards. The testing included three conditions in random order: control, imposed expiratory resistance of 7 cmH₂O/L/s, and imposed expiratory resistance of 11 cmH₂O/L/s. Resistance was imposed with a threshold inspiratory muscle trainer (Vacumed, Ventura, CA) installed in reverse in the spirometer (Parvo Medics, Sandy, UT). **RESULTS** FEV1 was reduced ($F[1.492, 38.79] = 66.94, p < 0.05$) with 7 and 11 cmH₂O/L/s of resistance vs control (3.21 ± 0.88 and 3.24 ± 0.91 L, respectively, vs. 4.05 ± 0.15 L). FVC was also reduced ($F[1.599, 41.57] = 68.11, p < 0.05$) at 7 and 11 cmH₂O/L/s vs control (4.11 ± 1.09 and 4.14 ± 1.12 L, respectively, vs. 5.07 ± 1.35 L). FEV1/FVC was not different ($F[1.865, 48.48] = 3.611, p < 0.05$) between resistance conditions. PEF was reduced ($F[1.534, 39.89] = 63.61, p < 0.05$) with 7 and 11 cmH₂O/L/s of resistance vs control (6.01 ± 1.73 and 6.20 ± 1.92 L, respectively, vs. 8.71 ± 2.83 L). **CONCLUSIONS** Imposed expiratory resistance reduced key variables (FEV1, FVC, PEF) measured during pulmonary function testing. FEV1/FVC ratio was not affected by either resistance condition. A concave expiratory flow-volume relationship was consistently absent – a key limitation for model comparison with pulmonary function in COPD. This is most likely due to the imposed resistance being applied outside of the airways, leading to higher airway pressures.

The higher airway pressures are likely to resist dynamic airway compression and either maintain the equal pressure point position or possibly move it proximally.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC132

Cardiovascular consequences of fatiguing expiratory muscle work

T.A. Hardy¹, M.P. Ribeiro², G.K. Lyall¹, K.M. Birch¹, C. Ferguson¹ and B.J. Taylor¹

¹*School of Biomedical Sciences, University of Leeds, Leeds, UK and* ²*Division of Translational Medicine, Federal University of Sao Paulo, Sao Paulo, Brazil*

Exhaustive high-intensity whole-body exercise ($>90\% \text{VO}_{2\text{peak}}$) elicits fatigue of the expiratory muscles that in turn impairs exercise tolerance (1, 2). A possible mechanism for this exercise limitation is a reflexively-mediated increase in sympathetic vasomotor outflow that compromises perfusion of limb locomotor muscles limiting their ability to perform work (i.e. 'the respiratory muscle metaboreflex'). We investigated the effect of fatiguing expiratory muscle contractions on: 1) leg blood flow (Q_L); 2) leg vascular resistance (LVR); and 3) mean arterial pressure (MAP), heart rate (HR) and cardiac output (Q) in otherwise resting humans. On two separate days, twelve healthy adults (2 f; mean $\pm$ SD; age = 25 ± 4 y) performed expiratory resisted loaded breathing (ERL) at 2% (ERL-CONTROL) or 65% (ERL-FATIGUE) of maximal expiratory mouth pressure (MEP); ERL-CONTROL was performed for 5 min and ERL-FATIGUE was performed to task failure. On a third day, a subset of individuals ($n = 5$) performed ERL at 2% of maximal gastric pressure (Pga_{MAX}) (ERL_{pga}-CONTROL) for 5 min before undergoing ERL at 65% of Pga_{MAX} (ERL_{pga}-FATIGUE) to task failure; subjects were instructed to 'breathe from their abdomen' during each resisted expiration to specifically target the expiratory abdominal muscles. Breathing frequency was set at 15 breaths/min with a 0.5 expiratory duty cycle (T_E/T_{TOT}) for all ERL trials. MAP, HR and Q were measured continuously via finger photoplethysmography. Q_L was measured every 1 min by Doppler ultrasound and LVR calculated as MAP/Q_L . For all trials, expiratory muscle fatigue was assessed by measuring the pre- to post-ERL reduction in MEP. In addition, the reduction relative to baseline values in magnetically evoked gastric twitch pressure (Pga_{tw}) was assessed in ERL_{pga}-CONTROL and ERL_{pga}-FATIGUE. There was no evidence of expiratory muscle fatigue in either control trial. Q_L , LVR, MAP, HR and Q were unchanged during ERL-CONTROL and ERL_{pga}-CONTROL ($P > 0.05$). By contrast, MEP decreased by $19 \pm 5\%$ ($P < 0.05$) from before to after ERL-FATIGUE. Similarly, there was a $16 \pm 6\%$ and $14 \pm 9\%$ decrease in MEP and Pga_{tw} , respectively, from pre- to post-ERL_{pga}-FATIGUE. At task failure, MAP and HR were increased relative to resting values by 31 ± 10 mmHg and 28 ± 4 beats/min for ERL-FATIGUE and 18 ± 9 mmHg and 31 ± 10 beats/min for ERL_{pga}-FATIGUE (all $P < 0.05$). Conversely, there was no significant change in Q during either fatiguing ERL trial. Group mean Q_L and LVR were unchanged from eupneic values during ERL-FATIGUE and ERL_{pga}-FATIGUE

($P < 0.05$), despite considerable variability in individual responses to both tasks (Figure 1). In conclusion, these data do not support a reflexively-mediated reduction in Q_L and increase in LVR during fatiguing expiratory muscle contractions, despite large increases in MAP and HR, even when the expiratory abdominal muscles are specifically targeted.

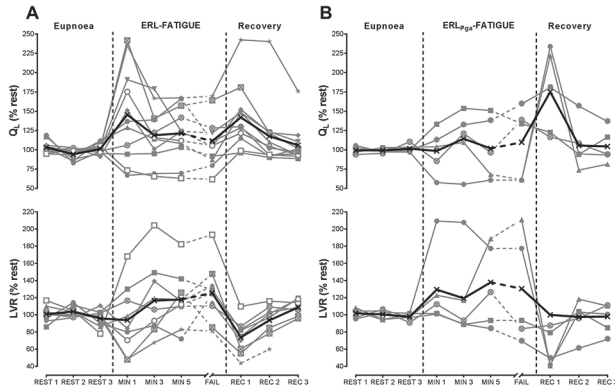


Figure 1. Leg blood flow (Q_L ; top panel) and leg vascular resistance (LVR; bottom panel) during (A) fatiguing expiratory resistive loading targeting 65% of maximal expiratory mouth pressure (ERL-FATIGUE; $n = 12$) and (B) fatiguing expiratory resistive loading targeting 65% of maximal gastric pressure (ERL_{pg}-FATIGUE; $n = 5$). Black lines represent group means; grey lines represent individual data. Female data are presented in open symbols.

Taylor BJ, How SC, Romer LM. Exercise-induced abdominal muscle fatigue in healthy humans. *Journal of Applied Physiology*. 2006 May;100(5):1554-62.

Taylor BJ, Romer LM. Effect of expiratory muscle fatigue on exercise tolerance and locomotor muscle fatigue in healthy humans. *Journal of Applied Physiology*. 2008 May;104(5):1442-51.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC133

Effects of Progressive Resistance Training Targeting Muscles Rich in Type One Fibers on Ventilatory Efficiency

E. Sahin² and A. AYAR¹

¹Physiology, Karadeniz Technical University, Trabzon, Turkey and ²Physiotherapy and Rehabilitation, Recep Tayyip Erdogan University, Rize, Turkey

Ventilatory efficiency is an indicator of ventilation-perfusion mismatch at lungs. It is independent of effort and can be measured in submaximal workloads. Ventilatory efficiency which can be calculated from ventilatory equivalent for CO₂ (VE/VCO₂) and O₂ ventilatory equivalent (VE/VO₂), more sensitive to ventilatory pathologies

in exercise, both increases in pulmonary and cardiac diseases. These parameters are associated with better fitness level and tend to increase as individuals get older. Although effects of aerobic exercise on parameters related to cardiorespiratory status including VE/VCO₂ are documented, in this study the effect of progressive resistance exercise on muscles rich in type 1 fiber ratio (soleus, biceps femoris, adductor magnus, tibialis anterior, vastus medialis) was investigated. University students aged 19-25 filled International Physical Activity Questionnaire to confirm that they were indeed sedentary. 12-LED cardiopulmonary exercise test (CPET) was performed on bicycle ergometer with breath by breath method before the resistance training program.

Then they performed 6 week of progressive resistance training targeting muscles rich in type fibers, as 3 days per week. After training VE/VO₂ and VE/VCO₂ at anaerobic threshold (VE/VO₂@AT and VE/VCO₂@AT) at CPET, calculated by V-slope method were determined. VE/VO₂@AT median (IQR) value at the anaerobic threshold before the training program was 28.1 (26.9-34.3) and it changed to 31.2 (26.9-36.1). But this change was not statistically significant ($p=0.484$). The VE/VCO₂ at anaerobic threshold (VE/VCO₂@AT) median (IQR) value was 33.3 (28.2-34.9) before training and it decreased to 29.1 (27.5-32.9) after training ($p=0.036$). This statistically significant change in VE/VCO₂ at anaerobic threshold indicates that by this local exercise protocol in individuals who are elderly, dependent on bed and cannot find opportunity to perform aerobic exercise can rehabilitate and improve their general condition.

This study was supported by Karadeniz Technical University Scientific Research Fund

(TYL-2016-5372)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC134

Novel low dose spironolactone protects experimentally-induced polycystic ovarian syndrome against insulin-resistant metabolic disturbances through anti-androgenic mechanism

O.A. Adeyanju and T. Falodun

Physiology, Afe Babalola, Ilorin, Nigeria

Background: Polycystic ovarian syndrome (PCOS) is the most common endocrinological disorder in women of reproductive age and hyperandrogenism is a prominent feature of PCOS resulting in infertility and increased risk of developing metabolic disorders including insulin resistance (IR), abdominal adiposity, glucose intolerance and cardiovascular diseases. Spironolactone (SPL), a non-selective mineralocorticoid receptor (MR) antagonist and potent diuretic, has been in wide clinical use for several decades.

Objective: We designed the present study to investigate the effects of SPL (at a very low dose that has not been used before for this condition) on IR and cardiometabolic disturbances in letrozole-induced PCOS rats.

Methods: Eighteen adult female Wistar rats were randomly divided into 3 groups and treated with vehicle, letrozole (LET; 1 mg/kg) and LET+SPL (SPL; 0.25 mg/kg), p.o. once daily for 21 consecutive days. Plasma as well as ovarian and heart tissues were analyzed. Results were shown as mean $\pm$ SEM (standard error of mean). Statistically significant differences were accepted at $p < 0.05$. Statistical group analysis was performed with SPSS statistical software.

Results: Results showed that LET treatment induced PCOS characterized by elevated plasma testosterone (3.1 ± 0.4 ng/ml) and luteinizing hormone (LH; 0.9 ± 0.1 mIU/ml) accompanied with increased body weight (195.7 ± 11.3 g) and visceral adiposity (13.5 ± 1.2 kg/bw), IR (6.5 ± 0.5), glucose intolerance, dyslipidemia and altered histomorphological ovaries and heart. Treatment with SPL however attenuated the elevated testosterone in LET-induced PCOS (0.9 ± 0.1 ng/ml) model accompanied with a reversal in all the observed alterations.

Conclusion: Taken together, analysis of the physical, biochemical and histological evidences shows for the first time that SPL at this low dose has protective effect on IR and other cardiometabolic disturbances and this may be through its anti-androgenic mechanism.

The authors acknowledge members of HOPE CRT

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC135

Magnesium prevent of atherosclerosis via decrease in LOX1 gene expression in diabetic vessel

N. Soltani¹, M. Fazeali², F. Kharazmi¹ and M. Kamran²

¹Physiology, Isfahan University of Medical Sciences, Isfahan, Iran (the Islamic Republic of) and ²Hormozgan University of Medical Sciences, Bandar Abbas, Iran (the Islamic Republic of)

Magnesium is the second intracellular and fourth common cation in the body. Magnesium deficit has been described in patients with type I diabetes. Hypomagnesemia can also be the cause for some clinical diseases such as atherosclerosis. The aim of the present study was determine the possible role of LOX1 receptor inhibition on decrease of atherosclerosis by oral magnesium sulphate administration in diabetic rats. **Method:** Animals divided into four groups diabetic, diabetic+ Mg, diabetic+ insulin and control after 16 weeks mesenteric artery was isolated and perfused. Perfusion pressure was recorded by power lab system in presence of phenylephrine, L-NAME and adenosine receptors inhibitor. Mg therapy also used for 32 atherosclerotic patients referred to angiography center and patients followed up for 6 months. Blood sample in animals and patients was taken to measure

plasma Mg, Ca, oxLDL and lipid profiles levels by micro plate reader and appropriate kits and aorta and common carotid arteries in all animal were isolated for LOX1 gene expression via Real time PCR method and pathology study. Blood LOX1 gene expression via PCR method was measured for all patients. One-way and two-way ANOVA with the Tukey post-hoc test and t-test were used for data analysis. **Results:** Our results showed that plasma magnesium level decreases after diabetes induction and magnesium deficiency is a risk factor for diabetic vessel complications. On the other hand, magnesium could decrease blood glucose, LDL, cholesterol, oxLDL and triglyceride levels in both animals and patients. Magnesium also could decrease atherosclerosis via decrease in LOX1 gene expression in animal model. Mg plays an important role in regulation of blood pressure and decrease the sensitivity of vessels to phenylephrine the mechanism of this actions are mediated by NO and adenosine A2a receptor. **Conclusion:** Mg has acceptable effect on prevention of atherosclerosis in diabetic vessel and it hopes that the more study about Mg mechanism can help diabetic patients to better Mg usage in future.

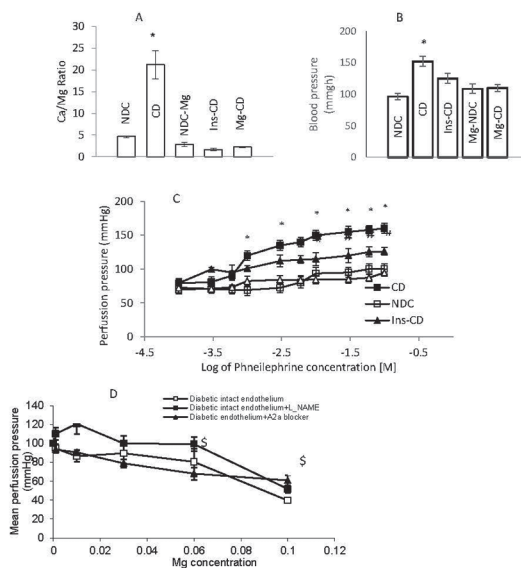
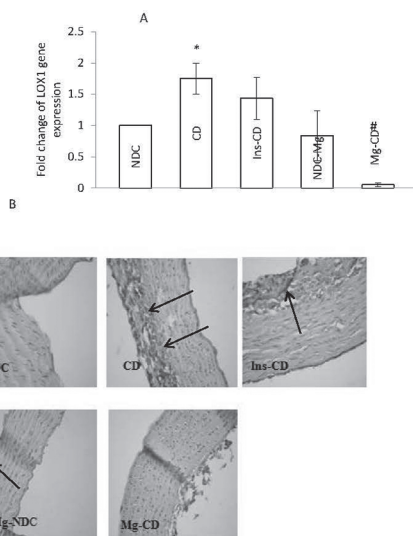


Figure 2: Ca/Mg ratio (A), mean arterial blood pressure (B) and dose response curve of phenylephrine in mesenteric vascular bed with intact endothelium (C) in non-diabetic control (NDC), chronic diabetic (CD), Mg-treated non diabetic control (Mg-NDC), insulin-treated chronic diabetic (Ins-CD) and Mg-treated chronic diabetic (Mg-CD) groups (10 rats in each group, data were expressed as mean $\pm$ SEM). *Significant difference between CD and other groups ($P < 0.001$). # Significant difference between Ins-CD and Mg-CD groups ($P < 0.001$). \$ Significant difference between diabetic intact endothelium and diabetic intact endothelium+A2a blocker group ($P < 0.01$)



C	NDC	CD	Ins-CD	Mg-NDC	Mg-CD
LOX1 Protein level	+	+++	+++	+	-

Figure 3: Comparison of LOX-1 gene expression (A) and Immunohistochemistry for LOX1 (B and C) in non-diabetic control (NDC), chronic diabetic (CD), Mg-treated non diabetic control (Mg-NDC), insulin-treated chronic diabetic (Ins-CD) and Mg-treated chronic diabetic (Mg-CD) groups (10 rats in each group, data are expressed as mean $\pm$ S EM and protein level of LOX1 was showed by black arrows). *Significant different between CD and other groups ($P<0.01$)
Significant different between Mg-CD and Ins-CD and Mg-NDC groups ($P<0.01$)

Liao F, Folsom AR, Brancati FL. Is low magnesium concentration a risk factor for coronary heart disease? The Atherosclerosis Risk in Communities (ARIC) Study. American heart journal. 1998;136(3):480-90.

Soltani N, Keshavarz M, Dehpour AR. Effect of oral magnesium sulfate administration on blood pressure and lipid profile in streptozocin diabetic rat. European journal of pharmacology. 2007;560(2):201-5.

Soltani N, Keshavarz M, Minaei B, Mirershadi F, Asl SZ, Dehpour AR. Effects of administration of oral magnesium on plasma glucose and pathological changes in the aorta and pancreas of diabetic rats. Clinical and experimental pharmacology and physiology. 2005;32(8):604-10.

Hormozgan University of Medical Sciences

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC136

Effects of Lunar Cycle on blood glucose level and autonomic functions in type 2 diabetic patients

S. DUTTA CHOWDHURY, S. PRAMANIK and R. GHOSH

DEPARTMENT OF PHYSIOLOGY, BASIRHAT COLLEGE, Kolkata, India

The position of the moon in its orbit at different phases of lunar month affects the human physiological functions. The purpose of the study was to explore the blood glucose level and autonomic functions by measuring resting heart rate and blood pressure in four specific phases of the lunar month in type 2 diabetic patients. The study was conducted on 40 randomly selected patients (21 males and 19 females) from Diabetes Clinic of Calcutta Medical College in four phases such as new moon (NM), first quarter (FQ), full moon (FM) and third quarter (TQ) of lunar month. Plasma glucose level of each subject was estimated by colorimetric end point test method. After overnight fasting, 3ml of venous blood sample was collected from each subject in ethylenediaminetetraaceticacid (EDTA) vacutainer. Then plasma was separated (by centrifugation at 3000 revolutions per minute for 10 minutes) and glucose concentration was measured using commercially available kit [Lab Care Diagnostics (India) Pvt. Ltd., India, Catalog No: GLU 500]. Blood pressure and heart rate of each patient were measured manually at rest and during static exercise in four lunar phases. Static exercise, in terms of hand grip strength, was performed using hand grip dynamometer (Lafco India Scientific industries, Ambala 133001, Haryana). The patients were asked to pull the handle maximally, hold the dynamometer at least for 5 seconds, and blood pressure and heart rate were recorded steadily during that period. The protocol and procedures employed were in accordance with the human ethical guidelines of the Institutional Human Ethical Committee, Institute of Post Graduate Medical Education and Research (IPGMER) and Seth Sukhlal Karnani Memorial Hospital (SSKM) (Ref. No. IHEC/SDC/P12/18 dated 16.03.2018). The fasting plasma glucose levels in NM and FM were significantly higher ($p < 0.001$) than that in TQ and FQ for both males and females. The mean values of resting systolic blood pressure (RSBP) and resting heart rate (RHR) for both males and females were significantly higher in NM and FM than that in FQ ($p < 0.05$) and TQ ($p < 0.05$). Similarly, the mean values of systolic blood pressure (EXSBP) and heart rate during static exercise (EXHR) for both males and females were significantly higher in NM and FM than that in FQ ($p < 0.01$) and TQ ($p < 0.01$). It appears from this study that there is a circalunar periodicity of blood glucose level and autonomic neural activity for type 2 diabetic patients which is efficiently adjusted during NM and FM in comparison to FQ and TQ. So the lunar phase is a factor that should be taken into concern when preparing or analyzing the data of autonomic neural activity for a type 2 diabetic patient.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC137

Sex Differences In Cardiovascular Responses To Squat Test In Diabetic And Non Diabetic Adults

A.O. Aiku^{1,2}, S. Ogbona¹, E. Adagbada¹ and A. Fasanmade¹

¹Physiology, University of Ibadan, Ibadan, Nigeria and ²Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, UK

Squatting elicits postural stress and increases in blood pressure. This elicits reflex bradycardia via the baroreceptor reflex. In disease conditions such as diabetes, there may be dysfunction of baroreceptor activity such that the greater pressor responses occur and bradycardia is blunted. The aim of this study was to study the effect of postural stress in diabetic patients and to determine whether sex influences the responses relative to non-diabetics.

Method.

40 non-diabetic adults (M/F:18 / 22, 43.30 ±1.07 years) were recruited from local community and 40 diabetic patients (M/F:11 /29, 52.56±1.53 years) were recruited. After subjects had rested in sitting position for 10 mins, they stood for 3 minutes and squatted for 2mins. Recording of systolic and diastolic blood pressure (SBP, DBP) and heart rate (HR) was done in each position using automated blood pressure monitor (Omron). Mean arterial blood pressure (MAP) was calculated using the formula (1/3 Pulse pressure + Diastolic blood pressure) and Pulse Pressure (PP) was calculated using the formula; SBP - DBP. Changes in BP and HR between sitting and standing and between standing and prompt squatting were calculated. Group comparisons were done and the effect of postural stress was determined using unpaired Student's T test. Statistical significance was determined at level of $p < 0.05$.

Result.

In diabetics and in non-diabetics, standing from sitting elicited increase in SBP of +4.63±1.41 vs +5.05±1.41 mmHg, DBP of +6.60±0.91 vs +4.50±0.70 mmHg, MABP of +7.44±1.75 vs +5.02±0.73 mmHg, PP of -1.98±1.19 vs +0.55±1.42mmHg and HR of +6.95±0.96 vs +4.78±0.96 bpm ($p > 0.05$ respectively).

Squatting elicited greater pressor responses in non-diabetic women relative to non-diabetic men with increase in SBP of +9.46±1.90 vs +1.44±2.26mmHg, $p = 0.009$; DBP of +3.00±1.38 vs -3.33±1.37mmHg, $p = 0.003$; MAP of +5.15±1.30 vs -1.82±1.44mmHg $p = 0.001$. The change in heart rate was similar in non-diabetic women (+1.36±2.77 bpm) and men (+1.83±1.11bpm, $p = 0.89$).

The diabetic men and women showed similar increase in SBP of +17.69± 2.57 vs +16.64± 4.26 mmHg, DBP of 5.48±1.49 vs 4.09±1.97mmhg and MABP of +8.77±1.90 vs +8.27±2.10 mmHg, ($p > 0.05$ respectively) in response to squatting. The difference in the HR between the diabetic women (-2.55±1.32bpm) and men (1.64±2.75bpm) was not significant ($p = 0.13$).

Conclusion.

In diabetic and non diabetic middle aged Nigerian adults, standing elicited pressor responses rather than a decrease. In the diabetic group, there were no

sex differences in the responses to squatting. However, the pressor responses to squatting were greater in the non-diabetic women relative to men, however there was no concurrent bradycardia, suggesting blunted baroreceptor activity. Unexpectedly, the results suggests greater vascular tone and autonomic dysregulation in the non-diabetic women who therefore may be at greater risk of developing stress induced cardiovascular disorder.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC138

Alanine scanning of exenatide reveals signalling bias at the recombinant GLP-1 receptor

J. Naylor¹, G. Geneci¹, J. Dodgson², S. Oldham¹, S. Will¹, J. Metcalfe², M. Bednarek² and D.C. Hornigold¹

¹Cardiovascular & Renal Metabolism, AstraZeneca, Cambridge, UK and ²Antibody Discovery and Protein Engineering, AstraZeneca, Cambridge, UK

The glucagon-like peptide-1 receptor (GLP1R) plays a key role in glucose homeostasis and as such is an important target for type 2 diabetes therapeutics. The endogenous ligand for the receptor, GLP-1, has a short half-life in vivo and is rapidly degraded by peptidases. GLP-1R agonists in the clinic must be stabilised against such degradation by amino acid substitutions, lipidation or fusion to immunoglobulins. Despite the method of stabilisation, GLP-1R agonists in the clinic retain a similar profile for gastrointestinal adverse events. Exenatide is a stable homologue of GLP-1 that was first approved in 2005, marketed under the brand name Byetta. We sought to investigate residues critical to function by performing alanine scanning of the exenatide peptide.

Alanine substitutions in positions 1, 4, 7, 9 and 22 caused a complete loss of β -arrestin1 binding and receptor internalisation. These residues are conserved in the related peptides glucagon and oxyntomodulin. Calcium signalling was also attenuated, however surprisingly, cAMP signalling was unaffected for these substituted peptides in terms of maximum effect (all were >90% of reference exenatide response). The alanine substituted peptides elicited an equivalent response to native exenatide peptide (70 ng/ml insulin) in glucose stimulated insulin secretion by the rat β -cell line INS-1 832/3, and were also equivalent at controlling glucose as measured by a lean mouse (C57Bl/6, n=7) ipGTT (Blood Glucose <200 mg/dL post 2 g/kg glucose bolus, p<0.0001 vs. vehicle by one-way ANOVA). This suggests that while critical for β -arrestin and Gq-coupled signalling, these key residues are not important for β -cell function. Substitution in position 1 with phenylalanine has been reported previously¹. In our hands, [Phe1]-Exenatide was a partial agonist in cAMP accumulation assays (maximum effect 41% of reference exenatide response), highlighting the importance of cell lines used in terms of 'method bias'.

The identification of key residues involved in β -arrestin binding, receptor internalisation and G protein signalling may aid the design of biased agonists for the treatment of diabetes, selecting beneficial outcomes and minimising adverse events. Alanine-mutated exenatide peptides will be progressed to rodent taste aversion tests to determine whether signalling bias at the GLP-1R is important in cell types other than the β -cell.

Jones, B; Buenaventura, T; Kanda, N; Chabosseau, P; Owen, BM; Scott, R; Goldin, R; Angkathunyakul, N; Corrêa, IR Jr; Bosco, D; Johnson, PR; Piemonti, L; Marchetti, P; Shapiro, AMJ; Cochran, BJ; Hanyaloglu, AC; Inoue, A; Tan, T; Rutter, GA; Tomas, A; Bloom, SR. Targeting GLP-1 receptor trafficking to improve agonist efficacy. *Nature communications* 2018, 9, 1602, 10.1038/s41467-018-03941-2

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC139

Suppression of (pro)renin receptor expression inhibits autophagy and decreases cell proliferation in cultured cancer cells

K. Takahashi, S. Sato, M. Endo, Y. Yokota and K. Ohba

Department of Endocrinology & Applied Medical Science, Tohoku University Graduate School of Medicine, Sendai, Japan

(Pro)renin receptor ((P)RR), a receptor for renin and prorenin, is expressed in various types of normal and cancer cells. (P)RR is related to both the renin-angiotensin system and vacuolar H-ATPase (V-ATPase) (1). Binding of renin and prorenin to (P)RR stimulates the intracellular signaling pathways, including ERK 1/2. An inhibition of V-ATPase function caused inhibition of autophagy. We have previously reported expression of (P)RR in the tumor tissues of breast cancers and cultured human breast cancer cells (2). Suppression of (P)RR expression by (P)RR-specific siRNA decreased cell proliferation of cultured breast cancer cells, suggesting that (P)RR acts as a stimulating factor for cancer cell proliferation. However, the relationship of (P)RR with ERK phosphorylation and autophagy has not been clarified in cancer cells. The aim of the present study was therefore to clarify the relationship of (P)RR with ERK phosphorylation and autophagy in proliferation of cancer cells. The experiments were performed in MCF-7 human breast cancer cells, T47D human breast cancer cells and HepG2 human liver cancer cells. Cell proliferation was examined by the WST-8 method. The expression levels of (P)RR and autophagy-related proteins, LC3 and p62, and ERK phosphorylation were examined by western blot analysis. Each experiment was repeated at least 3 times. Statistical analysis was performed by ANOVA, followed by Tukey–Kramer test. Suppression of (P)RR expression using (P)RR-specific siRNA decreased cell proliferation in MCF-7, T47D and HepG2 cells (36%, 49% and 81% of control, respectively; $p < 0.0001$ in all three cell lines, $n = 6$). By suppressing the expression of (P)RR, phosphorylation of ERK was activated about 5-fold in MCF-7 cells, whereas it was suppressed in T47D and HepG2 cells (about

40% and 60% of control). Furthermore, the intracellular amount of LC3 and p62, autophagy-related proteins, increased by suppressing the expression of (P)RR in all three types of cell lines (LC3, about 2-3-fold; p62, about 1.3-1.5-fold), suggesting that autophagy was inhibited by suppression of the (P)RR expression, and LC3 and p62 accumulated in the cancer cells. These findings suggest that proliferation of cancer cells by (P)RR may be mediated by signaling pathways other than the ERK signaling, possibly the regulation of autophagy via the V-ATPase function.

Nguyen G. Clin Sci (Lond) 120:169-178; 2011.

Ohba K., et al. Biomed Res (Tokyo) 35:117-126; 2014.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC140

L-ergothioneine supplement protect testicular functions in cisplatin-treated wistar rats

A. Dare¹, O.T. Olaniyan², M.A. Salihu¹ and L.K. Ilesanmi¹

¹Physiology, Bingham University, Karu, Nigeria and ²Physiology, Edo state University, Iyamho, Nigeria

The clinical efficacy of cisplatin (cis) has been compromised due to organ toxicity (particularly the kidney, ear and testis) via the generation of reactive oxygen species. This suggests that a potent antioxidant could serve as an adjuvant to prevent cisplatin-induced toxicity during chemotherapy. L-ergothioneine (L-erg) is a diet-derived, natural antioxidant with potent cytoprotective activities in-vivo. It is well-tolerated and its safety evaluation has been well-documented. This study aimed to investigate the role of L-ergothioneine supplement against cisplatin-induced reproductive toxicity in male Wistar rats.

Twenty male Wistar rats (180±10g) were randomly divided into four groups (n=5) and subjected to treatment as follows: Group I (control)- distilled water (10mL/kg *oral*) for 14-days; Group II (Cis alone) - cisplatin (7-mg/kg *ip*) on the 10th day. Group III (L-erg alone)- L-erg (10mg/kg *oral*) for 14-days and group IV (Cis+erg) received both L-erg (for 14 days) and Cis (on the 10th day). The animals were euthanized after 14 days of treatment under thiopentone anesthesia (30 mg/kg BW, *ip*). Body weight and reproductive organ weight were recorded using a calibrated weighing scale. Semen analysis was done according to standard protocols via microscopy. Enzyme-Linked Immunosorbent assay was used to measure the serum level of Testosterone, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Biomarkers of lipid peroxidation (MDA) as well as antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GPx) were measured using respective bioreagent kit. Histological examination of the testicular tissues was done using the hematoxylin and Eosin (H&E dye) staining technique. Data were reported as mean ± sem compared by ANOVA with p<0.05 as a level of significance.

Cis+L-erg administration to male animals caused a non-significant difference in body weight, testicular weight, testosterone, FSH and LH when compared with the Cis-treated group. Cis+L-erg reduces abnormal sperm morphology (16 ± 2.45 vs 24 ± 2.45 Cis alone), percentage dead sperm cells (24 ± 2.91 vs. 38 ± 4.89 Cis) and increases sperm motility (60 ± 7.58 vs 39 ± 2.45 Cis). Cis+L-erg reduced MDA (7.63 ± 0.73 vs 14.07 ± 1.29 Cis) but increases SOD (1.13 ± 0.06 vs 0.79 ± 0.03 Cis alone), CAT (19.16 ± 2.41 vs 3.92 ± 0.61 Cis) and GPx (0.56 ± 0.01 vs 0.52 ± 0.01 Cis) activities in the testicular homogenates. Cis+L-erg also prevented the histological damage in the testes caused by cis alone.

The data from this study showed that L-erg supplement during cisplatin administration may protect the testes against cis-induced damages via increasing antioxidant enzyme activities and inhibiting lipid peroxidation.

CONCLUSION: L-erg supplement may be used as a therapeutic adjuvant against male reproductive toxicity during cisplatin therapy.

Soni KK et al (2016). Drug Design, Development and Therapy. 10, 3959–3968.

Paul BD and Snyder SH (2010). Cell Death and Differentiation. 17, 1134–1140.

Forster R et al (2015). Food chemical toxicology. 80, 85-91.

Rezvanfar MA et al (2013). Toxicology and Applied Pharmacology. 266, 356–365.

Sylla BS & Wild CP (2012). International Journal of Cancer. 130 (2):245-50.

The authors acknowledge the assistance of Mr. J. Egene during the semen analysis procedure.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC141

AMP-activated protein kinase inhibits insulin-stimulated glucose and fatty acid uptake in adipocytes

F. Alghamdi and I. Salt

Institute of Cardiovascular & Medical Sciences, university of glasgow, Glasgow, UK

Insulin plays a critical role in regulating cellular metabolism by promoting cellular glucose and fatty acid (FA) uptake, lipid synthesis and inhibiting gluconeogenesis and lipolysis. Although mechanisms of FA uptake have been described in myocytes, FA uptake in adipocytes remains poorly characterised. AMP-activated protein kinase (AMPK) has been proposed to be a potential therapeutic target for obesity and type 2 diabetes due to its critical role in the maintenance of cellular and whole-body energy balance. AMPK activation by AICAR (5-Aminoimidazole-4-carboxamide ribonucleotide) is associated with an increase in GLUT4 translocation and glucose uptake in skeletal muscle. However, AICAR has been previously demonstrated to increase basal, yet inhibit insulin-stimulated glucose uptake. AMPK activation has been reported to increase FA uptake in myocytes in a manner analogous to glucose

uptake. This study aimed to characterise the effect of AMPK activity on FA uptake and the expression of FA transporter proteins.

The effect of AMPK activators on glucose or FA uptake was assessed in 3T3-L1 adipocytes incubated with AICAR (2 mM, 60 min) and/or the direct AMPK activator A769662 (300 μ M, 60 min) prior to stimulation with insulin. 2-[^{3} H]-deoxy-D-glucose or [^{3} H]palmitate uptake was subsequently assessed. To examine whether lack of AMPK activity influenced expression of glucose and FA transporters, the levels of glucose transporter (SLC2A1 and SLC2A4) and FA transporter (SLC27A1, SLC27A4 and CD36) mRNA were assessed by RT-PCR in epididymal adipose tissue from wild-type and AMPK α 1 knockout mice fed chow or high fat diet.

Insulin significantly increased glucose uptake ~2.5-fold (mean $\pm$ SEM, n=5, p<0.0001, 2-way ANOVA), an effect that was significantly inhibited by preincubation with A769662 (~70%, n=5, p<0.0001, 2-way ANOVA) and tended towards inhibition with the less-selective AMPK activator AICAR (~30%, n=5, p=0.13, 2-way ANOVA). Recent studies have indicated that insulin-stimulated FA uptake is mediated by mTOR, yet inhibition of mTOR with rapamycin had no effect on insulin-stimulated glucose uptake. Insulin stimulation significantly increased [^{3} H]palmitate uptake ~2-fold in 3T3-L1 adipocytes, an effect inhibited by A769662 (n=4, p<0.01, 2-way ANOVA). There were no significant differences in the levels of mRNA encoding FA and glucose transporters in adipose tissues between wild type and AMPK α 1 knockout mice or between diets.

These data indicate that AMPK activation inhibits insulin-stimulated FA uptake in 3T3-L1 adipocytes, analogous to the inhibitory effect of AMPK on insulin-stimulated glucose uptake. Furthermore, lack of AMPK α 1 has no effect on levels of glucose or FA transporter mRNA in adipose tissue. These data highlight the contrasting actions of AMPK activation on nutrient influx in adipocytes compared with myocytes, where AMPK activation has been reported to increase both glucose and FA uptake under basal and insulin-stimulated conditions.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC142

Maintenance Impact of Large doses of Vitamin C in Pancreatized Rat Model

O.M. Azeez¹, A. Adetayo S.², Z.S. Abdulkadir² and K.T. Biobaku³

¹Department of Veterinary Physiology and Biochemistry, University of Ilorin, Ilorin, Nigeria, ²Veterinary Surgery and Radiology, University of Ilorin, Ilorin, Nigeria and ³Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Nigeria

Background

The pancreas is an organ which secretes digestive juices for digestion of food. It harbours Islets of Langerhans which secrete insulin for maintaining blood sugar levels. Pancreatic cancer is an aggressive cancer. Surgery to remove the cancer improves survival. However a third of patients with pancreatic cancer have the fear

of increased complications after surgery. Resection (removing part of pancreas) has been suggested but to the best of our knowledge randomized controlled trials done so far have not given any conclusion.

Materials and Method: Forty Wistar rats were used in two phases. The two phases were the same procedure but in addition phase two rats were given 1000mg/kg of vitamin C as primer before and after the surgery. Each phase had four groups of five rats each. Groups 1-3 had resection done as follows: one quarter ($\frac{1}{4}$), half ($\frac{1}{2}$), three quarter ($\frac{3}{4}$) resection of pancreas while group 4 served as control. Rats were anesthetized with 90mg/kg ketamine and 10mg/kg Xylazine. Midline laparotomy incision was made on the lineal alba to expose the contents of abdominal cavity. One quarter, half and three quarter of each pancreas was excised without interfering with the pancreatic duct. The duodenal loop was then returned back into the abdominal cavity. Linea alba was closed with 5-0 chromic catgut using simple continuous suture pattern while the skin was closed with 5-0 nylon using simple interrupted suture pattern.

Results: First two weeks our report showed 100% survival of $\frac{1}{4}$ resection group in both phases, 40% of survival of $\frac{1}{2}$ resection group and 100% mortality of $\frac{3}{4}$ resection group in the non-vitamin C supplemented phase. There was 80% survival of ($\frac{1}{2}$) resection group and 60% survival ($\frac{3}{4}$) resection group in the vitamin C supplemented group. Our ECG result showed no significant difference in heart rate of all groups in 'P', PR, QRS after two weeks of resection but QTc in $\frac{3}{4}$ resection was significantly higher than the other groups. Blood glucose level checked every day post operation remained within normal range in all the groups. Serum cortisol level was significantly higher in $\frac{3}{4}$ group (33.959 ± 1.04) ng/ml than $\frac{1}{2}$ (18.49 ± 0.66) ng/ml, $\frac{1}{4}$ (22.409 ± 1.02) ng/ml and control (16.88 ± 0.97) ng/ml of the vitamin c group. No significant difference was seen in T3 values of $\frac{3}{4}$ and $\frac{1}{2}$ but the two were insignificantly higher than $\frac{1}{4}$ and control groups. No significant difference was observed in the T4 of all the groups.

Discussion and conclusion: Vitamin C is a strong antioxidant that can strengthen body's natural defenses that boost the immune system. It protects cells from free radicals, encourage the production of white blood cells. It also helps these wbc function more effectively while protecting cell from damage by potentially harmful molecules. Studies have also shown that vitamin C may shorten wound healing time. Conclusively, vitamin C could offer longevity to the life of pancreatectomized individual regardless of the size of the pancreas resected.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Bioinformatics guided study on the anticancer effect of aspirinZ. Zhang¹ and L. Shang^{1,2}¹Northwest University, Xi'an, China and ²Bradford University, Bradford, UK

Use of aspirin for cancer prevention and treatment have been reported extensively in recent years. But inconsistent results and unclear molecular mechanism of aspirin on cancer argue that it should be more cautious of using aspirin for long-term prevention for cancer [1-4]. In this study, we took aspirin as bait and put it in the comparative toxicogenomics database to search for all potential protein targets of aspirin against disease. These protein targets were subjected for GO enrichment analysis using the metascape online website. Meanwhile, p-value was corrected by false discovery rate (FDR). The threshold of p-value less than 0.5 was set as significantly related biological process. Finally, obtained differentially expressed genes were subjected to KEGG pathway enrichment analysis to find significant differences biological regulatory pathways. Human breast cancer cells were then cultured in vitro with aspirin to observe the changes in cancer cell phenotype and to select the signal pathway to be studied.

Based on the results of KEGG pathway enrichment analysis and in vitro cell culture phenotypic changes study, we chose two downstream key molecules of the MAPK signaling pathway, VEGF and MMP, as candidates to study. The effects of aspirin on breast cancer cells were verified by cell experiments and molecular experiments. Meanwhile, we added platelets as experimental variables in the experimental group to investigate whether platelets can interfere with this effect of aspirin. In vivo breast cancer mouse model was also used.

A total of 714 aspirin protein targets were obtained through searching the comparative toxicogenomics database. The results of GO enrichment analysis showed that the top 10 biological processes with the highest correlation with aspirin were cytokine-mediated signaling pathway, positive regulation of cell death, response to lipopolysaccharide, inflammatory response, response to inorganic substance, fluid shear stress and atherosclerosis, apoptosis, response to wounding, response to toxic substance, and pathways in cancer.

In vitro results showed that aspirin has an inhibitory effect on the proliferation and migration on tumor cells. Aspirin can block the in vitro epithelial-mesenchyme transformation of breast cancer cells and down-regulate the expression of VEGF and MMP-9. Platelets however played the opposite effect. After co-cultivation of aspirin, platelets and cancer cells, aspirin and platelets could interfere with each other. In vivo breast cancer mouse model experiments showed the similar results. In conclusion, our results indicate that aspirin can affect multiple biological processes in the organism. Aspirin can inhibit the development of breast cancer at the cellular level or organism to some extent. The mechanism to inhibit the development of cancer may be due to inhibiting the epithelial-mesenchymal transition of tumor cells and down-regulating the expression of VEGF and MMP-9.

P.M. Rothwell, J.F. Price, F.G. Fowkes, A. Zanchetti, M.C. Roncaglioni, G. Tognoni, R. Lee, J.F. Belch, M. Wilson, Z. Mehta, T.W. Meade, Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials, *Lancet* 379(9826) (2012) 1602-12.

K. Bibbins-Domingo, Aspirin Use for the Primary Prevention of Cardiovascular Disease and Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement, *Annals of internal medicine* 164(12) (2016) 836-45.

K.A. Orrell, A.D. Cices, N. Guido, S. Majewski, E. Ibler, T. Huynh, S.M. Rangel, A.E. Laumann, M.C. Martini, A.W. Rademaker, D.P. West, B. Nardone, Malignant melanoma associated with chronic once-daily aspirin exposure in males: A large, single-center, urban, US patient population cohort study from the "Research on Adverse Drug events And Report" (RADAR) project, *J Am Acad Dermatol* 79(4) (2018) 762-764

The Associated Press. Aspirin disappoints for avoiding first heart attack, stroke. The Associated Press, 2018-8-27

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC144

Skeletal myogenesis is accelerated by key growth factors contained in the human platelet secretome

D. Scully¹, P. Sfyri¹, S. Verpoorten¹, R. Mitchell², L. Gutierrez³, K. Patel² and A. Matsakas¹

¹Centre for Atherothrombosis & Metabolic Diseases, Hull York Medical School, Hull, UK, ²School of Biological Sciences, University of Reading, Reading, UK and ³Dept. of Medicine, Universidad de Oviedo, Instituto de Investigación Sanitaria del Principado de Asturias, Oviedo, Spain

Introduction: Biomaterials such as autologous platelets have recently gained intense research interest (1). However, the mechanisms regarding platelet mediated skeletal myogenesis remain to be established. The aim of this study was to determine the role of platelet releasate in skeletal myogenesis and muscle stem cell fate *in vitro* and *ex vivo* and *in vivo*.

Methods: The effects of platelet releasate on proliferation and differentiation of skeletal myoblasts by means of cell proliferation assays, immunohistochemistry, gene expression and cell bioenergetics were assessed. We expanded *in vitro* findings on single muscle fibres by determining the effect of platelet releasate on murine skeletal muscle stem cells *ex vivo* using protein expression profiles for key myogenic regulatory factors. Most importantly, we validated our findings on an *in vivo* model of acute muscle injury *via* cardiotoxin. Statistical analysis was performed by one way ANOVA followed by Tukey's post hoc test.

Results: We have shown that platelet releasate dose dependently stimulates myoblast proliferation and temporally stimulates differentiation *via* upregulation of MyoD and an increase in muscle stem cell commitment to differentiation.

Proteomics revealed that the abundance of PDGF and VEGF ligands varies according to the platelet agonist used. Inhibiting these ligand binding sites dose dependently inhibited myoblast proliferation. Skeletal muscle stem cell markers for *ex vivo* proliferation and cell fate lineage (Cyclin D1 and Scrib, respectively) were robustly upregulated when exposed to platelet releasate. Most importantly, the translational aspect of platelet releasate to an *in vivo* model promoted myogenic regeneration. We have shown novel data that supra physiological levels of the platelet secretome can further increase myoblast proliferation in a dose dependent manner.

Conclusion: Platelet releasate is a powerful driver of skeletal myoblast proliferation (2). This study provides novel mechanistic insights on the role of platelet releasate in skeletal myogenesis by directing muscle stem cell fate and sets the physiological basis for exploiting platelets as biomaterials in regenerative medicine.

Scully, D., Naseem, K., Matsakas, A. Platelet Biology in Regenerative Medicine of Skeletal Muscle. *Acta Physiologica*. (2018) Apr 06:e13071

Scully, D., Sfyri, P., Verpoorten, S., Papadopoulos, P., Muñoz-Turrillas MC., Mitchell, R., Aburima, A., Patel, K., Gutiérrez, L., Naseem, KM., Matsakas, A. Platelet releasate promotes skeletal myogenesis by increasing muscle stem cell commitment to differentiation and accelerates muscle regeneration following acute injury. *Acta Physiologica*. 19 Oct 2018:e13207

The study was supported by the European Union, The Royal Society and The British Heart Foundation (Grants: FP7-PEOPLE-PCIG14-GA-2013-631440, RG140470 Research Grant and BHF RG/16/5/32250 respectively).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC145

Hepatic transcriptome reveals novel gene targets of the synthetic retinoid Fenretinide in mouse models of obesity

S. Mahmood¹, N. Morrice^{1,2}, D. Thompson¹ and N. Mody¹

¹Aberdeen Cardiovascular & Diabetes Centre, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK and ²Centre for Genome Enabled Biology and Medicine (CGEBM), University of Aberdeen, Aberdeen, UK

Within cells, retinol is converted to retinoic acid (RA), the direct ligand for the nuclear RA-receptors (RARs) that bind to promoters of target genes to modulate transcription. Alterations in retinoid levels and RA-responsive genes have been observed in several models of obesity and type-2 diabetes. The synthetic retinoid Fenretinide (FEN), which can signal through RAR, inhibits adiposity and improves glucose homeostasis in mice fed a high-fat diet (HFD)¹. However, the mechanisms behind these effects remain poorly understood. To identify target genes of FEN responsible for its beneficial effects, we performed global RNA-sequencing analysis in livers from male C57BL/6 mice fed 45% HFD (D12450B, Research Diets) +/- 0.04% FEN for both 7 days and 20 weeks (n=4, per group). Differential gene expression

statistical analysis performed by DESeq2². Animal procedures were approved by University of Aberdeen Ethics Review Board and performed under project and personal licences approved by the UK Home Office. Chronic HFD feeding led to major alterations in gene expression with induction of genes regulating hepatic lipid metabolism e.g. very low density lipoprotein receptor, *Vldlr* (up 1.8-fold log₂ scale, adjusted p<0.001) and repression of several members of the major urinary protein gene family such as *Mup2*, repressed (down 2.4-fold log₂ scale, adjusted p<0.001). Chronic FEN treatment normalized the expression of many of these genes, including completely repressing levels of the metabolic regulator FGF21³. *Cyp2c70*, an enzyme involved in bile acid metabolism, was identified as a novel induced hepatic FEN target, later confirmed by qPCR of livers from several FEN treated models. Data analysed by Pfaffl method, mean+/-SEM calculated and statistical analysis by one-way ANOVA. Particularly, genetically obese male C57BL/6 *Lep^{db/db}* mice had 80% repressed *Cyp2c70* expression compared to lean counterparts (n=7-8, p<0.001) which was rescued by FEN treatment to 47% (n=7, p<0.001). Other bile acid metabolism genes *Cyp39a1* and *Cyp46a1* were also amongst genes significantly altered, suggesting that FEN has a major effect on this pathway. Obesity and metabolic dysregulation led to major alterations in many more genes with diverse physiology e.g. arginine vasopressin receptor 1A (*Avpr1a*) was repressed in multiple disease models e.g. ~90% (n=6-7, p<0.001) decreased in genetically obese BTBR female *Lep^{ob/ob}* mice, with and without FEN compared to lean controls (n=7). Our findings confirm the liver as a target tissue of FEN treatment in multiple disease models of obesity. Furthermore whole transcriptome analyses in gene expression associated with HFD feeding +/- FEN may have identified novel pathways of disease, giving scope for possible targets for the intervention of impaired lipid and glucose homeostasis.

McIlroy G, et al. *Diabetes*. 2013;62(3):825-836. doi: 10.2337/db12-0458

Morrice N, et al. *Sci Rep* 2017 Mar 3;7:43782 doi: 10.1038/srep43782

This study was supported by a BHF Fellowship FS/09/026 and Tenovus Scotland grant G14/14 to N. Mody; James Mearns Trust PhD studentship to S. Mahmood and PhD studentship to N. Morrice (Centre for Genome Enabled Biology and Medicine, CGEBM); CGEBM is supported by Friends of Anchor

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC146

Vitamin D receptor regulation of skeletal muscle mass through coordination of muscle remodelling and energy metabolism genesets

J.J. Bass¹, D. Wilkinson¹, K. Smith¹, A. Philp², N.J. Szewczyk¹, M.E. Cleasby³, I.J. Gallagher⁴ and P. Atherton¹

¹MRC/ARUK Centre for Musculoskeletal Ageing Research and National Institute for Health Research (NIHR), Nottingham Biomedical Research Centre (BRC), The University of Nottingham, Derby, UK, ²Mitochondrial Metabolism & Ageing Laboratory, Diabetes and Metabolism Division, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia, ³Dept. of Comparative Biomedical Sciences, Royal Veterinary College, London, UK and ⁴Faculty of Health Sciences & Sport, University of Stirling, Stirling, UK

Vitamin D (VitD) deficiency is common in ~25% of the population, and is considered to have actions upon skeletal muscle metabolism and health, and is associated with age-related sarcopenia (1). VitD regulates and exerts its effects through the ubiquitously expressed VitD receptor (VDR); a potent transcription factor, controlling the expression of multiple genes at VitD response elements initiating transcription (2). Recent investigations have established a VDR autonomous role in muscle function and regeneration (3). Thus, we aimed to investigate whether VDR has a functional role in modulating key regulatory pathways/ genesets important to muscle growth and homeostasis.

To examine the role of VDR in muscle mass regulation, *Tibialis Cranialis*(TC) muscles of Wistar rats were electroporated (under 2.5% isoflurane, 50mg/kg carprofen) to continuously over-express (VDR-OE) or knockdown (VDR-KD) VDR by cDNA or shRNA lentiviral transfection; contralateral TC's were sham treated internal controls. RNA from muscles underwent RNA-Seq using the Illumina HiSeq system, before analysis of differential gene expression, geneset enrichment and pathway analysis. False discovery rates were applied throughout.

VDR-OE stimulated fibre hypertrophy (cross-sectional area (CSA) +17±7%, $P<0.05$) and enhanced muscle protein synthesis (+69±7%, $P<0.05$). This was matched by an upregulation in key growth-related extracellular remodeling, *Paxand integrin*-pathway genesets. Comparatively, VDR-KD induced myofibre atrophy(CSA -8±2%, $P<0.001$)and autophagy related processes (e.g. LC3B-II +84±43%, $P<0.05$). This was complemented by an upregulation in multiple lysosome and vesicle biogenesis genesets. Moreover, many energy metabolism related genes were downregulated, i.e. multiple electron transport complex, oxidative phosphorylation, glycolysis and citric acid cycle genesets.

Thus, the VDR acts autonomously to regulate muscle mass; operating through reciprocal geneset modulation to facilitate hypertrophy by enhanced remodeling and anabolic signalling pathways, whilst limiting atrophy through regulating energy metabolism and autophagy. Therefore, the VDR has an essential role in the coordination of genesets key to the maintenance of muscle mass.

Cangussu LM, Nahas-Neto J, Orsatti CL, Bueloni-Dias FN, Nahas E a. P. Effect of vitamin D supplementation alone on muscle function in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial. *Osteoporos Int.* 2015

Toell A, Polly P, Carlberg C. All natural DR3-type vitamin D response elements show a similar functionality in vitro. *Biochem J.* 2000

Srikuea R, Zhang X, Park-Sarge O-K, Esser K a. VDR and CYP27B1 are expressed in C2C12 cells and regenerating skeletal muscle: potential role in suppression of myoblast proliferation. *Am J Physiol Cell Physiol.* 2012

This work was supported by the Medical Research Council [grant number MR/J500495/1]. Additionally, the MRC/ARUK (MRC: MR/K00414X/1 and ARUK: 19891) and NIHR Nottingham Biomedical Research Centre for providing centre funding and supporting our work.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC147

Caveolin-1 K.O. mice exposed to a high fat diet exhibit a lipotoxic milieu but less beta pancreatic damage compared to wild type mice

P.M. Lillo¹, S. Wehinger², P. Llanos^{1,3} and O. Nuñez²

¹Facultad de Medicina, Universidad de Chile, Chile, Chile, ²Department of Clinical Biochemistry and Immunohematology, Universidad de Talca, Talca, Chile and ³Instituto de investigación en ciencias odontológicas, Universidad de Chile, Santiago, Chile

Background: Deleterious effects of high levels of free fatty acids (FFA) lead to a phenomenon known as lipotoxicity, associated with insulin resistance and beta pancreatic cell damage, key events in development of type 2 diabetes (T2D). Lipotoxicity has been associated with cellular oxidative stress and beta-cell apoptosis, however, many aspects of cellular mechanisms involved remains to be defined. Caveolin-1 (CAV1) is a membrane protein normally present in beta pancreatic cells, but its role in these cells has been poorly explored. We previously reported that the expression of the membrane protein CAV1 promotes palmitate-induced apoptosis in vitro in a mouse beta cell line. However, remains to be elucidated if this phenomenon is relevant in vivo, in a whole organism subjected to lipotoxic conditions induced by a high fat diet (HFD). Our aim was to elucidate whether in mice lacking CAV1 expression, their pancreatic islets present a greater resistance to lipotoxic effects of a HFD. Materials and methods: We used C57BL6J WT mice and K.O. mice lacking expression of Caveolin-1 (CAV1 K.O. mice). Mice were anesthetized by an intraperitoneal injection of ketamine/xylazine (100/10mg/kg) before surgery. The pancreases were removed by laparotomy. We evaluated the FFA, triglycerides and insulin levels in blood in fasting conditions, oral glucose tolerance test (GTT), carbonylated proteins in serum and C-peptide in WT mice and CAV1-K.O. mice exposed to HFD 60% for 3 months, and they were compared with mice fed with a

control diet (CD). Also, the presence of apoptosis was evaluated by TUNEL staining in beta pancreatic islets from K.O. and WT mice. All the experiments had groups of animals >5 and <8 individuals. Results: We found that HFD WT mice, showed higher levels of triglycerides ($p<0.05$), cholesterol ($p<0.01$), FFA (N.S.), insulin ($p>0.05$) and carbonylated proteins ($p<0.001$) compared to CD WT mice. CAV1 K.O. mice showed an altered profile even in C.D, but there were not significant differences compared with HFD. The levels of FFA were high with C.D., but the HFD did not change these levels significantly. The HFD worsened the response to GTT in WT mice, increasing AUC ($p<0.01$). In contrast, K.O. mice presented an altered GTT with C.D., but the HFD did not make it significantly worse. Interestingly, the C-peptide levels were significantly higher in K.O. mice fed with HFD compared with K.O. CD ($p<0.001$) and also even with WT HFD. Importantly, islets from HFD K.O. mice showed lower levels of apoptosis compared with HFD WT mice, evidenced by TUNEL staining (9.6 vs 15.5% TUNEL positive nuclei). Conclusion: Our results suggest that their pancreatic islets were more resistant to HFD deleterious effects over beta cells, accordingly with our previous results in vitro. We think that this could be a very interesting aspect of beta cell biology that could help to identify targets for advanced therapies in preventing T2D.

P. Lillo³, S.A. Wehinger^{1,2}, P. Llanos^{3,2}, O. Núñez⁴;

¹Department of Clinical Biochemistry and Immunohematology, University of Talca, Talca, Chile, ²Center for studies of Exercise, Metabolism and Cancer (CEMC), Santiago, Chile, ³Institute of Research in Odontological Sciences, University of Chile, Santiago, Chile, ⁴Magister in Biological Sciences, University of Talca, Talca, Chile.

Supported by FONDECYT Grant 11160257

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC148

Biosensors HyPer and HyPer2 to monitor intracellular hydrogen peroxide in skeletal muscle cells and fibres

E. Fernández-Puente^{1,2,3}, M. Sánchez-Martín^{4,5,3}, L. Méndez⁵ and J. Palomero^{1,2,3}

¹Physiology and Pharmacology, University of Salamanca, Salamanca, Spain, ²Institute of Neurosciences of Castilla y León (INCYL), Salamanca, Spain, ³Institute of Biomedical Research of Salamanca (IBSAL), Salamanca, Spain, ⁴Medicine, University of Salamanca, Salamanca, Spain and ⁵Unit of Transgenesis, University of Salamanca, Salamanca, Spain

Hydrogen peroxide (H_2O_2) is one of the Reactive Oxygen Species (ROS) that seems to play an essential role in pathophysiological processes. H_2O_2 might act as a signalling molecule and modulate different crucial cellular signalling pathways. However,

identification of location where H_2O_2 is generated and quantification of H_2O_2 flux in cells and cellular compartments are virtually impossible with traditional methodology.

The aim of this work was to implement and develop methodological strategies based on the use of a hydrogen peroxide biosensor, HyPer, in skeletal muscle cells, such as myoblasts, myotubes and individual muscle fibres isolated from the flexor digitorum brevis (FDB) mouse muscle. The expression of HyPer biosensor in the cytosol of these cells followed by live cell fluorescence microscopy image analysis permits to monitor and register intracellular H_2O_2 in situ and in real time in those cells.

HyPer biosensor is synthesised intracellularly in cells. Thus, the coding DNA sequence of HyPer, inserted in a cloning vector, had to be incorporated into the cell to achieve biosensor expression. For this purpose, we carried out transfection techniques based in chemical agents for HyPer transfection in C2C12 myoblasts and C2C12 myotubes. In the case of skeletal muscle fibres, a combination of microinjection and electroporation techniques were undertaken to incorporate the coding sequence of HyPer into muscle fibres of the FDB muscle. Analysis of live cell fluorescence microscopy images confirmed both expression and functionality of HyPer as H_2O_2 biosensor. We monitored fluorescence emitted by cells expressing HyPer during an experimental period in which cells or isolated fibres were exposed to extracellular hydrogen peroxide in the medium or exposed to a reducing agent, dithiothreitol (DTT). An improved biosensor, HyPer2, potentially with higher H_2O_2 sensitivity than HyPer, was assayed in muscle fibres and proved to detect intracellular H_2O_2 generated by an extracellular enzymatic system, glucose oxidase (GOX) / catalase, which produces and hydrolyzes H_2O_2 . Statistical analysis: paired Student's t test for single comparisons and significance set at $p < 0,05$.

Results indicate that (i) It is possible the transfection and intracellular expression of hydrogen peroxide biosensor HyPer in C2C12 myoblasts and myotubes and in single isolated skeletal muscle fibres, ii) HyPer biosensor is functional and detects changes in the intracellular concentration of hydrogen peroxide, and (iii) HyPer2 biosensor is more sensitive and detects intracellular changes of H_2O_2 in skeletal muscle fibres where H_2O_2 had been generated by an extracellular enzymatic system, GOX/catalase.

In conclusion, using chemical transfection techniques and a combination of electroporation and transfection, it is possible to incorporate HyPer and HyPer2 biosensors into skeletal muscle cells and register intracellular H_2O_2 flow in these cells and fibres in situ and in real time.

Thanks to grant support in Spain by Ministerio de Economía, Industria y Competitividad (SAF2017-85762-R), Junta de Castilla y León – Consejería de Sanidad (BIO/SA73/14) and Universidad de Salamanca (18KA7D/463AC01).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC149

Autophagy Regulates Extracellular Vesicle Formation in Cells under Metabolic Stress.

A. Muniz¹, M. Romero², A. Zorzano² and S. Mora¹

¹Physiology, University of Liverpool, Liverpool, UK and ²IRB, Barcelona, Spain

Extracellular vesicles (EVs) are vesicles released by cells that carry proteins, nucleic acids and lipids. They have been widely described as important for cell-to-cell communication, causing physiological changes in recipient cells. Despite the increase of their study, molecular mechanisms responsible of their biogenesis and release are still largely unknown. We previously reported that hypoxia increases the release of EVs in adipose cells. On the other hand, autophagy is a cellular homeostasis maintenance response also activated upon low oxygen conditions.

Aim: To determine whether hypoxia and autophagy increase EVs release.

Methods: Several cell lines (HEK293, 3T3 fibroblasts and SK1-N neuroblastoma cells) were exposed to normoxic or hypoxic conditions (1% O₂) for different periods of time. Autophagy was induced by nutrient starvation with Hank's Balanced Salt Solution (HBSS). EVs release profile and autophagy activation were determined in control normoxic, hypoxia exposed or nutrient starved cells. Autophagy progression was independently verified by over expression of an autophagy-related protein: TP53INP2. EVs were isolated from culture media supernatants by differential ultracentrifugation and quantified by Nanoparticle Tracking analysis. Induction of autophagy was confirmed by western blot of total cellular lysates. Data was statistically analysed running One-way ANOVA tests using GraphPad Prism 6.

Results: Hypoxia induced EVs release upon prolonged hypoxic stimulus and peaked at a time coincident with activation of autophagy response. Starvation-induced autophagy showed an increase on EVs release parallel to autophagy progression. Enhanced EVs release was also confirmed following the overexpression of TP53INP2.

Conclusion: Our results suggest that autophagy increases EVs release and that this may be one of the molecular mechanisms involved in EVs release under low oxygen conditions.

Work funded by the Ramon Areces Foundation and University of Liverpool.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC150

Comparative study of protein tyrosine phosphatase 1B (PTP1B) inhibitors in high-fat/high-cholesterol diet fed C57/B6 and ApoE^{-/-} mouse models of diabetes and cardiovascular diseases

S. Kamli-Salino, D. Thompson, N. Mody and M. Delibegovic

School of Medecine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterised with hyperglycaemia due to a loss of insulin production and/or action on its receptor and is often associated with obesity and cardiovascular disease. Protein-tyrosine phosphatase 1B (PTP1B) has been identified as a negative regulator of the insulin receptor and its expression is increased in T2DM patients. Moreover, PTP1B also plays a role in the modulation of inflammatory response via the JAK/STAT pathway. MSI-1436 and CPT157633 are PTP1B inhibitors which, separately, demonstrated promising effect on the insulin signalling pathway and in-vivo glucose homeostasis (Krishnan *et al.*, 2015; Thompson *et al.*, 2017). Inhibition of PTP1B with MSI-1436 also demonstrated protective and reversal effect against the development of atherosclerotic plaques via its anti-inflammatory action (Thompson *et al.*, 2017). The aim of this project is to compare the effects of both inhibitors at different concentrations on high-fat/high cholesterol diet (HFD) fed C57/B6 mice, then use the optimal dose in APOE^{-/-}, a mouse model of atherosclerosis. All animal procedures were performed under a project license approved by the U.K. Home Office under the Animals (Scientific Procedures) Act 1986. After 8 weeks on HFD, mice were injected weekly intraperitoneally for 5 weeks with saline (n=4) or different concentrations of PTP1B inhibitors (0.5, 1 and 5 mg/kg of animal; MSI 1mg/kg: n=4; others: n=5). Daily weight measurements and metabolic status assessments were performed during the treatment period. Preliminary data suggested that increasing dose of MSI-1436 promotes significant body weight loss after 5 weeks of treatments (MSI 5mg/kg compared to saline: two-way ANOVA, Bonferroni *post-hoc* test, $p<0.0001$), when CPT157633 did not affect body weight. This has been confirmed using the Echo-MRI, where a significant fat mass loss in MSI-1436 5mg/kg treated mice was observed (two-tailed t-test, $\alpha=0.05$, $p<0.05$) without any change in lean mass. Glucose test tolerance showed an improved glucose homeostasis in mice treated with higher dose of MSI-1436 (two-tailed t-test, $\alpha=0.05$, $p<0.05$). These preliminary data suggest that both drugs have a beneficial effect on glucose homeostasis and insulin signalling. The absence of weight loss using CPT157633 indicates that the drug does not cross the blood-brain barrier and does not have an effect on central leptin receptors but has a peripheral effect only while MSI-1436 is known to have an effect on both peripheral and central tissues. Further investigations are required to investigate PTP1B activity in brain tissues to assess the central effect of both drugs. Furthermore, improvement in glucose homeostasis, insulin signalling, and

atherosclerotic plaques formation will be assessed at the end of the study after 5 weeks of treatment with PTP1B inhibitors.

Krishnan, N. *et al.* (2015) *The Journal of Clinical Investigation*, 125(8), 3163–3177

Thompson, D. *et al.* (2017) *Clinical Science*, 131(20), 2489 LP-2501

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC151

Cbl/CAP are essential for mitochondria respiration complex assembly and bioenergetics efficiency in muscle cells.

C. Aye¹, D.E. Hammond¹, S. Rodriguez-Cuenca², M.K. Doherty³, P.D. Whitfield³, M. Phelan¹, C. Yang¹, R. Perez-Perez⁴, X. Li¹, A. Diaz-Ramos⁵, A. Vidal-Puig², A. Zorzano⁵, C. Ugalde⁴ and S. Mora¹

¹Physiology, University of Liverpool, Liverpool, UK, ²University of Cambridge, Cambridge, UK, ³University of Highlands and Islands, Inverness, UK, ⁴Hospital 12 Octubre, Madrid, Spain and ⁵IRB, Barcelona, Spain

Casitas b-lymphoma (CBL) is an adaptor protein that rapidly becomes phosphorylated following insulin receptor activation in adipose and muscle cells. While CBL null mice exhibits improved insulin sensitivity and enhanced glucose clearance, the molecular mechanisms have not been fully elucidated. Here we depleted CBL or the associated protein SORBS1 also known as Cbl Associated Protein (CAP) in C2C12 myocytes via lentiviral delivery of specific shRNAs to investigate the molecular mechanisms.

Methods: We determined AMPK activation by western blotting, mitochondrial mass by flow Cytometry, 2-deoxyuglucose uptake, mitochondrial respiration with SeaHorse analyser. We determined mitochondrial OXPHOS complex activities by spectrophotometric methods and mitochondrial respiratory complex assembly by Blue Native gel electrophoresis and western blot. Cellular proteome and metabolome was analysed by MS and NMR. ANOVA Statistical analysis of biochemical assays was performed using GraphPad Prism6. Bioinformatic analysis of proteome and metabolome data was carried out in R/Bioconductor package.

Results: CBL depletion increased AMP- regulated kinase activation in C2C12 cells, Consistently, CBL KD myocytes showed significantly increased basal and insulin-stimulated glucose uptake, increased expression of transcriptional coactivators PGC1 α and PGC1 β , and higher mitochondrial mass compared to control cells. Cellular respiration rates in CBL and CAP KD cells showed greater proton leak than control cells. Respiratory chain complex I (CI) activity and assembly into super-complexes I+III+IV were significantly reduced. Furthermore, cellular proteome and metabolome analyses revealed alterations affecting proteins involved in CI biogenesis, fatty acid degradation and glycolysis.

Conclusions: Our findings demonstrate that the CBL/CAP pathway couples insulin signalling to efficient mitochondrial respiratory function and metabolic adaptation in muscle cells.

This work was supported by a research grant from the European Commission (grant HEALTH-F4-2008-223450 to SM, AZ, AVP), by NIH-RO1 grant GM105781 (to CU) and by Instituto de Salud Carlos III-MINECO/European FEDER Funds grant PI17-00048 (to CU). MKD and PDW acknowledge funding from European Regional Development Fund, Scottish Funding Council and Highlands and Islands Enterprise.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC152

Diet-induced obesity impairs muscle stem cell function and redox homeostasis, which is linked to CD36 in mice

S. Verpoorten, D. Scully, P. Sfyri and A. Matsakas

Hull York Medical School, Hull, UK

The prevalence of obesity is one of today's greatest health challenges. Caused by a sedentary lifestyle, obesity is leading to a pandemic development of cardio-metabolic disorders, collectively described as the metabolic syndrome.

The recently described correlation between diet-induced obesity (DIO) and impaired muscle regeneration suggests an altered muscle stem cell response upon injury. In fact, ectopic lipid infiltration has been associated with metabolic deficits, further contributing to lipotoxicity and increased oxidative stress in skeletal muscle, possibly altering the muscle stem cell function. Muscle stem cells, also called satellite cells (SC), are of crucial importance for muscle regeneration. Given the importance of lipid accumulation in the context of impaired muscle metabolism, the fatty acid translocase CD36 has recently received a great amount of attention. CD36 deficiency has been shown to protect mice from DIO. However, the underlying protective mechanism of abrogated CD36 signalling is still largely unknown. Here we investigated the impact of CD36-deficiency on skeletal muscle oxidative stress and stem cell function in response to DIO.

Methods: All experiments were in accordance with UK Animals Scientific Procedures Act 1986. This study was carried out on male Wild-type (WT; C57/Bl6) and CD36 knockout (KO) mice (N= 5 animals/group). Mice were maintained in a temperature- and humidity-controlled facility with 12/12 h light/dark cycle and had ad libitum access to water and food. At 2-3 months of age, a subset of mice was randomly distributed into a standard laboratory chow group (EURodent Diet, 5LF) and a high-fat diet (SDS 824053 High Fat diet, 45%) group for a period of 13 weeks. Single muscle fibres and muscle tissue were studied for stem cell recruitment, fibre type composition and markers of oxidative stress (≥ 2 replicates). SC bioenergetic capacity was assessed using the Seahorse extracellular flux analysis (3 replicates).

Statistical analysis was performed by two-way ANOVA followed by Tukey's post-hoc test. Data are mean $\pm$ SD. A p-value of *p<0.05 was interpreted as significant.

Results/Conclusion: CD36-deficient mice on a high-fat diet were protected against adiposity (reduced body- and adipose tissue weight), intramuscular lipid accumulation (by means of reduced Bodipy signal and Oil Red O staining) and showed attenuated skeletal muscle oxidative stress levels (by means of DHE-staining intensity and protein-adduct quantification; ≥ 3 replicates). Obese mice had impaired SC proliferation and differentiation, which was assessed by in-vitro quantification of SC number at day 1-4 after fibre isolation. Interestingly, CD36 deficiency rescued SC proliferation along with an altered bioenergetic profile but led to impaired differentiation capacity independent of diet. It remains to be established whether CD36 inhibition represents a therapeutic target to tackle obesity and obesity-related impaired muscle regeneration.

This study was funded by the European Union, Marie Curie Actions, FP7-PEOPLE-PCIG14-GA-2013-631440.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC153

Osteoblast-specific *Enpp1* deficiency engenders increased bone mass and insulin resistance

F.L. Roberts¹, N. Rashdan¹, I. Orriss², E. Milne¹, N. Morton³, C. Farquharson¹ and V.E. Macrae¹

¹Developmental Biology, Roslin Institute, Edinburgh, UK, ²Department of Comparative Biomedical Sciences, Royal Veterinary College, London, UK and ³Department of Cardiovascular Sciences, Queen's Medical Research Institute, Edinburgh, UK

The principal bone mineralisation inhibitor ectonucleotide pyrophosphatase/phosphodiesterase-1 (NPP1) has recently emerged as a pathogenic factor predisposing to insulin resistance and type 2 diabetes mellitus. We have previously demonstrated that *Enpp1*^{-/-} mice exhibit pronounced resistance to obesity and insulin resistance following chronic high-fat diet (HFD) feeding. We also demonstrated that *Enpp1*^{-/-} mice present with significant ectopic mineralisation (e.g. of the vasculature) and a paradoxical hypomineralisation of the long bones. Here, we determined the impact of osteoblast-specific *Enpp1* ablation on HFD-induced obesity and skeletal phenotype.

Mice lacking NPP1 specifically in osteoblasts (Ob-KO) were generated by crossing *Enpp1*^{flox/flox} and *Osteocalcin*-Cre mice. Male mice were reared on a control diet (6.2% fat) or HFD (58% fat) up to 16-weeks of age. Metabolic tests, micro-computed tomography of long bones, dissection, gait, and histological analysis were performed. For data of two groups, *t*-test analysis was conducted. For data

of more than two groups, one-way analysis of variance (ANOVA) using a general linear model and incorporating Tukey pairwise comparisons was performed.

Under control dietary conditions, Ob-KO mice exhibited increased bone mass, indicated by increased femoral bone volume/total volume (149%; $P<0.01$), and reduced trabecular spacing (84%; $P<0.01$) at 6-weeks of age. Adult Ob-KO mice exhibit increased muscle mass (13.3%; $P<0.05$), likely resultant of increased gait instability (increased base of support (115%; $P<0.05$), girdle support (166%; $P<0.05$), initial (117%; $P<0.05$) and terminal (128%; $P<0.05$) forelimb dual stance). Adult mice showed normal glucose and insulin tolerance tests (GTT/ITT) comparable between genotypes. No notable differences in body weight or gross anatomy were observed.

Following chronic HFD-challenge, no significant differences in body weight gain, muscle or white fat mass were observed between genotypes. However, Ob-KO mice showed significantly increased liver (130.1%; $P<0.05$) and brown fat mass (122.2%; $P<0.05$). These Ob-KO mice showed decreased glucose and insulin tolerance ($P<0.05$) following GTT and ITT respectively. No significant difference in pancreatic islet size or number between genotype were observed.

These data indicate that the protection against diabetes noted in *Enpp1*^{-/-} mice is likely due to the actions of non-skeletal NPP1. Indeed, osteoblast-specific NPP1 deficiency drives an unexpected worsening of insulin sensitivity relative to control mice on HFD. Together, these data indicate that NPP1 inhibition at its major site of expression is metabolically detrimental. Further studies are required to establish the mechanisms through which NPP1 regulates its actions within and out-with bone.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC154

Hypoglycaemic, Antihyperglycaemic and Anti-Lipid Peroxidation Potential of Avocado Peel in Rat Model

O. Ilochi², A.N. CHUEMERE¹, D.V. Dapper², M. Anyiyeloye³ and T. Kolawole¹

¹human physiology, university of port harcourt, Port Harcourt, Nigeria, ²Department of Human Physiology, Madonna University, Elele, Nigeria and ³Department of Pharmacology, University of Port Harcourt, Port Harcourt, Nigeria

Globally, diabetes mellitus is increasingly becoming public health concern that care and prevention would be of paramount importance. Avocado peel has been exploited as folk remedies linked to all kinds of health problems including diabetes. This work was carried out to evaluate the therapeutic efficacy of hydroethanolic extract of avocado peel as hypoglycaemic, antihyperglycaemic and anti-lipid peroxidation. Six groups of 10 healthy male wistar rats were used for the study. Control Group 1 were given drinking water; Group 2 glibenclamide (0.6mg/kg/day); Group

3 Avocado peel extract (50mg/kg⁻¹); Group 4 streptozotocin (60 mg/kg⁻¹) diabetic; Group 5 diabetic + glibenclamide; Group 6 diabetic + avocado peel extract. Both blood glucose and glycated haemoglobin levels were assayed at age 0, 21, and 42 days respectively. Pancreas was analyzed for lipid peroxidation production of malondialdehyde (MDA) and isoprostanes (F₂isoP). Like glibenclamide, avocado peel extract time-dependently significantly (<0.05) lowered blood glucose and glycated haemoglobin, as well as significantly down-regulated MDA and F₂isoP in comparison to non-diabetic and diabetic control respectively. Correlation analysis revealed possible link between oxidative stress and impairment of glucose regulation. In conclusion, avocado peel extract can ameliorate blood glucose regulation possibly through its antioxidant mechanisms. So, we can recommend that avocado peel extract should be considered in the management plan of diabetics.

Treatment /day	Blood glucose level (mmol/l)					
	0	% change 0→21	21	%change 21→42	42	% change 0→42
Control	4.14±0.14	1.0	4.18±0.91	-1.19	4.13±0.14	-0.24
Glibenclamide	4.17±0.12	-9.6	3.77±0.41 ^a	-27.5	2.73±0.23 ^a	-34.5
Avocado peel	4.22±0.11	-10.2	3.79±0.24 ^a	-25.0	2.84±0.17 ^a	-32.7
Streptozotocin diabetic	16.20±0.14	13.0	18.31±0.47 ^a	26.3	23.14±0.01 ^a	42.8
Diabetic + glibenclamide	13.41±0.81	-28.2	9.63±0.02 ^a	-24.9	7.23±0.12 ^a	-46.0
Diabetic + avocado peel	12.70±0.03	-23.1	9.77±0.13 ^a	-24.0	7.42±0.24 ^a	-41.5

Table 1: Time-dependent effect of treatment on blood glucose level

Treatment	Lipid peroxide (µg/ml)			
	MDA	% change to control	F ₂ isoP	%change to control
Control	90.6±0.12	-	40.1±0.41	-
Glibenclamide	40.2±2.12 ^a	-55.6	23.2±1.12 ^a	-42.1
Avocado peel	51.2±1.41 ^a	-43.4	32.3±1.32 ^a	-19.4
Streptozotocin diabetic	123.2±1.32 ^a	35.9	78.1±1.21 ^a	94.7
Diabetic + glibenclamide	60.3±0.02 ^a	-33.4	43.1±1.31 ^a	7.4
Diabetic + avocado peel	67.2±0.41 ^a	-25.8	41.4±1.21	3.2

Table 2: Effect of treatment on products of lipid peroxidation

Nwafor A & Owhoji A. (2001) J. Appl. Sci. Environ. Mgt. 5(1); 75-77.
 Ogadinma I & Chuemere AN (2019). W W J M Res Dev: 5(1): 70-74
 Tremocoldi MA, et al (2018). PLoS One. 13(2): e0192577

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC155

Insulin sensitivity was enhanced by *Ocimum gratissimum* leaf extract in Wistar rats with dexamethasone-induced metabolic syndrome

S.T. Shittu, T.J. Lasisi, S.A. Shittu, A. Adeyemi and A. Alada

Department of Physiology, University of Ibadan, Ibadan, Nigeria

Ocimum gratissimum (OG) leaf extract was consistently shown to possess antidiabetic properties in animal models of diabetes mellitus. This was linked to its ability to promote peripheral glucose utilization while conserving glycogen by inhibiting glycogen phosphorylase activity in the diabetic animals. The models of diabetes mellitus used in the earlier studies were characterized by pancreatic beta cell destructions which created an hypoinsulinemic condition. The effect of OG is therefore not known in conditions like metabolic syndrome characterized by hyperglycemia and hyperinsulinemia. This study was carried out to investigate the effects of *ocimum gratissimum* on glucose metabolism in dexamethasone-induced metabolic syndrome in male Wistar rats.

Twenty male Wistar rats (130 ± 20 g) were randomly grouped into four ($n=5$ each) as control, normal+OG, Dex and Dex+OG. Dexamethasone (1 mg/kg) was administered intraperitoneally to Dex and Dex+OG groups followed by normal saline and OG (400 mg/kg), respectively; while control and normal+OG were administered normal saline and OG, respectively. Body weight and fasting blood glucose level were monitored before and after the treatments. After 10 days of treatments, under anaesthesia by sodium thiopental (50 mg/kg, i.p.), blood samples were obtained for fasting blood glucose (FBG) and insulin levels; liver and soleus muscle biopsies were taken for the determination of glycogen content and hexokinase activity; pancreas was obtained for histology; and HOMA-IR was calculated using FBG and insulin level.

Values are mean $\pm$ SEM, compared using ANOVA and post-test analysis at $\alpha_{0.05}$. Body weight was significantly reduced in the Dex and Dex+OG group compared with the control. The FBG (147.8 ± 9.93 mg/dL), insulin (2.98 ± 0.49 μ U/ml) and HOMA-IR (1.11 ± 0.22) of Dex animals were significantly higher than the control (FBG = 89.22 ± 6.53 mg/dL; insulin = 1.70 ± 0.49 μ U/ml; HOMA-IR = 0.37 ± 0.04); this was however reduce by OG treatment in the Dex+OG (FBG= 115.31 ± 5.93 mg/dL; insulin = 1.85 ± 0.11 μ U/ml; HOMA-IR = 0.53 ± 0.08) compared with Dex. Liver and muscle glycogen contents significantly depleted in the Dex were reversed in the Dex+OG while; hexokinase activity was reduced in the liver and muscle of the Dex but increased in the muscle of the Dex+OG.

It was concluded that *ocimum gratissimum* leaf extract may promote cellular insulin actions on glucose metabolism in Wistar rats with metabolic syndrome induced by dexamethasone.

Aguiyi JC *et al.* (2000). *Fitoterapia*, 71:444-6.

Egesie UG *et al.* (2006). *Niger J Physiol Sci.*, 21:31-5.

Shittu ST *et al.* (2018). *Niger Med Pract.*, 73:10-7.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC156

Iron uptake and utilization in copper-toxic female Wistar rats

A.O. Odetola^{1,2}, O.F. Eddie-Anunobi¹, B.O. Adele¹, I.E. Emediong¹, A.O. Ige¹ and E.O. Adewoye¹

¹*Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan, Ibadan, Nigeria and* ²*Department of Human Physiology, Nnamdi Azikiwe University, Nnewi, Nigeria*

Copper, within physiological limits, is needed for iron homeostasis and its deficiency has been reported to disrupt iron metabolism. There is however paucity of information on the effect of copper toxicity on iron metabolism. This study investigated iron metabolism in copper-toxic female Wistar rats. Twenty rats (160-180g) were assigned into 4 groups and were treated with 0.3mls normal saline (control), Copper sulphate (100mg/kg), Copper sulphate (100mg/kg) + Ferrous sulphate (1mg/kg) and Ferrous sulphate (1mg/kg) respectively. Rats were orally treated for 5weeks. Blood, collected retro-orbitally after thiopentone sodium anaesthesia (50mg/kg i.p.) into EDTA and plain bottles, was analysed for haematocrit (Hct), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) using Sysmex Haematology Analyser. Liver was excised and homogenized for copper and iron analysis. Bone marrow from femur was harvested for myeloid and erythroid count. Serum copper, iron, ferritin, total iron binding capacity (TIBC), liver copper and iron were estimated with assay kits (Fortress Diagnostics, UK). Values (Mean±SEM, n=5), compared by ANOVA. Copper toxicity significantly decreased PCV (28±0.66 %), Hb (9.3±0.07 g/dl), RBC (4.5±0.01 ×10³/mm³), WBC (3630±589 ×10³/mm³) compared to control (34±1.4 %, 11±0.44 g/dl, 5.6±0.20 ×10³/mm³, 6310±741 ×10³/mm³ respectively, *P*<0.05). Also, serum copper (figure 1), iron (fig. 2), ferritin (fig. 3), TIBC (fig. 4), myeloid/erythroid ratio (fig. 5) were significantly lowered (*P*<0.05) while Liver copper (fig. 6) and iron (fig. 7) were significantly elevated (*P*<0.05) in copper toxic rats compared to control. These data suggest that copper toxicity altered iron uptake, storage and utilisation to cause haematological alterations.

TABLE 1

GROUPS	Hct (%)	Hb (g/dl)	RBC (×10 ³ /mm ³)	PLATELET
CONTROL	34±1.4	11±0.44	5.6±0.20	190000±18091
COPPER	28±0.66*	9.3±0.07*	4.5±0.01*	211200±917
COPPER + IRON	40±1.60*	13±0.51*	6.9±0.48*	163600±36953
IRON	37 ±1.3	13±0.47*	6.3±0.26	158000±5292

Values are Mean±SEM, n=5, *p*<0.05

* indicates significant difference compared to control

indicates significant difference compared to copper toxic group

TABLE 2

GROUPS	WBC ($\times 10^3/\text{mm}^3$)	LYMPHOCYTES (%)	NEUTROPHILS (%)	MONOCYTES (%)	EOSINOPHILS (%)
CONTROL	6310 $\pm$ 741	67 $\pm$ 1.90	29 $\pm$ 2.30	2.2 $\pm$ 0.49	1.8 $\pm$ 0.37
COPPER	3630 $\pm$ 589*	60 $\pm$ 2.90	37 $\pm$ 3.60	2.4 $\pm$ 0.40	1.2 $\pm$ 0.49
COPPER + IRON	4970 $\pm$ 639	63 $\pm$ 4.20	31 $\pm$ 3.90	2.2 $\pm$ 0.37	3.0 $\pm$ 0.32
IRON	6520 $\pm$ 527	63 $\pm$ 4.40	33 $\pm$ 4.30	2.0 $\pm$ 0.32	2.0 $\pm$ 0.55

Values are Mean $\pm$ SEM, n=5, p<0.05

* indicates significant difference compared to control

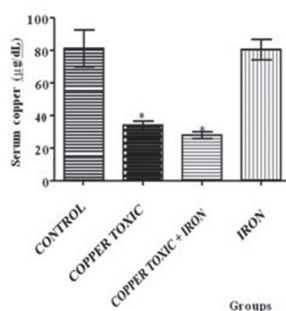


Figure 1: Serum copper levels in control and treated rats

Values are Mean $\pm$ SEM, n=5, p<0.05

* indicates significant difference compared to control

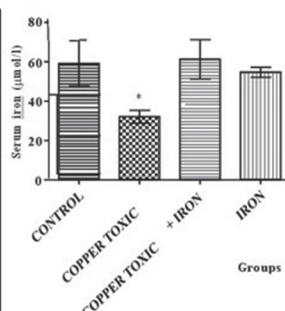


Figure 2: Serum iron levels in control and treated rats

Values are Mean $\pm$ SEM, n=5, p<0.05

* indicates significant difference compared to control

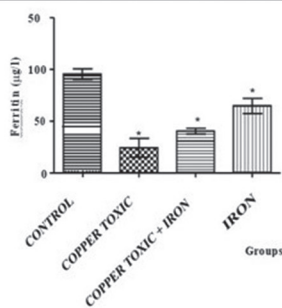


Figure 3: Ferritin levels in control and treated rats

Values are Mean $\pm$ SEM, n=5, p<0.05

* indicates significant difference compared to control

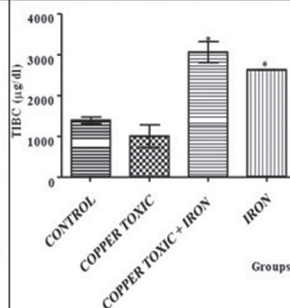


Figure 4: Total iron binding capacity in control and treated rats

Values are Mean $\pm$ SEM, n=5, p<0.05

* indicates significant difference compared to control

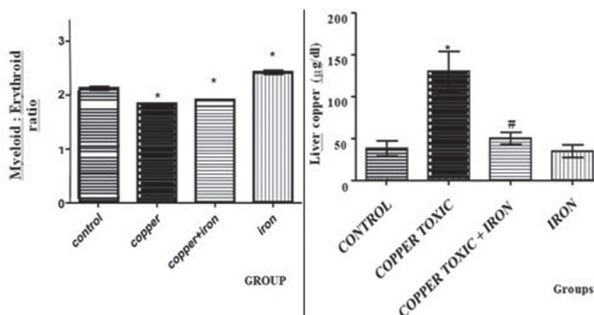


Figure 5: Myeloid : Erythroid ratio in control and treated rats
Values are Mean±SEM, n=5, p<0.05
* indicates significant difference compared to control

Figure 6: Liver copper levels in control and treated rats
Values are Mean±SEM, n=5, p<0.05
* indicates significant difference compared to control
indicates significant difference compared to copper toxic group

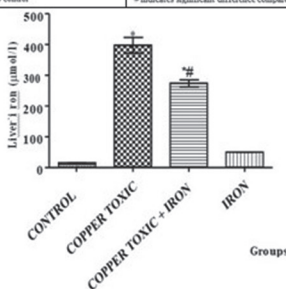


Figure 7: Liver iron levels in control and treated rats
Values are Mean±SEM, n=5, p<0.05
* indicates significant difference compared to control
indicates significant difference compared to copper toxic group

Musacco-Sebio R et al. (2017). *Liver Res Open J.* 2(1), 9-13.

Tandara L & Salamunic I (2012). *Biochem Med* 22, 311–328.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC157

Investigating enhancer activity of a finemapped *WARS2-TBX15* GWAS locus associated with waist-hip ratio

R. Dumbell¹, M. Muso¹, L. Zolkiewski¹, S. Laber^{1,2,3}, L. Bentley¹ and R. Cox¹

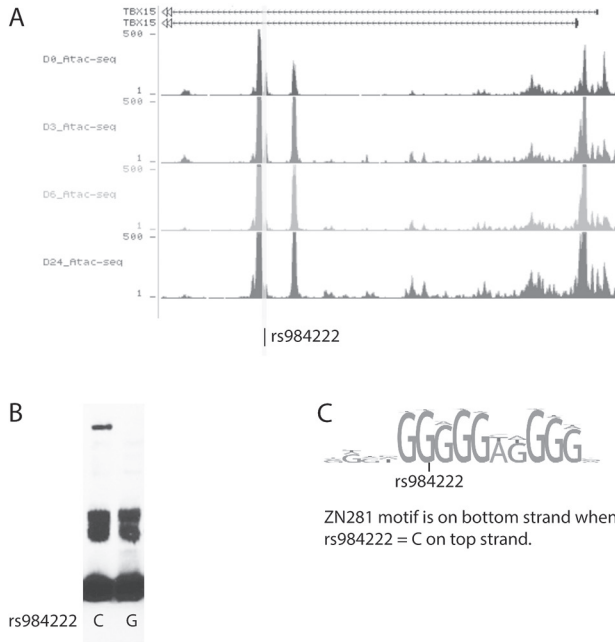
¹Mammalian Genetic Unit, MRC Harwell Institute, Didcot, UK, ²The Broad Institute, Cambridge, MA, USA and ³The Big Data Institute, University of Oxford, Oxford, UK

While overweight and obesity carry increased risk of metabolic disease, the distribution of excess body fat, measured indirectly by waist-hip ratio and independent of body mass index, is a predictor of metabolic disease risk. Genome-wide association studies (GWAS) have so far identified 346 loci associated with waist-hip ratio,

including the *WARS2-TBX15* locus (1, 2). We aim to determine the mechanism(s) by which this locus may alter adipose tissue development and function in different fat depots. The *WARS2-TBX15* locus has been further fine-mapped in a study combining populations of European and African descent to an approximately 1.5 kb region within the first intron of *TBX15*, which includes the lead single nucleotide polymorphism (SNP) from the initial publication of this locus, rs984222 (3). We show that this SNP overlays a peak in an assay for transposase-accessible chromatin (ATAC)-seq experiment in a human pre-adipocyte cell line (hWAT) throughout differentiation and in mature cells (figure 1A); indicating that this region is accessible to transcription factor binding. We demonstrate altered binding of nuclear protein from differentiating (day 4) hWAT cells to DNA oligos containing either version of rs984222 with electrophoretic mobility shift assays (figure 1B; 4 independent replicate experiments, band is lost when SNP=G), corresponding to predicted loss of a ZN281 binding motif (figure 1C).

The human 1.5kb fine-mapped region corresponds to an 841bp region in intron 1 of *Tbx15* in the mouse and we demonstrate enhancer activity of this region in a mouse pre-adipocyte cell line (3T3L1) throughout differentiation using luciferase enhancer assays. N=8, fold change luciferase activity vs scrambled control; differentiation day -2, 1.97 ± 0.18 $P < 0.0001$; day 0, 1.76 ± 0.17 $P = 0.015$; day 1, 1.58 ± 0.10 $P = 0.014$; day 4, 1.93 ± 0.28 $P = 0.0001$ (arbitrary units, mean $\pm$ SEM, unpaired t-test, representative data of three experiments). These data suggest a role of this fine-mapped region in transcriptional regulation, and supports the use of mouse models for investigating human GWAS identified loci.

We have produced a CRISPR mouse model lacking this enhancer region and will present preliminary metabolic phenotyping data from this line; including body weight, fat mass and lean mass (EchoMRI), in comparison to a spontaneously occurring *Tbx15*^{-/-} mouse where the deletion begins in intron 1 downstream of the 841bp fine-mapped region (4, 5). This is along with planned RNA-seq experiments on tissue from different fat depots in these mice which will help inform the regulatory network of *TBX15* and that potentially regulated by this putative enhancer region in intron 1 of the gene.



A: ATAC-seq peaks surround rs984222 in intron 1 of *TBX15* at in a human preadipocyte cell line (hWAT) on differentiation day (D) 0, 3, 6, and 24 (mature adipocytes). B: Representative electrophoretic-mobility shift assay of rs984222 biotin labelled oligo with C and G version of SNP and day 4 differentiated hWAT cell nuclear protein. C: Predicted ZN281 motif on bottom strand is not predicted when rs984222=G.

1. Shungin D, et al. *Nature*. 2015; 518(7538):187-96.
2. Heid IM, et al. *Nature genetics*. 2010;42(11):949-60.
3. Liu CT, et al. *Human Molecular Genetics*. 2014;23(17):4738-44
4. Candille SI, et al. *PLoS biology*. 2004;2(1):E3.
5. Curry GA. *Journal of embryology and experimental morphology*. 1959;7(1):39-65.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC158

Improvement of the indicator amino acid oxidation technique for the assessment of methionine requirements in dogs

J. Lewis¹, R. Bednall², R. Haydock², D. Allaway², K. Smith¹, B. Phillips¹ and M. Harrison²

¹MRC-ARUK Centre for Musculoskeletal Ageing Research, Schools of Medicine, University of Nottingham, Derby, UK and ²WALTHAM Centre for Pet Nutrition, Melton Mowbray, UK

Companion animals may receive all their nutrition from commercially-prepared pet foods and responsible food manufacturers ensure their diets are nutritionally balanced and complete. The current recommendations for dietary methionine, an essential amino acid (EAA) often found in limited amounts in raw materials, is however based upon limited evidence (1). The indicator amino acid oxidation (IAAO) method assesses EAA requirements by monitoring the rate of amino acid oxidation of a ¹³C-labelled EAA (the indicator). This is based on the principle that if an amino acid (AA) is rate limiting for protein synthesis, then other AAs present in excess are oxidised (2). This rate of oxidation is measured from the appearance of ¹³CO₂ in the breath, derived from, in this case, 1-¹³C phenylalanine added to the diet. By providing different amounts of methionine in the diet and measuring the rate of appearance of ¹³CO₂ from the indicator AA, breakpoint analysis on a two-phase linear regression can be used to identify the minimum amount of methionine required to overcome limited substrate availability for protein synthesis. The aim of this study was to improve the IAAO method used previously (3) by reducing variability, using fewer dogs, acquiring fewer breath samples and studying fewer diets.

After obtaining all required ethical approvals, thirteen adult dogs maintained at a normal body condition score, were fed 5 semi-purified diets: one basal and four test diets each varying in methionine concentration (1.7g and 0, 0.3, 0.6 and 1.5 g/1000kcal, respectively). Each week the dogs were fed the basal diet (1 meal/day) for 4-days, followed by 3-days on a test diet (2-days with 4 meals/day and 1-day with 8 meals/day- the study day). All dogs received each test diet 3 times. On the study day (after 6 months of animal training) meals were given hourly and breath samples were collected prior to tracer dosing and during the last 2 hours. The daily phenylalanine requirement was provided with the first meal. Meal 4 contained a priming dose of 1-¹³C phenylalanine, with "top-ups" added to each subsequent meal. Breath was collected using a respiratory mask and collection bags. The ratio of ¹³CO₂:¹²CO₂ in the breath was measured, by continuous flow isotope ratio mass spectrometry to calculate the rate of appearance of ¹³CO₂.

Breakpoint analysis estimated a minimum methionine requirement of 0.337g/1000kcal with 95% CI (0.330, 0.345). This value is similar to previous estimates (3), but with greater precision (±2.2% vs. 27.1%). This approach reduced the numbers of dogs needed (12 vs. 21) whilst requiring less sampling points (7 vs. 11) and fewer diets to be tested (4 vs. 9).

Altering the diet to an extruded pellet (vs. mash (3)) facilitated the development of a method with more robust estimates of EAA requirements in dogs, which will contribute to improving canine diets.

Allaway, D. *et al.* (2016) 'Determination of the methionine requirement of large breed adult dogs using the indicator amino acid oxidation (IAAO) technique', in *WINSS*.

Elango, R., Ball, R. O. and Pencharz, P. B. (2008) 'Indicator Amino Acid Oxidation: Concept and Application', *The Journal of Nutrition*, 138(2), pp. 243–246. doi: 10.1093/jn/138.2.243.

National Research Council (2006) *Nutrient Requirements of Dogs and Cats*. fifth. Washington, DC: The National Academies Press.

We would like to acknowledge the BBSRC for providing iCASE study to support this PhD and thank all colleagues at WALTHAM for their care and expertise in training, welfare and sampling the dogs used in this study. *BP and MH are joint senior authors

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC159

High fat diet and small intestine: morphological and immunocytochemical changes in a murine experimental model

A. Obeso¹, A. Rocher¹, E. Olea², S. Conde³, J. Prieto-Lloret¹ and E. Gonzalez-Obeso⁴

¹*Departamento de Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid, Spain. Instituto de Biología y Genética Molecular, IBGM, CESIC. Spain, Valladolid, Spain,* ²*Departamento de Enfermería, Universidad de Valladolid, Facultad de Enfermería, Valladolid, Spain,* ³*CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisbon, Portugal and* ⁴*Servicio de Anatomía Patológica, Hospital Clínico Universitario de Valladolid. Spain, Valladolid, Spain*

Obesity is a chronic disease of multiple etiologies, dependent on genetic, environmental, metabolic and endocrine factors. An important factor is the energy imbalance between calories consumed and spent. Obesity is a risk factor for metabolic syndrome; both pathologies present mild chronic systemic inflammatory state, with increased cytokines, TNF α and C-reactive protein. Some studies show intestinal modifications derived from a systemic inflammatory state. Until now it has not been determined if a high-fat diet produces histological changes in the small intestine, nor if these are related to the generalized inflammatory state that obesity produces. Our objective is to elucidate whether there is a relationship between obesity produced by high-fat diet intake and the possible histological/morphological alterations in the small intestine. Also, modifications of local inflammation markers IL-1R and TNF α R have been studied.

In handling the animals, we followed the European Union Directive for Protection of Vertebrates Used for Experimental and Other Scientific Ends (2010/63/EU).

Protocols were approved by the University of Valladolid Institutional Committee for Animal Care and Use (Project Approval Ethical Code: 4505502). Swiss male mice, 3 months old, were used. Mice (n=10) were randomly divided into two groups: Control group (n=5), fed standard diet and experimental group (n=5), fed a high-fat diet, for 5 weeks. At the end of diet period, animals were weighed. After overnight fasting, basal glucose and insulin were determined in plasma; animals were euthanatized with lethal *i.p.* doses of sodium pentobarbital; segments of small intestine were extracted, fixed in 4% paraformaldehyde in 0.1M PB, and embedded in paraffin. Cuts of tissue 5 μ m thick were stained with hematoxylin and eosin. The presence of TNF- α R and IL-1R was identified by fluorescent immunohistochemistry in control and experimental small intestine sections, examined with a fluorescent and white light microscope with coupled camera, the captured images were processed with Metamorph 6.3 software.

Mice average weight after 5 weeks of treatment was 32.4g in controls vs 41.4g experimental ($p < 0.05$). Blood glucose was 67.2mg/dL in controls vs 102.8mg/dL experimental ($p < 0.05$); insulinemia was 0.16 μ g/L in controls vs 0.19 μ g/L experimental ($p = n.s.$). Height of intestinal villi: $409 \pm 18\mu$ m vs $312 \pm 47\mu$ m, ($p \leq 0.001$); width at medium level: $74 \pm 12\mu$ m vs $94 \pm 25\mu$ m, ($p = n.s.$); distal width: $72 \pm 13\mu$ m vs $114 \pm 27\mu$ m, ($p < 0.05$). Values are means $\pm$ S.E.M., compared by ANOVA. We found increased goblet and neuroendocrine cells and qualitative increase of fluorescence for both receptors, TNF- α and IL-1, in the intestine of mice with a high-fat diet vs controls.

Conclusion: High-fat diet induces obesity and alterations in glucose homeostasis that run with morphological changes and signs of inflammation in the small intestine.

This research was funded by BFU2015-70616-R (MINECO/FEDER) (DGICYT), VA106G18 (JCyl)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC160

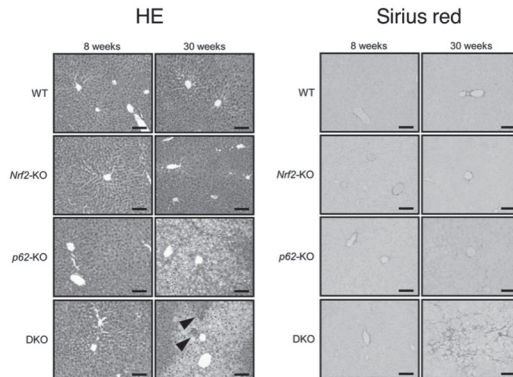
Sqstm1/p62 and Nrf2 double knockout mice spontaneously develop nonalcoholic steatohepatitis

E. Warabi, K. Akiyama, K. Okada, T. Yanagawa, S. Takahashi, T. Ishii and J. Shoda

University of Tsukuba, Tsukuba, Japan

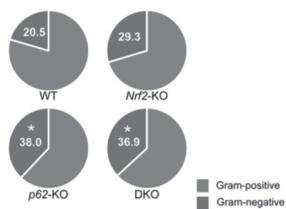
Nonalcoholic steatohepatitis (NASH) is one of the leading causes of chronic liver disease worldwide. However, details of pathogenetic mechanisms remain unknown. In this study, we found deletion of both Sequestosome1 (Sqstm1)/p62 and Nrf2 genes spontaneously led to the development of NASH in mice fed a normal chow and was associated with liver tumorigenesis. The pathogenetic mechanisms underlying the NASH development was investigated in p62:Nrf2 double-knockout (DKO) mice. DKO mice showed massive hepatomegaly and steatohepatitis with

fat accumulation. DKO mice had hyperphagia[1] and enhanced adipocyte differentiation-induced obesity [2] coupled with insulin resistance and adipokine imbalance. We found that DKO mice also showed dysbiosis associated with an increased proportion of gram-negative bacteria species (wild type:20.5±4.8% vs. DKO:36.9±8.1%, $p<0.05$, $n=8$) by fecal 16S rDNA analysis. An increased lipopolysaccharide (LPS) level in the feces was also found in DKO (wild type:15.4±7.7 EU/mg vs. DKO: 176±27 EU/mg, $p<0.05$, $n=8$). The intestinal permeability was elevated (wild type:3.8±1.2 dextran-FITC $\mu\text{g/mL}\cdot\text{min}$ vs. DKO: 17.3±2.7 dextran-FITC $\mu\text{g/mL}\cdot\text{min}$, $p<0.05$, $n=8$) in association with both epithelial damages and decreased expression levels of tight junction protein zona occludens-1 (Zo-1) (relative expression in wild type:1.00±0.08 vs. DKO: 0.67±0.04, $p<0.05$, $n=8$), and thereby LPS levels were increased in the serum (wild type:3.1±0.1 EU/mL vs. DKO: 9.0±0.5 EU/mL, $p<0.05$, $n=8$) at 8 weeks of age. These results suggest that innate immunity activation by excessive LPS flux from the intestines, occurring both within and outside the liver, is central to the development of hepatic damages in the form of NASH in *Sqstm1/p62* and *Nrf2* double knockout mice [3].



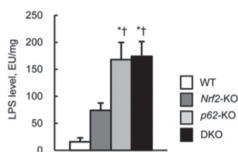
p62:Nrf2 gene double-knockout (DKO) mice developed nonalcoholic steatohepatitis and liver tumors. Hematoxylin and eosin (HE)-stained sections of representative liver specimens from wild-type (WT), *Nrf2*-knockout (KO), *p62*-KO, and DKO mice at 8 and 30 weeks of age. Arrowheads indicate infiltration of inflammatory cells (scale bar, 100 μm).

Ratio of gram-negative bacteria in feces



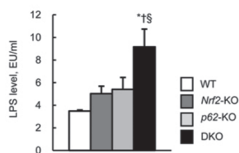
The relative abundance of gram-negative bacteria was calculated as the percentage of bacteria compared with total bacteria for fecal bacteria (n = 8 per group).

Fecal LPS levels

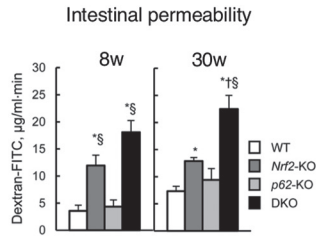


LPS levels in feces in each group (n = 8).

Serum LPS levels

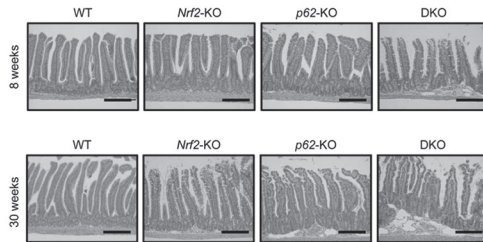


LPS levels in serum each group (n = 8).



Intestinal permeability increased in p62:Nrf2 gene double-knockout (DKO) mice.

Area under the plasma concentration curve of fluorescein isothiocyanate (FITC)-dextran after administration of the marker by gavage (n = 8 per group).



HE-stained sections of representative intestine specimens from wild-type (WT), *Nrf2*-knockout (KO), *p62*-KO, and DKO mice at 8 and 30 weeks of age (scale bar, 100 μ m).

Harada H, Warabi E, Matsuki T, Yanagawa T, Okada K, Uwayama J, Ikeda A, Nakaso K, Kirii K, Noguchi N, Bukawa H, Siow RC, Mann GE, Shoda J, Ishii T, Sakurai T. Deficiency of p62/Sequestosome 1 causes hyperphagia due to leptin resistance in the brain. *J Neurosci.* 2013;33:14767-14677.

Rodriguez A, Durán A, Selloum M, Champy MF, Diez-Guerra FJ, Flores JM, Serrano M, Auwerx J, Diaz-Meco MT, Moscat J. Mature-onset obesity and insulin resistance in mice deficient in the signaling adapter p62. *Cell Metab.* 2006;3(3):211-222.

Akiyama K, Warabi E, Okada K, Yanagawa T, Ishii T, Kose K, Tokushige K, Ishige K, Mizokami Y, Yamagata K, Onizawa K, Ariizumi S, Yamamoto M, Shoda J. Deletion of both p62 and Nrf2 spontaneously results in the development of nonalcoholic steatohepatitis. *Exp Anim.* 2018;67(2):201-218.

Supported by JSPS and Great Britain Sasakawa Foundation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC161

Perinatal programming of intestinal homeostasis following exposure to low dose of chlorpyrifos in male rats offspring

M. Guibourdenche^{1,2}, H. El Khayat El Sabbouri¹, A. Bouzerara³, N. Djekkoun¹, H. Khorsi-Cauet¹, J. Guibourdenche³, V. Bach¹, P. M Anton² and J. Gay-Quéheillard¹

¹PériTox, Périnatalité & Risques Toxiques, UMR-I 01, UPJV, Amiens, France, Amiens, France, ²Equipe PETALES – EA 7519 - Unité Transformations & Agro-Ressources, UniLaSalle, Beauvais, France and ³Biologie hormonale, CHU Cochin, Université Paris Descartes, AP-HP, Paris, France

OBJECTIVE

Perinatal period is characterized by phases of development with high sensitivity to environmental factors. Among the risk factors, pesticide exposure are now recognized to program children's metabolism and promote the occurrence of obesity or type 2 diabetes. This study purposes the peri-gestational exposure of mothers (animal model: rat) to a low dose of pesticide (chlorpyrifos, CPF). This exposition might increase the occurrence of obesity, associated with metabolic disorders and disturbances of digestive function in male offsprings.

METHODS

8 female Wistar rats were exposed to CPF, and 8 female rats to its vehicle (controls), supplemented or not with inulin. Female rats were exposed to these experimental conditions during a pre-gestational period of 4 months as well as during the gestation and lactation periods.

After weaning, 56 male pups were studied at young adulthood (D60), without any treatment during the experiment. Different segments of the digestive tract were studied for histological analysis, metabolic assays, inflammation and intestinal permeability.

RESULTS

Rats from mothers exposed to CPF have lower birth weights than control pups ($p < 0.05$), but this difference appears to decrease at young adult age (J60). Lipid and glycemic assays did not show significant differences. We noticed a decrease of IGF1 ($p < 0.01$) in animals from CPF-exposed mothers as well as a decrease in leptin plasma levels but not significantly. FITC assays didn't show any perturbation of the intestinal permeability. LPS and pro inflammatory cytokines assays (IL6, IL1 β , TNF α) didn't show any tissue inflammation. Intergroup comparisons were performed using one-way ANOVA with Bonferroni's post test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com".

CONCLUSIONS

Preliminary results reveal that pups from mothers in contact with a pesticide during the peri-gestational period have developmental disabilities characterized by a lower birth weight, but also a decrease in plasma levels of factors involved in growth and metabolism (IGF1 and leptin).

We suggest that the pesticide slows down fetal and postnatal development. This hypothesis remains to be confirmed after analysis of tight junction proteins of the intestinal barrier, other inflammatory markers and morphological alterations of the digestive system.



Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC162

Probiotics Reduce Oxidative Stress Parameters in The Liver of Wistar Rats (*Rattus Norvegicus*) Induced by High-Fat Diet

T. Nurmasitoh¹, A. Widyaningrum², R. Lusiantari¹ and M.D. Pramaningtyas¹

¹Physiology, Faculty of Medicine, Islamic University of Indonesia, Sleman, Indonesia and

²Faculty of Medicine, Universitas Islam Indonesia, Sleman, Indonesia

Hypercholesterolemia is a risk factor for non-alcoholic fatty liver disease (NAFLD) due to excess fat deposits in the liver. The excess fat deposits in the liver will cause a buildup of free radicals which can damage hepatocytes.⁽¹⁾ Components in probiotics have been proven to reduce cholesterol levels through the increase of excretion,⁽²⁾ inhibition of absorption,⁽²⁾ and inhibition of free radical formation.⁽³⁾ Probiotics are expected to reduce MDA (malondialdehyde) levels of hepar as the end product of lipid peroxidation and the indicator of cell damage due to oxidative stress. Probiotics are also expected to increase antioxidant levels of SOD (superoxide dismutase) in liver which protect from the negative effects of free radicals. This research was approved by the ethics committee, i.e. no. 9/Ka.Kom.Et/70/KE/XI/2018. The researcher applied a post test only control group design. The research subjects were 25 male Wistar rats (*Rattus norvegicus*) which were divided into 5 groups, namely C- (negative control), C+ (positive control, induced by high-fat diet or HFD), T1 (induced by HFD and given probiotic dose of 6.5×10^6 cfu/g), T2 (induced by HFD and given probiotic dose of 5.5×10^6 cfu/g), and T3 (induced by HFD and given probiotic dose of 1.65×10^7 cfu/g).⁽⁴⁾ Induction of HFD was performed by giving 3 g/day of white butter.⁽⁵⁾ Probiotic used in this study was over the counter probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*. Induction and treatment were given orally using gavage for 10 weeks. At the end of treatment, rats were euthanized under anaesthesia (ketamine HCl 100 mg/kg body weight). Immediately, rat liver was taken and the MDA level and SOD activity were examined using spectrophotometric methods. The obtained data were analyzed using software to see differences in mean MDA levels and SOD activities between groups.

The average levels of MDA $\pm$ SD in group C-, C+, T1, T2, and T3 were 1.43 ± 0.13 nmol/g; 4.98 ± 0.43 nmol/g; 3.63 ± 0.16 nmol/g; 3.11 ± 0.10 nmol/g; and 1.83 ± 0.06 nmol/g. One-way ANOVA analysis showed statistically differences of liver MDA levels between the groups ($p < 0.05$). The average activity of SOD $\pm$ SD in group C-, C+, T1, T2, and T3 were 66.34 ± 3.05 %; 19.46 ± 6.60 %; 35.83 ± 2.72 %; 45.36 ± 3.86 %; and 58.30 ± 4.92 %. Analysis using one-way ANOVA showed statistically differences in liver SOD activity between the groups ($p < 0.05$). Pearson correlation analysis showed a significant relationship between MDA levels and liver SOD activity ($p < 0.05$).

In conclusion, probiotics administration could reduce oxidative stress parameters in the liver of Wistar rats induced by HFD. There was significant decrease in liver MDA levels and significant increase in liver SOD activity due to administration of probiotics in Wistar rats induced by HFD.

(1) Boyer JL (2013). Bile formation and secretion. *Comprehensive Physiology* 3(3), 1035-78.

(2) Ooi LG & Liong MT (2010). Cholesterol-lowering effects of probiotics and prebiotics: a review in vivo and in vivo finding. *International Journal of Molecular Sciences* 11(6), 2499-522.

(3) Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, Wang Y, Li W (2017). Antioxidant properties of probiotic bacteria. *Nutrients* 9(521), 1-15.

(4) Kim SJ, Sang HP, Sin HS, Jang SH, Lee SW, Kim SY, Kwon B, Yu KY, Kim SY, Yang DK (2017). Hypocholesterolemic effects of probiotic mixture on diet-induced hypercholesterolemic rats. *Nutrients* 9(293), 1-10.

(5) Nurmasitoh T & Pramaningtyas MD (2015). Honey improves lipid profile of diet-induced hypercholesterolemic rats. *Universa Medicina*. 34(3), 177-86.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC163

***In vitro* suppression of adipogenic markers in adipose cells treated with rilpivirine and quercetin.**

S. behl

Forensic and applied sciences, University of central lancashire, Dubai, United Arab Emirates

Rilpivirine is a non-nucleoside reverse transcriptase inhibitor recently developed as a drug of choice for initial treatment of HIV infection. It may however, be associated with the aggravation of metabolic syndrome. Quercetin, a natural flavanoid, is well known for its anti-adipogenic and lipolytic properties. In an attempt to develop treatment and prevention strategies to overcome the metabolic alterations in HIV-infected patients, human subcutaneous pre-adipose cells were used as a model to study the effect of rilpivirine (15 μ M) or quercetin (50 μ M) either alone or in combination. Immunostaining and qPCR techniques were used to study key adipogenic markers involved in the process of adipogenesis. Data were analyzed statistically using SPSS Version 25 software. Results were analyzed using one-way ANOVA and values of $p < 0.05$ were considered significant.

Quantitative measurement of protein markers show a marked reduction in the levels of PPAR γ , CEBP α and its related genes for adipose cells treated with 15 μ M of rilpivirine and 50 μ M of quercetin individually. Concomitant treatment of adipose cells with these two drugs further reduced these levels. The results from qPCR show that the expression of peroxisome proliferator-activated receptor gamma (PPAR γ), CCATT/enhancer-binding protein alpha (C/EBP α) and their related genes. In parallel, the expression of SREBP-1 and its target genes like FAS, aP2 and LPL were all significantly decreased displaying the lipoatrophic and anti-adipogenic effects of rilpivirine and quercetin respectively. The results indicate that rilpivirine alters adipogenesis through the down-regulation of adipogenic transcriptional factors and their target genes. The study also, for the first time, reports that co-administration of rilpivirine and quercetin further down regulated the expression of these markers and quercetin failed to overcome the lipoatrophic affects of rilpivirine. In conclusion, the results have demonstrated that both rilpivirine and quercetin can down regulate PPAR γ , C/EBP α and SREBP-1 together with their target genes, FAS, aP2 and LPL. However, further studies are required to investigate the mechanism(s) of interaction of the two drugs and more clinical research studies are needed to develop quercetin as a proven natural and effective agent for the antiretroviral therapy-induced prevention or treatment of metabolic disturbances.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC164

Effects of rilpivirine and quercetin on triglyceride, adipocytokines and on oxidative stress markers in adipose cells *in vitro*

S. behl

Forensic and applied sciences, University of central lancashire, Dubai, United Arab Emirates

Highly active anti-retroviral therapy (HAART) has significantly improved the prognosis of HIV-1-infected patients, but it is associated with the aggravation of a metabolic syndrome with potentially severe consequences like dyslipidemia, insulin-resistance and overt lipodystrophy (re-distribution of body fat). The HAART-induced inflammation is normally exerted in the form of altered cytokine release, adipocyte dysfunction, metabolic disturbances and oxidative stress as part of the body's effort to fight the virus. Rilpivirine is a non-nucleoside reverse transcriptase inhibitor recently developed as a drug of choice for initial anti-retroviral treatment of HIV infection. Quercetin, a natural flavanoid, is well known for its anti-adipogenic and lipolytic properties. This study investigated the anti-adipogenic properties of quercetin to overcome the adverse metabolic side effects incurred due to the anti-retroviral therapy, using rilpivirine. *In vitro* studies using human subcutaneous pre-adipose cells were used to model the adverse effects of rilpivirine and the

beneficial physiological role of quercetin either alone or in combination to combat the adverse effects of rilpivirine.

The results show that either rilpivirine (10 μ M and 20 μ M) or quercetin (50 μ M) either alone or when combined had little or no significant effect on adipose cell viability over 15 days, as measured by lactate dehydrogenase (LDH) release compared to untreated cells. However, there were significant ($p<0.05$; ANOVA) reductions in the accumulation of triglyceride levels and evidenced by morphological changes of the cells on the 5th, 8th, 11th and 15th day of the differentiation process with both drugs. Rilpivirine at 20 μ M alone or in combination with quercetin severely inhibited triglyceride accumulation during adipocyte differentiation. In the second set of experiments, pre-adipocytes were treated with either rilpivirine or quercetin alone and in combination for the quantitative measurement of adipocytokines as well as oxidative stress markers. The results also show that rilpivirine (15 μ M) can significantly ($p<0.05$) reduced the release of adiponectin and increased the release of resistin and IL-8, but with no effect on leptin level. On the other hand quercetin (20 μ M) can reverse these effects of rilpivirine even alone or when combined compared to untreated cells. The data also reveal that rilpivirine can induce significant ($p<0.05$) decreases in the activities of the antioxidant enzymes, superoxide dismutase, catalase and glutathione from adipose cells, accompanied by a significant increase in the nitric oxide levels. Again, quercetin treatment reversed these effects of rilpivirine either alone or in combination. The results have clearly demonstrated the beneficial physiological role of quercetin to combat the adverse effects of rilpivirine during the process of adipogenesis.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC165

Beneficial use of bitter melon (*Momordica charantia*) to treat diabetes and hypertension in Guyana

J. Singh

Forensic and applied sciences, University of Central Lancashire, Lancashire, UK

Diabetes mellitus (DM) is a major global health disorder affecting 450 million people, with 72,000 in Guyana. It can elicit several long-term complications including the heart, kidney, eye, nerve and other organs. DM is classified as type 1 DM (T1DM) and type 2 DM (T2DM) and 90% of diabetics suffer from T2DM. This is related to genetic and life-style habits leading to end-organ failures. As a developing country, Guyana is cursed with the 'obese-diabetic time bomb'.

This study recruited 8 newly T2DM and 40 hypertensive patients to investigate a cost-effective way to treat T2DM and high blood pressure (BP) compared to orthodox medicines using moderate regular exercise, diet modification and bitter melon (BM; 5 gm twice daily) either as a salad or as a juice over a period of 6

weeks. In other experiments, 30 healthy subjects and 30 T2DM patients undertook a glucose tolerance test (GTT) combined with BM. The study had ethical clearance from University of Guyana and UCLAN and data were subjected to statistical analysis for significance (ANOVA) and presented as mean ($\pm$ SEM).

Time-course treatment of newly diagnosed T2DM patients (8 per group) with either diet modification and regular exercise, BM alone, diet modification, exercise and BM or all three parameters combined with orthodox medicine over 6 weeks revealed significant ($p < 0.05$) decreases in fasting blood glucose (FBG) compared to week 1, when mean ($\pm$ SEM) FBG was 205.5 ± 5.4 mg/dl (100%). After 6 weeks of treatment, FBG decreased significantly (based on ANOVA the p values were $6.99E-20$, $1.59E-21$, $3.87E-21$ when compared to $p = 0.05$) to 145.3 ± 5.2 mg/dl (29.3%), 105.3 ± 6.2 mg/dl (48.4%), 102.5 ± 5.3 mg/dl (50.1%) and 96.2 ± 4.4 mg/dl (53.2%) for diet modification and regular exercise, BM alone, diet modification, exercise and BM and all three parameters combined with orthodox medicine, respectively. BM alone also reduced BMI, HBA1c, lipids and triglycerides after 6 weeks compared to week 1. During GTT, BM alone intake (10 gm) exerted significant ($p < 0.05$) decreases in FBG after 2 hours after intake of a glucose solution, followed by BM. In healthy subjects, blood glucose (BG) reduced from 117.5 ± 1.8 mg/dl to 107.6 ± 1.6 mg/dl. Likewise, for diabetic patients BG reduced from 139.8 ± 5.2 mg/dl to 108.3 ± 5.4 mg/dl. Daily intake of BM significantly ($p < 0.05$; $n = 10$ per group) reduced systolic and diastolic BP in hypertensive patients either alone or combined with regular exercise after 6 weeks compared to week 1 (based on ANOVA the p values were $1.11E-12$ and $5.12E-09$). BM had no effect on BP combined with orthodox medicine. Chemical analysis of BM revealed that it possesses phenolic contents and anti-oxidant properties. The results show that BM, combined with diet modification and exercise, is beneficial and cost-effective to treat DM and hypertension without interacting with orthodox medication.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC166

Repeated dosing of absorbable concentrations of (poly)phenols induces lipolysis in differentiated human-derived adipocyte cells

R. Little^{1,2}, M. Houghton¹, M. Wabitsch³, A. Kerimi¹ and G. Williamson^{1,4}

¹Food Science and Nutrition, The University of Leeds, Leeds, UK, ²Biomedicine, The University of Aarhus, Aarhus, Denmark, ³Department of Pediatrics and Adolescent Medicine, The University of Ulm, Ulm, Germany and ⁴Monash University, Melbourne, VIC, Australia

Obesity has become a worldwide epidemic. Excess body fat is associated with a range of metabolic and cardiovascular disorders that are placing an ever increasing strain on the provision of public health. However, a controlled reduction in fat mass and maintaining appropriate body weight remains elusive for many obese people.

Dietary bioactive components of the (poly)phenol class, including flavonoids such as quercetin and phenolic acids such as ferulic acid, have been shown to prevent body fat accumulation and to be associated with positive markers of cardiovascular health. Such compounds are naturally present in edible plants and may present part of a new strategy for personal health. However, importantly, the long-term effects of these pure compounds on mature adipocytes remain uncharacterised. To address this, we differentiated human-derived Simpson-Golabi-Behmel syndrome (SGBS) cells to a lipid storing phenotype (2 weeks) and then chronically treated the cells with serum attainable doses of quercetin (Q), ferulic acid (FrA) or both (QFrA). Storage of neutral lipids, quantified by solubilisation of oil red O stain, was significantly reduced after 3 days of daily dosing with 1 μ M Q, 1 μ M FrA or 1 μ M of both combined (reduction of $27.6 \pm 4.8\%$, $19.8 \pm 7.1\%$ and $42.1 \pm 4.3\%$ respectively, compared to vehicle treated cells. $n \geq 9$ /treatment. Multiple T-testing). For each treatment a single dose for 3 days did not significantly reduce lipid storage compared to vehicle treatment. 3 days of repeated treatments did not significantly affect the viability of differentiated SGBS cells. In conditions promoting further lipogenesis (18 days differentiated), only QFrA at 10 μ M had a significant effect ($P < 0.01$. Multiple T-testing) in attenuating oil red O accumulation. Lipidomic sequencing analysis revealed that repeated treatment significantly altered the distribution of cellular lipid species, most noticeably for phospholipids in the combined treatment group ($n=5$ separate experiments).

We show that (poly)phenols, at post prandial bioavailable concentrations, exert significant effects on lipid handling by differentiated SGBS cells only with repeated treatment. This model can support public health dietary advice. Phosphatidylcholine, species of which were highlighted in our lipidomic screening, has recently been shown to dose dependently promote lipolysis specifically in adipocytes. Further investigation is required to elucidate the precise molecular mechanisms mediating (poly)phenol effects in adipocytes. Understanding such pathways may potentially contribute to the development of novel anti-obesity strategies.

Phosphatidylcholine causes adipocyte-specific lipolysis and apoptosis in adipose and muscle tissues.

Jung TW, Park T, Park J, Kim U, Je HD, Kim HD, Cho SW, Abd El-Aty AM, Song JH, Kim HC, Shin YK, Jeong JH.

PLoS One. 2019 Apr 8;14(4):e0214760. doi: 10.1371

The authors thank Metablon, Potsdam, Germany, for the determination of a cellular lipid profile in treated and control cells.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC167

Fructose-induced hepatic steatosis is prevented by daily dietary quercetin supplementation in male Sprague Dawley rats, post-weaning

M.C. Molopo, K.H. Erlwanger and J. Donaldson

School of Physiology, University of the Witwatersrand, Johannesburg, South Africa

Consumption of fructose-rich diets, early in life, has been implicated in metabolic syndrome (MetS) and non-alcoholic fatty liver disease (NAFLD) development. Biologically active phytochemicals are being investigated as alternatives in the treatment of metabolic abnormalities associated with MetS and NAFLD. The plant-derived flavonoid quercetin has been found to improve signs associated with MetS and NAFLD; however most studies used quercetin therapeutically and not prophylactically. Thirty-eight, 21 day old, male Sprague Dawley rats (66.13 ± 12.89 g) were used to investigate the effects of quercetin supplementation in the prevention of fructose-induced NAFLD development, post weaning. Group 1 (C) (n=8) received normal rat chow (NRC), plain gelatine cubes (GC) and tap water (TW) to drink; Group 2 (Q) (n=8) received NRC, GC with quercetin (100 mg.kg^{-1} body mass daily) and TW to drink; Group 3 (F) (n=8) received NRC, plain GC and a 20 % fructose solution (FS) to drink; Group 4 (FQ) (n=7) received NRC, GC with quercetin (100 mg.kg^{-1} body mass daily) and a 20 % FS to drink; Group 5 (FFe) (n=7) received NRC, GC with fenofibrate (100 mg.kg^{-1} body mass daily) and a 20 % FS to drink. Rats were fed *ad libitum* for ten weeks after which they were euthanized using an anaesthetic overdose of sodium pentobarbital and blood and liver samples were collected. Blood metabolic markers of health, liver mass, liver lipid content and liver NAFLD scores were investigated. All rats grew significantly over the ten week period ($p < 0.0001$, ANOVA), however there were no significant differences between groups in percentage body mass gain throughout the treatment period ($p > 0.05$, ANOVA). Fasting blood glucose and serum triglycerides, alanine transaminase, urea and creatinine were not significantly different between groups ($p > 0.05$, ANOVA). Liver mass (relative to body mass) was significantly higher in all rats fed fructose (F, FQ, FFe) versus the control group (C) ($p < 0.05$, ANOVA). Total liver lipid content (ranging between 10.0 ± 1.8 % and 13.1 ± 2.6 %) was not different between groups ($p > 0.05$, ANOVA). The livers of rats fed fructose alone (F) had significantly higher ($p < 0.05$, Kruskal Wallis) micro-steatosis scores (correlating to 33-66 % of liver area covered by steatosis in camera field) compared to those fed quercetin (Q, FQ) or fenofibrate (FFe) (correlating to < 5 %). Liver inflammation scores were significantly higher in rats fed fructose (F) compared to those fed quercetin alone (Q) or fenofibrate with fructose (FFe) ($p < 0.01$, Kruskal Wallis). Thus, NAFLD was induced in the rats fed fructose following the ten week treatment period. The development of NAFLD was reduced by daily quercetin supplementation, evident in the significantly reduced micro-steatosis scores observed in quercetin-treated rats. The use of quercetin from an early age as a natural dietary supplement should thus be considered in the prevention of NAFLD in individuals at risk of developing the condition.

The authors would like to acknowledge the National Research Foundation (Thuthuka Research Grant) for funding the research and Dr Pilani Nkomozepi (University of Johannesburg) for his assistance with the histological procedures.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC168

Protective Effect of Allium Cepa against Coffee Induced Oxidative Stress on Impairment of Blood Glucose

A.N. CHUEMERE¹, O. Ilochi², D.V. Dapper¹, M. Anyiyeloye³ and O. Olorunfemi¹

¹human physiology, university of port harcourt, Port Harcourt, Nigeria, ²Department of Human Physiology, Madonna University, Elele, Nigeria and ³Department of Pharmacology, University of Port Harcourt, Port Harcourt, Nigeria

Coffee has been linked to all kinds of health problems- from diabetes to increasing blood pressure to weight gain and increased risk for a heart attack. Allium cepa (onion) is a special delicacy that contains physio-pharmacological active ingredients that for centuries has been exploited as folk remedies for the treatment of diseases. In this study, the protective effect of Allium cepa on impairment of blood glucose formed by coffee was investigated. Five groups of 10 healthy male wistar rats were used for the study. Hydromethanolic extract of Allium cepa (AC) 100mg kg⁻¹; coffee (C) 500mg kg⁻¹; vitamin E (VE) 400mg kg⁻¹; and combinations of Allium cepa and coffee (ACC); and vitamin E and coffee (VEC) respectively were administered orally to each group of rats daily. While blood glucose levels were estimated at age 0, 21 and 42 days with electronic glucometer, the corresponding intrinsic oxidative stress markers- superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and malondialdehyde (MDA) were analyzed after age 42 days using standard methods. GSH, GPx, SOD, CAT and MDA activities between control, coffee, Allium cepa, combinations of Allium cepa and coffee; and vitamin E and coffee groups were found to be statistically significant (<0.05). Compared to normal control and coffee groups, ACC>AC>VE>VEC produced a significant time-dependent glucose lowering efficacy with up regulations of GSH, GPx, CAT and SOD and diminution in MDA. Allium cepa as one of the most important nutrient sources worldwide were observed to ameliorate some increases in blood glucose oxidative damage induced with coffee. Additionally, combination of Allium cepa and coffee improve glycaemic control, and provided additional metabolic benefits, not achieved with either coffee or Allium cepa alone.

Treatment /biomarker/ 42day	SOD	GPx	CAT	MDA	GSH
Control	21.7±0.32	29.4±5.04	202.0±15.93	97.51±6.88	11.9±0.84
Vitamin E	237.2±20.13*	31.9±4.43*	224.0±32.75*	80.2±18.46*	23.4±1.33*
<i>Allium cepa</i>	337.3±23.20*	38.9±7.50*	230.0±37.70*	73.9±16.80*	28.0±15.6*
Coffee	121.5±32.0*	20.2±15.7*	91.8±11.14*	156.1±21.0*	9.1±1.90*
<i>Allium cepa</i> + coffee	487.6±46.22*	42.0±5.04*	220.0±19.40*	74.7±3.70*	19.2±1.33*
Vitamin E + coffee	457.6±32.8*	32.9±6.44*	206.0±29.80*	86.4±10.80*	10.6±0.90*

Table 1: Effects of *Allium cepa*, coffee, and vitamin E and combinations on serum biomarkers

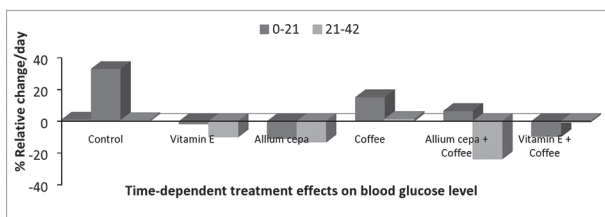


Figure 1: Effects of *Allium cepa*, coffee, and vitamin E and combinations on blood glucose level

Koloverou E, et al. (2015) Eur J Clin Nutr 69(11): 1220-1225.

Ogunmodede OS, et al (2012) Inter J Pharm 8: 21-29

Cano-Marquina A, et al (2013) Maturitas 75(1): 7-21

Jacobs S, et al (2014) Am J Clin Nutr 100(3): 891-900.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC169

Effect of Gum Arabic (*Acacia Senegal*) supplementation on serum Adiponectin level in patients with type 2 diabetes mellitus: a randomized and placebo-controlled clinical trial

R. Babiker¹ and A.M. Saeed²

¹Physiology, University of Medical Sciences and Technology, Khartoum, Sudan and

²Physiology, University of Khartoum, Khartoum, Sudan

Background: Adiponectin is the most abundant hormone secreted by adipose tissue, and it has role in the maintenance of normal body weight. Adiponectin sensitize the cells to insulin and has anti-inflammatory effects. Gum Arabic (GA) is a safe dietary fiber, an exudate of *Acacia Senegal* tree; it had shown weight and lipid lowering effect in both humans and animals.

Objective: To determine the effect of GA oral supplementation on serum Adiponectin level in patients with type 2 diabetes mellitus (DM).

Design, Setting, and Participants: This randomized, double blinded, placebo controlled trial recruited a total of 91 type 2 diabetic patients (73 females, 18

males), age (mean $\pm$ SD) 50.09 $\pm$ 9.3 years; BMI (mean $\pm$ SD) 28.8 $\pm$ 6.05 Kg/m²; HbA1c (mean $\pm$ SD) 9.4 $\pm$ 2.4 %; Adiponectin (mean $\pm$ SD) 5.5 $\pm$ 1.15 (μ g/ml), all patients on hypoglycemic agents and were randomly assigned into two groups, either to consume 30 g of GA (46 patients) or 5 g of placebo (45 patients), daily for 3 months. Serum level of Adiponectin was measured using enzyme-linked immunosorbent assay (ELISA) for each participant pre and post intervention.

The Elisa Kits were used at this research from Abcam's Human Eliza (ab99968). All procedures (**Illustration 1-10**) were performed following the manufacturer's instructions.

Anthropometric obesity markers were measured and lipid profile was determined in fasting blood samples at the start and end of the study period.

Main outcome measures: Mean percent change of serum Adiponectin level from baseline (pre-intervention) level to post-intervention level compared to mean percent change in placebo group.

Results: After intervention, 91 patients were analyzed. Mean percent change of Adiponectin was increased by 5.6% from baseline level among GA with a decrease by 7.6% in placebo group. The 95% CI of Adiponectin mean difference were (-0.58, 0.06; $P = 0.22$) and (-0.62, 0.69; $P = 0.89$) in GA and placebo groups, respectively. There was no statistical significance of mean difference between the two groups ($P = 0.18$). Gum Arabic significantly ($P < 0.05$) decreased HbA1C by 8.9% in GA group (8.42 $\pm$ 1.73 vs 9.23 $\pm$ 2.10 pre-intervention). Gum Arabic decreased BMI and VAI significantly ($P < 0.05$) in GA group by 2% and 23.7% respectively while there were no significant changes in waist circumference or waist-to-hip ratio (WHR).

Conclusion: Although the increase in serum Adiponectin in type 2 DM in this trial is not significant but Gum Arabic consumption at a dose of 30 g/d for 3 months may play an effective role in preventing weight gain and modulating adipose tissue dysfunction in type 2 diabetic patients. Further experimental studies on animals are needed to elaborate the actual physiological mechanism of GA in adipose tissue at cell level.

Trial registration: The trial had been registered as prospective interventional clinical trial in the Pan African Clinical Trial Registry (PACTR) PACTR201403000785219, on 7th March 2014.

Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. Effects of Gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. *Nutrition journal*. 2012;11(1):111.

Ali BH, Ziada A, Blunden G. Biological effects of gum arabic: a review of some recent research. *Food and Chemical Toxicology*. 2009;47(1):1-8.

Ushida K, Hatanaka H, Inoue R, Tsukahara T, Phillips GO. Effect of long term ingestion of gum arabic on the adipose tissues of female mice. *Food hydrocolloids*. 2011;25(5):1344-1349.

Glover DA, Ushida K, Phillips AO, Riley SG:Acacia (sen) SUPERGUM™(Gum Arabic). an evaluation

389 of potential health benefits in human subjects. *Food Hydrocolloids*. 2009;23(8):2410-2415.

Mohamed Ahmed A, Hassan Ahmed N. Diabetes mellitus in Sudan: the size of the problem and the possibilities of efficient care. *Practical Diabetes*. 2001;18(9):324-327

Funding: TWAS; Organization for Women in Science for the Developing World (OWSD) scholarship to Rasha Babiker. The authors would like to thanks participants and investigators involved in this study. Special thanks to Dr. Ahmed Dirar as consultant physician in this study and we grateful to Dar Savanna Ltd (safast)-Sudan for providing Gum Arabic (*Acacia Senegal*) powder.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC170

***Annona muricata* Linn. ethanolic leaf extract ameliorates reproductive dysfunctions in streptozotocin-induced diabetic Wistar rats**

O.E. Adeleye¹, N.A. Abojah¹, A.I. Adeleye², E.A. Sogebi³, A.S. Adetomiwa⁴ and J.O. Olukunle¹

¹Veterinary Physiology and Pharmacology, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria, ²Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria, ³Veterinary Medicine and Surgery, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria and ⁴Veterinary Public Health and Reproduction, Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria

Diabetes mellitus, an endocrine and metabolic disorder characterized by hyperglycemia and low blood insulin levels or insensitivity of target organs to insulin (Maiti *et al.*, 2004) affecting quality of life due to its complications. Infertility is a major complication in diabetes (Koroglu *et al.*, 2015; Vlad *et al.*, 2016) due to histomorphological damage of epididymis and testes (Ghanbari *et al.*, 2015), decreased sperm motility (Saumya *et al.*, 2016), semen volume (Adedara *et al.*, 2015), abnormal sperm morphology (Afifi *et al.*, 2015) and disruption of seminiferous tubule morphology (Rashid *et al.*, 2015). Though various agents have been used for intervention studies on diabetes-induced infertility globally, there are no current treatments for diabetes associated infertility in humans (Shi *et al.*, 2017). *Annona muricata* leaf extract (AMELE) have been demonstrated to have anti-oxidant and anti-diabetic activities in animals (Feng 1962; Vasquez, 1990; Oladipo *et al.*, 2018)). This study was performed to investigate the effects of AMELE on reproductive dysfunctions of male diabetic rats.

Thirty-five male rats (150-200g) were randomly assigned to 4 groups (n=5): CTRL (control); DNT, DT1 and DT2 (diabetic, intraperitoneal injection, streptozotocin, 60 mg/kg once). DT1 and DT2 received AMELE orally at 100 mg/kg and 200 mg/kg respectively daily for fourteen days. Data were analysed using ANOVA at $\alpha_{0.05}$. The animals were sacrificed after 2 weeks and testicular weights were recorded. Fasting blood glucose was determined using a digital glucometer. Sperm count, motility, viability and morphology were assessed microscopically. Testes were histologically evaluated.

Blood glucose levels were significantly higher in DNT (267.3 ± 6.6 mg/dL), DT1 (98.3 ± 1.3 mg/dL) and DT2 (87.5 ± 1.8 mg/dL) compared with CTRL (97.5 ± 1.0 mg/dL). Testes weights increased in DT1 and DT2 (1.0 ± 0.04 , 1.0 ± 0.03 g) compared with DNT (0.7 ± 0.03 g). Sperm concentration increased in DT1 and DT2 (130.0 ± 5.9 , $130.7 \pm 7.3 \times 10^6$ cell/mL) compared with DNT ($98.3 \pm 2.3 \times 10^6$ cell/mL). Abnormal sperm morphology decreased in DT1 and DT2 (13.0 ± 0.9 , 8.8 ± 0.4 %) compared with DNT (19.8 ± 0.9 %). Sperm motility increased in DT1 and DT2 (77.7 ± 1.5 , 80.0 ± 0.0 %) compared with DNT (57.0 ± 1.5 %). Sperm viability increased in DT1 and DT2 (70.0 ± 0.0 , 80.0 ± 0.0 %) compared with DNT (52.7 ± 1.3 %). Testes showed degenerated cells in DNT and no lesions in DT1 and DT2.

Reduction in blood glucose, improved testicular functions and morphology showed that AMELE can be used to manage diabetes and its associated reproductive dysfunctions in male Wistar rats.

Key words: *Annona muricata*, diabetes, streptozotocin, reproductive dysfunctions.

Afifi, M., Almaghrabi, O. A. and Kadasa, N. M. (2015). Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin- induced diabetic rat testes. *Biomedical Research International*, 2: 1–6.

Koroglu, P., Senturk, G. E., Yucel, D., Ozakpinar, O. B., Uras, F. and Arbak, S. (2015). The effect of exogenous oxytocin on streptozotocin (STZ) induced diabetic adult rat testes. *Fertility and Sterility*, 2: 47–54.

Maiti, R., Jana, D., Das, U. K. and Ghosh, D. (2004). Antidiabetic effect of aqueous extract of seed of tamarindus indica in streptozotocin induced diabetic rats. *Journal of ethnopharmacology*, 2: 85-91.

Rashid, K. and Sil, P. C. (2015). Curcumin ameliorates testicular damage in diabetic rats by suppressing cellular stress-mediated mitochondria and endoplasmic reticulum-dependent apoptotic death. *Biochimica et Biophysica Acta*, 3: 70–82

Adedara, I. A., Awogbindin, I. O., Anamelechi, J. P. and Farombi, E. O. (2015). Garcinia kola seed ameliorates renal hepatic, and testicular oxidative damage in streptozotocin-induced diabetic rats. *Pharmaceutical Biology*, 1: 695–704.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC171

Chronic consumption of high and low salt diet impairs female reproductive cycle in Sprague-Dawley rats

O.M. Olumide and I.I. Olatunji-Bello

Physiology, Lagos State University College Of Medicine, Ikeja, Nigeria

The reproductive outcome of salt intake as presumed by the ancient myths is being continuously contested. We therefore investigated the effects of high and low salt consumption on reproductive cycle in female rats.

Thirty six female rats (104-118 g) with regular estrus cycle of 4-5 days were divided into 3 groups of control; normal diet of 0.3% salt, low salt diet (LSD); 0.15% and high salt diet (HSD); 8% salt for 6 weeks. Estrus cycle was monitored daily from week 2 to 6 by collecting vagina smear from the rats between 7 and 9am. After the 6 weeks study period, rats were anaesthetized with chloroform and sacrificed following cervical dislocation on diestrus, proestrus, estrus and metestrus. Blood samples were collected to measure the level of estrogen, progesterone, Luteinizing hormone (LH) and Follicle Stimulating Hormone (FSH) using an Enzyme-linked Immuno Assay (ELISA) kit. Malondialdehyde (MDA) and activities of the antioxidants enzymes; catalase (CAT), glutathione reductase (GSH) and superoxide dismutase were measured in the ovary and the uterus. Histology of the ovary and the uterus were also done. Values were represented as $\pm$ SEM and compared with ANOVA using Newman-Keul as posthoc test.

Results showed prolonged ($P<0.05$) dietstrus phase (8.00 ± 0.19 vs 7.0 ± 0.27 days) and a reduced estrus phase (6.38 ± 0.46 vs 7.50 ± 0.19 days) in HSD than control. Metestrus was prolonged ($P<0.05$) in the LSD than in control (8.00 ± 0.21 vs 5.50 ± 0.38 days). Serum level of FSH was significantly lower at diestrus (0.30 ± 0.0323 miu/ml vs 0.40 ± 0.058 miu/ml) and proestrus (0.26 ± 0.050 miu/ml vs 0.52 ± 0.058 miu/ml) in HSD than control. Estrogen level was higher ($P<0.05$) at proestrus (42.46 ± 7.15 pg/ml vs 27.22 ± 2.77 pg/ml) and estrus (42.90 ± 6.232 pg/ml vs 26.48 ± 2.94 pg/ml) in HSD than control. At diestrus, proestrus, estrus and metestrus, CAT (4.260 ± 0.28 mg/ml vs 6.158 ± 0.480 mg/ml; 3.620 ± 0.334 mg/ml vs 5.860 ± 0.42 ; 3.928 ± 0.282 mg/ml vs 6.034 ± 0.512 mg/ml; 4.024 ± 0.389 vs 6.662 ± 0.714 mg/ml), GSH (2.916 ± 0.442 μ mol/ml vs 4.98 ± 0.508 μ mol/ml; 2.46 ± 0.424 μ mol/ml vs 4.89 ± 0.699 μ mol/ml; 3.382 ± 0.596 μ mol/ml vs 5.324 ± 0.543 μ mol/ml; 3.688 ± 0.453 μ mol/ml vs 6.064 ± 0.591 μ mol/ml) and SOD (3.812 ± 0.543 mg/ml vs 6.668 ± 0.788 mg/ml; 3.960 ± 0.433 mg/ml vs 6.754 ± 0.587 mg/ml; 3.242 ± 0.238 mg/ml vs 4.722 ± 0.329 mg/ml; 2.922 ± 0.566 mg/ml vs 5.198 ± 0.592 mg/ml) were lower ($P<0.05$) in HSD than control. MDA was higher ($P<0.05$) at diestrus, proestrus, estrus and metestrus (0.348 ± 0.048 nmol/ml vs 0.145 ± 0.037 nmol/ml; 0.316 ± 0.035 nmol/ml vs 0.167 ± 0.034 nmol/ml; 0.32 ± 0.053 nmol/ml vs 0.118 ± 0.027 nmol/ml; 0.296 ± 0.0323 nmol/ml vs 0.114 ± 0.0296 nmol/ml) in HSD than control. Histology of the ovary and uterus showed a distortion in the HSD compared to the control. In conclusion, chronic consumption of high salt diet will disrupt the reproductive cycle in Sprague dawley rats.

Iranloye B.O., Oludare G.O., Morakinyo A.O., Esume N.A., Ekeh L.C. (2013). Reproductive parameters and oxidative stress status of male rats fed eith low and high salt diet. J Hum Reprod Sci.6(4): 267-272.

Paccola C.C., Resende C.G., Stumpp T., Miraglia S.M., Cipriano I. (2013). The rat estrous cycle revisited: a quantitative and qualitative analysis. Anim. Reprod. 10(4):677-683.

Tertiary education trust fund (TETFUND)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC172

The impact of age-induced hyperglycaemia on structural remodelling in the left ventricle of the streptozotocin-induced type 1 diabetic rat

J. Singh¹, F.C. Howarth², E. Adeghate², K. Bidasee³ and T. Waqar¹

¹University of Central Lancashire, Preston, UK, ²United Arab Emirates University, Al Ain, United Arab Emirates and ³University of Nebraska Medical Centre, Omaha, NE, USA

Chronic hyperglycaemia (HG) induced by diabetes mellitus (DM) is known to elicit adverse structural remodelling in the heart. This study investigated the impact of age-induced hyperglycaemia (2 and 4 months) on structural remodelling in the left ventricle (LV) of the streptozotocin (STZ)-induced type 1 diabetic (T1DM) rats compared to age-matched Wistar controls. The study measured the general characteristics of the animals, structural changes, fibrosis, apoptosis and gene expressions for extracellular matrix (ECM) factors, transforming growth factor beta 1 (TGF-beta1), brain natriuretic peptide (BNP), atrial natriuretic factor (ANP) and calcium transporting proteins using immune-histochemical, immunoblotting and quantitative gene expression analysis. The project had the relevant ethical clearance from each institution to undertake the study.

The results revealed that STZ-induced diabetic rats weighed significantly (Student's t-test; $p < 0.01$) less than their age-matched controls and these reductions in weight were more severe with increasing age ($270 \pm 5.78^{**}$ vs. 343 ± 9.99) at 2 months and ($221 \pm 13.02^{**}$ vs. 378 ± 10.63) at 4 months post STZ-treatment. Similarly, heart weights were also significantly ($p < 0.01$) lower along with an increase in the HW/BW ratio at 2 and 4 months indicating signs of hypertrophy. Plasma blood glucose levels were significantly elevated in T1DM animals after 2 months ($443 \pm 12.2^{**}$ vs. 98 ± 3.79) and 4 months ($446 \pm 18.8^{**}$ vs. 97 ± 3.04) of treatment. Cell viability measured by the percentage (%) of viable myocytes was significantly decreased ($p < 0.01$) at 2 months ($46.2 \pm 7.5\%$ vs. $69.0 \pm 5.8\%$) and at 4 months ($29.5 \pm 1.2\%$ vs. $51.4 \pm 3.5\%$) in LV from STZ-treated rats compared to age-matched controls. These changes were accompanied by structural alteration of the heart and also by significant ($p < 0.05$) increases in fibrosis, apoptosis and gene expressions for BNP, ANP, TGF-beta 1, ECM factors and their regulators, and calcium transporting proteins in the LV of rats induced with T1DM for 2 and 4 months compared to the respective Wistar controls. These results reveal that age-induced hyperglycaemia can evoke profound adverse remodelling of the LV with more severity at 4 months of T1DM.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC173

EFFECT OF CHRONIC CAFFEINE CONSUMPTION ON CARDIAC TISSUE METABOLISM IN RABBITS

A. Alada¹ and W. Nabofa²

¹Physiology, University of Ibadan, Ibadan, Nigeria and ²Physiology, Babcock University, Remo-Ilishan, Nigeria

Previous studies on caffeine-induced effects on endurance and performance have been on skeletal muscle and brain metabolism but not on cardiac tissue metabolism. This study investigated the effect of caffeine on cardiac tissue substrate metabolism in the rabbit. The study was carried out on adult male New Zealand rabbits divided into 3 groups (n=5). Group I rabbits served as control and were given distilled water orally while groups II and III rabbits were administered orally with 2mg/Kg and 6mg/kg of caffeine respectively for 28 days. Blood samples were collected by retro orbital puncture for biochemical analyses of serum myeloperoxidase activity, nitrites, total cholesterol, triglycerides and glucose concentrations. Animals were sacrificed by cervical dislocation and cardiac tissue biopsies were collected for biochemical and immunohistochemical analyses. Cardiac tissue glycogen concentration was determined by anthrone reagent method. Cardiac tissue CPT1 activity and cAMP concentration were determined by immunohistochemistry and colorimetry techniques respectively, with assay kits obtained from Biovision Inc. Values are means $\pm$ SEM, compared by ANOVA and Tukey Post Hoc test for honestly significant difference at $\alpha_{0.05}$. The results showed that Caffeine (2mg/kg and 6mg/kg) significantly reduced MPO activity from 0.72 ± 0.05 to 0.164 ± 0.045 and 0.46 ± 0.12 U/L respectively and significantly increased serum nitric oxide only at 6mg/Kg, from 28.01 ± 6.53 to $45.25 \pm 3.88 \mu\text{M}$ of nitrite. Caffeine also increased serum total cholesterol and triglycerides at the two doses but the serum glucose increased significantly only at 6 mg/kg of caffeine. Also, both doses of caffeine increased cardiac tissue glycogen from 15.62 ± 0.73 to 40.69 ± 6.35 and 38.82 ± 6.91 mg/100g respectively while carnitine palmytol transferase 1 activity increased from 18.3% to 20% and 25.2% respectively. The study showed that caffeine exhibits strong effects on cardiac tissue metabolism most probably by causing cardio protection, cardiac hyperaemia, glycogen sparing, increased cAMP concentration and cardiac utilization of fatty acids as source of energy.

Graham TE, and Spriet LL (1995). J Appl. Physiol. 78:867-74

Nehlig, A. (1999) Neuroscience and Biobehavioral Reviews 23.4: 563–576.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC174

The impact of coupling beta-hydroxy-beta-methylbutyrate (HMB) ingestion to an oral glucose tolerance test in relation to glucose/insulin kinetics across age

P.J. Herrod¹, N. Gharahdaghi¹, H. Phillips¹, R. Ranat¹, E. Hardy¹, J. Rathmacher², P. Atherton¹ and B. Phillips¹

¹University of Nottingham, Derby, UK and ²Metabolic Technologies Inc, Ames, IA, USA

Background

Impaired glucose homeostasis is a key feature in the development of a variety of metabolic diseases including type 2 diabetes (T2D), metabolic syndrome facets, and ageing. The cornerstone for treatment of risk-factors for insulin resistance remains diet and exercise prescription (prior to medicating), however these have poor rates of adherence. As such, there remains a need to identify safe and effective interventions which could help to combat insulin resistance. The leucine metabolite, beta-hydroxy beta-methylbutyrate (HMB), is a bio-active nutraceutical with anabolic properties and contentious effects on insulin sensitivity in animal models (1,2). Therefore the aim of this study was to evaluate the impact of HMB adjuvant to an oral glucose challenge upon plasma glucose/insulin kinetics in older and younger men.

Methods

Groups of 10 younger (18-35y) and 10 older (65-85y) healthy men were recruited. Subjects underwent two 3-hour 75g oral glucose tolerance tests (OGTT's), spaced >7d apart. During one OGTT, glucose was administered with 3g oral HMB and during the other OGTT glucose was administered alone in a randomised-order. Blood was drawn periodically for the quantification of blood glucose (YSI) and plasma insulin (ELISA). Statistical analysis was performed via 2-way ANOVA by age and treatment, with Sidak's multiple comparison post-hoc testing. Data is presented as mean (SD). Significance was set at $p < 0.05$.

Results

In younger men, HMB reduced the insulin area-under-the-curve (AUC) (4158 (SD:2753) vs. 5836 (4451) mU/L/180min; $p = 0.02$) with no difference in glucose AUC (1127 (147) vs. (1142 (122) mmol/Lx180min; $p = 0.8$). This led to a trend towards an increase in the Cederholm index of insulin sensitivity (62.7 (13.9) vs. 55.1 (11.0); $p = 0.08$) with HMB. In older men, HMB had no effect on the insulin (5712 (2488) vs. 5980 (2854) mU/Lx180min; $p = 0.88$) or glucose (1426 (194) vs. (1409 (180) mmol/Lx180min; $p = 0.84$) AUC, and as such there was no difference in their Cederholm index (57.9 (14.2) vs. 55.6 (10.7); $p = 0.72$).

Conclusions

HMB improved insulin sensitivity following a single glucose load in young men; however this does not appear to be sustained into older age, limiting the potential for HMB as a supplement to combat insulin resistance (given the incidence of this rises sharply with advancing age). Nonetheless, the positive effects in younger people indicate the possibility that HMB may form a potential treatment for younger 'at-risk'/T2D populations.

Sharawy et al., The ergogenic supplement beta-hydroxy beta-methylbutrate (HMB) attenuates insulin resistance through suppressing GLUT-2 in rat liver. *Can J Physiol Pharmacol.* 2016; 94(5):488.

Yonamine et al., Beta-hydroxy beta-methylbutrate supplementation impairs peripheral insulin sensitivity in healthy Wistar rats. *Acta Physiologica.* 2014; 212:1.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC175

Association between haematological parameters and body mass index among an adult population in Kaduna, Northwestern Nigeria

F.L. Ciroma

Human Physiology, Kaduna State University, Kaduna North, Nigeria

Background

Obesity is a pro-inflammatory state that increases susceptibility to infections because of impaired granulopoiesis and reduced bacterial clearance upon infection. There is gap about this knowledge in the African population. Therefore, we conducted this study to determine the association between haematological parameters and obesity among adults in an urban area of Northwestern Nigeria.

Method

A total of 280 apparently healthy respondents comprising of 186 males and 94 females, average age of 32 years were recruited from Kaduna State University community after informed consent was obtained. The study was permitted by the research ethics committee, Kaduna State Ministry of Health (reference number MOH/ADM/744/Vol.1/61). Height and weight were measured by standard methods for each participant and BMI was calculated. Participants were divided into normal ($BMI \geq 18.5 \leq 24.9$), overweight ($BMI > 24.9 \leq 29.9$) and obese ($BMI > 29.9$). Blood sample for each subject was analyzed for full blood count by automated haematology analyzer. Results were presented as mean $\pm$ SEM. Analysis of variance was used to compare means and Pearson's correlation was used to test for correlation. The study was conducted in line with the World Medical Association's Declaration of Helsinki guiding principles for research involving humans and animals.

Results

The total leucocyte count was significantly ($P < 0.01$) higher in obese subjects ($5.7 \pm 0.15 \times 10^3/\mu L$) when compared to normal subjects ($5.1 \pm 0.12 \times 10^3/\mu L$). Obese male participants had significantly higher PCV ($46.1 \pm 0.57\%$) and WBC ($5.8 \pm 0.21 \times 10^3/\mu L$) counts when compared to BMI ($44.3 \pm 0.33\%$) and ($5.0 \pm 0.13 \times 10^3/\mu L$) respectively. Overweight females had insignificantly ($P = 0.05$) lower neutrophils ($37.0 \pm 2.26\%$) compared to normal weight ($44.5 \pm 1.24\%$). A significant relationship exists between BMI and haematological parameters.

Conclusion

Higher WBC in obese males and the positive association between WBC and BMI may suggest an increased risk of infections in the obese population (Raghavan et al., 2016). Also, obese males in the present study may be at higher risk of developing cardiovascular complications due to higher PCV (Salih et al., 2016).

Raghavan, V., Gunasekar, D. and Rao, K. R. (2016). Relevance of haematologic parameters in obese women with or without metabolic syndrome. *Journal of Clinical and Diagnostic Research*, 10(5): Ec11-Ec16.

Salih, K., Jouda, J. and El-Haboby, B. (2016). Obesity average and its relation with packed cell volume (pcv) value in a section of students and employees of Al-Mustansiriyah University in Iraq. *Journal of Biology, Agriculture and Healthcare*, 6(6): 24- 29.

Chisale, MR., Kumwenda, P., Ngwira, M., M'baya, B., Chosamata, Bl., Mwapasa, V. (2015). A pilot study to determine the normal haematological indices for young Malawian adults in Blantyre, Malawi. *Malawi Medical Journal*, 27(3). Doi.org/10.4314/mmj.v27i3.5.

Serbulea, V., Upchurch, C.M., Schappe, M.S., Voigt, P., DeWeese, D.E., Desai, B.N., Meher, A.K. and Leitinger, N (2018). Macrophage phenotype and bioenergetics are controlled by oxidized phospholipids identified in lean and obese adipose tissue. *Proceedings of the National Academy of Sciences*, 115 (27): E6254 DOI: 10.1073/pnas.1800544115.

The authors would like to acknowledge all the volunteers who participated in the study, medical students who served as research assistants and laboratory technologists who helped with laboratory analyses.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC176

The effects of treadmill, elliptical and rowing exercise on fat and CHO oxidation in healthy males

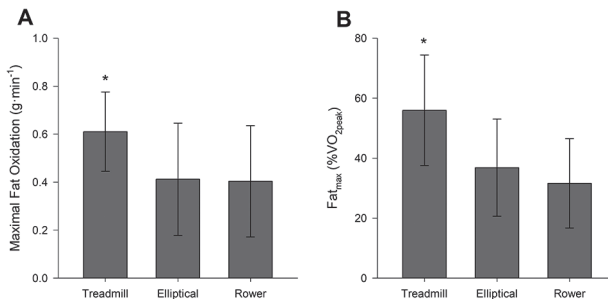
D. Gagnon^{1,2}, S. Munten¹ and M. Filipovic^{1,3}

¹Human Kinetics, Laurentian University, Sudbury, ON, Canada, ²Center for Research in Occupational Safety and Health, Laurentian University, Sudbury, ON, Canada and

³Northern Ontario School of Medicine, Sudbury, ON, Canada

During aerobic exercise, utilization of carbohydrates (CHO) and fat in exercising muscles, can be influenced by intrinsic biochemical factors, as well as extrinsic factors, such as exercise modality (Edgan et al., 2016). Substrate metabolism has previously been shown to be different between modes of exercise, specifically during running and cycling (Achten et al., 2003; Chenevière et al., 2010). Whether substrate oxidation differs across other exercise modalities remain unclear. This study investigated the effects of treadmill, elliptical and rowing exercise on fat and CHO oxidation, maximal fat oxidation rates (MFO), the exercise intensities at which they occur (Fat_{max}), and the crossover point where the energy contribution of fat and CHO were equal. Nine males (22 ± 0.5 years, 77.5 ± 3.71 kg,

178.2 ± 2.94 cm) participated in three adapted maximal oxygen consumption tests on a treadmill, elliptical and rowing ergometer. Indirect calorimetry was used to assess substrate oxidation variables during exercise. Venous blood samples were used to determine changes in blood gases, lactate, and pH. Multiple linear mixed-effect regression analyses were performed to assess differences in substrate oxidation curves. Two-way RM ANOVA were used to assess differences in blood gases across conditions, before and after exercise. Maximal fat oxidation rates were significantly higher with the treadmill ($0.611 \pm 0.0552 \text{ g}\cdot\text{min}^{-1}$) compared to both the elliptical ($p=0.022$, $0.41 \pm 0.078 \text{ g}\cdot\text{min}^{-1}$) and the rower ($p=0.017$, $0.40 \pm 0.078 \text{ g}\cdot\text{min}^{-1}$). Fat_{max} values were significantly higher with the treadmill ($56 \pm 6.2 \% \text{VO}_{2\text{peak}}$) compared to both the elliptical ($p=0.049$, $37 \pm 5.4 \% \text{VO}_{2\text{peak}}$) and rower ($p=0.021$, $32 \pm 5.0 \% \text{VO}_{2\text{peak}}$). Mean crossover points were not different between exercise conditions ($p=0.388$). Lactate concentration was higher in rower ($17.39 \pm 2.79 \text{ mmol}\cdot\text{L}^{-1}$) vs. treadmill after exercise ($p=0.021$, $13.09 \pm 2.26 \text{ mmol}\cdot\text{L}^{-1}$), while partial pressure of O_2 was lower in rower ($37.7 \pm 10.8 \text{ mmHg}$) vs. treadmill after exercise ($p=0.007$, $44.2 \pm 19.9 \text{ mmHg}$). No differences were observed between conditions for blood pH. Regression analyses revealed that the fat but not CHO oxidation curve with treadmill was different compared to rower ($p<0.001$). Exercise on the treadmill maximizes fat oxidation to a greater extent than elliptical and rowing exercises. The treadmill remains a modality that can be used during exercise to help improve cellular and metabolic health. Future investigations should investigate the influence of exercise modality in metabolically-diseased populations.



Edgan B et al. (2016) *Scand. J. of Med. & Sci. in Sports* 26(6), 630–37

Achten J et al. (2003). *Int. J. of Sports Med.* 24(8), 603–8

Chenevière X et al. (2010) *Eur. J. of Appl. Physiol.* 109(6), 1037–45

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC177

Association between Birth Weight and Metabolic Syndrome among Healthy Medical Student in Al Neelain University Faculty of Medicine

T.A. Alnoor^{1,2}, L. Kaddam¹, M. Mohammed¹, H. Humeda² and F. Altekena¹

¹Physiology, Alneelain University, Khartoum, Sudan and ²Physiology, International University of Africa, Khartoum, Sudan

Abstract:

Introduction:

Epidemiological evidences suggest a strong relation between birth weight and some diseases in adult life (hypertension, diabetes, cardiovascular diseases (CVD)). It is thought that an adverse intrauterine environment provokes adaptive response to ensure fetal survival which if persist into adulthood may cause metabolic and CVD disease.

Objectives:

The aim of this study was to study association between birth weight and metabolic syndrome parameters among medical students, aiming to avail information to build the natural history of weight gain during early adulthood.

Methodology:

This descriptive cross-sectional study conducted in Al-Neelain public University; done as part of a larger study that examined the prevalence of obesity among medical, 50 students who have birth weight data were involved in this study. Ethical approval was obtained and Data (collected by Questionnaire, blood pressure, anthropometric measurements and blood sample) were analyzed using SPSS (version23).

Results:

In this study metabolic syndrome prevalence was 2% and 4.1% using IDF and ATPIII definitions respectively. Metabolic syndrome risk factors were highly prevalent 32.6%; for obesity and overweight, 48.1% for hypertension and pre hypertension. There was gender base difference in blood pressure which was statistically significant p value = 0.02, with higher prevalence of pre hypertension and hypertension among male. The relationship between birth weight and adulthood obesity show inverted J shape relation with higher BMI among lower birth weight. Mean value for BP was higher among large for gestational age LGA and low birth weight LBW compared to appropriate birth weight individuals. Birth weight show statistical significance in relation to uncontrolled eating habit P value= 0.004 LBW compared to appropriate birth weight).

Conclusion:

High prevalence of overweight/obesity as well as pre-hypertension/ HTN. An insignificant inverted J shape relationship between birth weight and adulthood obesity.

Recommendation:

Prevention of abnormal birth weight development through proper antenatal care is recommended.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC178

Phenotypic screening reveals diverse gene expression signatures between different browning agents using high throughput Openarray qPCR in human adipocytes

G.R. Davies¹, F. Krause², M. Chodorge³, J. Dodgson³, J. Griffin², Y. Tseng⁴, D. Baker¹ and C. Church¹

¹CVRM, AstraZeneca, Cambridge, UK, ²Department of Biochemistry and Cambridge Systems Biology Centre, University of Cambridge, Cambridge, UK, ³ADPE, AstraZeneca, Cambridge, UK and ⁴Joslin Diabetes Center, Boston, MA, USA

Obesity is a major risk factor for many pathologies including type 2 diabetes and cardiovascular disease and is characterised by an excessive accumulation of white adipose tissue (WAT) which stores excess energy as triglyceride. Conversely, brown adipose tissue plays a role in increasing energy expenditure through uncoupling protein 1 (UCP1). Several compounds have been shown to induce brown-like adipocytes within WAT in a process known as browning. The stimulation of browning in human adipose tissue is a potential novel therapeutic for obesity and diabetes. High throughput phenotypic screening using human adipocytes is an effective method to assess the effects of multiple compounds that can induce browning. However, many pathways in addition to UCP1 can improve adipocyte health, such as insulin signalling, inflammation and adipokine release for systemic benefits on metabolic health. Assessing gene expression changes across multiple pathways provides a comprehensive assessment of adipocyte browning and function.

Here, we used a high throughput Openarray qPCR with a panel of 52 genes involved in diverse gene expression pathways including adipocyte browning, mitochondrial function, fatty acid metabolism and adipogenesis on human white adipocytes. We successfully used this high throughput platform to profile adipocytes treated with a diverse selection of 25 literature supported browning agents including small-molecule drugs, metabolites and peptides, some of which have not previously been investigated in human adipocytes.

We show differential gene expression signatures and clustering across browning agents with effects on multiple pathways with a subset of compounds. For example, forskolin increased genes involved in mitochondrial pathways including UCP1 (33 fold, $p < 0.05$), CKMT2 (6 fold, $p < 0.05$), CKMT1b (7.7 fold, $p < 0.05$) as well as adipokine genes such as adiponectin (3.7 fold, $p < 0.05$) and leptin (3.6 fold, $p < 0.05$). Peroxisome proliferator activated receptor gamma coactivator 1

alpha (PGC1 α) gene expression was unaltered by forskolin treatment. In contrast, lactate did not significantly increase UCP1 ($p>0.05$) but increased the mitochondrial genes PGC1 α (2.8-fold, $p<0.05$) and CKMT2 (3.3 fold, $p<0.05$) as well as the adipokine adiponectin (3.3 fold, $p<0.05$). Differences between individual browning agents may allow combination approaches providing additive or synergistic effects between compounds to further enhance adipocyte health and energy expenditure. The functional effect of gene expression changes in response to diverse browning agents were confirmed by cellular respiration and metabolic flux analysis.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC179

Combined administration of omega-3 fatty acids and metformin had more desirable effects on indices of bone mineralization in experimental diabetic rats

T.A. Abdussalam, W. Johnson and L.A. Olayaki

Department of Physiology, University of Ilorin, Nigeria, Ilorin, Nigeria

Dietary supplementation with omega-3 fatty acids (N-3) has been widely recommended because of the global preference for Western-style diet which is rich in omega-6 fatty acids (Wang et al, 2008). Therefore, we investigated the effects of N-3 and/or metformin in male diabetic Wistar rats. It was hypothesized that the co-administration of N-3 and metformin would not have significant effect on bone mineralization markers in diabetic state. Twenty-five rats (N=5) (Average weight: 120 g) were divided into the following: control; diabetic untreated; diabetic + N-3; diabetic + metformin and diabetic + N-3 + metformin. Diabetes was induced by the administration of streptozotocin (65 mg/kg b.w., *i.p.*), 15 min after the administration of nicotinamide (110 mg/kg b.w., *i.p.*) (Masiello et al., 1998). Five days afterwards, N-3 and metformin were administered daily at 200 and 180 mg/kg b.w., *p.o.* respectively for four weeks (Adeyemi and Olayaki, 2018), after which the rats were sacrificed under ketamine anaesthesia (50 mg/kg, *i.m.*). Data were analysed using analysis of variance and Turkey *post hoc* test at $p<0.05$. The results showed that the induced diabetes was accompanied with significant increases in calcium to phosphorus ratio (Ca:P) and tartrate acid phosphatase (TRAP), but significant decreases in parathormone and phosphorus. Relative to diabetic untreated, treatments with N-3 and metformin caused significant elevations in total alkaline phosphatase (TALP), osteocalcin, parathormone, oestrogen and calcium; however, a significant decrease in TRAP. Co-administration of N-3 and metformin showed more desirable effects on TALP, c-terminal telopeptide of type 1 collagen (CTX-1), oestrogen and Ca:P compared to single administration. There was no significant difference in the effects of N-3 relative to metformin in all the biomarkers, with the exception of Ca:P, where metformin had a more favourable effect. In conclusion, co-administration of N-3 and metformin could be preferably used in the maintenance of bone health diabetic state.

Table 1. Effects of omega-3 fatty acids and/or metformin on calcium, phosphorus, calcium to phosphorus ratio, testosterone, oestrogen and parathormone in induced diabetes mellitus in rats

Groups/ Parameters	Calcium	Phosphorus	Calcium to phosphorus ratio	Testosterone	Oestrogen	Parathormone
Control	1.85±0.03	1.46±0.04	1.24±0.10	1.25±0.09	8.65±0.94	136.76±6.07
Diabetic untreated	1.76±0.01	1.08±0.01*	1.67±0.13*	0.61±0.05	8.23±0.20	64.76±3.10*
Diabetic + N-3	1.92±0.05#	1.10±0.05*	1.68±0.03*	1.27±0.32	13.98±1.69*#	118.61±18.17#
Diabetic + Metf	1.91±0.01#	1.22±0.02*#	1.54±0.05	1.25±0.18	13.10±0.68*#	115.52±18.26#
Diabetic+N-3+Metf	1.90±0.02#	1.22±0.02*#	1.36±0.14	1.21±0.27	17.77±0.81*#c	114.94±3.08#

Values (n=5) are expressed as mean ± SEM. *p< 0.05 is significant compared to control group; #p< 0.05 is significant compared to Diabetic untreated group; cp< 0.05 is significant – Diabetic + Metfm (metformin) vs Diabetic + N-3 + Metfm

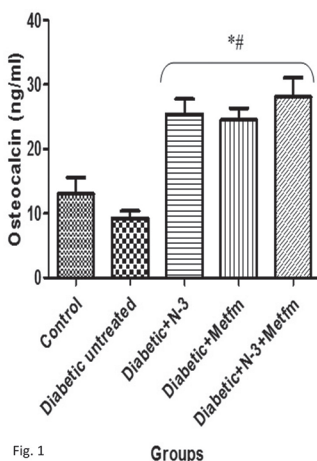


Fig. 1

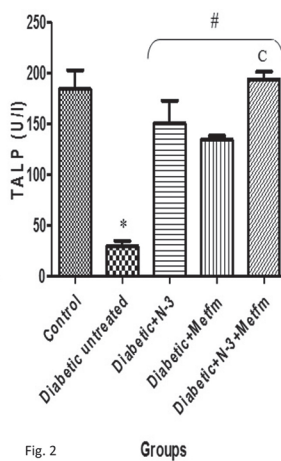


Fig. 2

Figure A and B

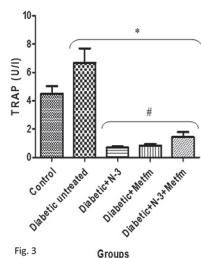


Fig. 3

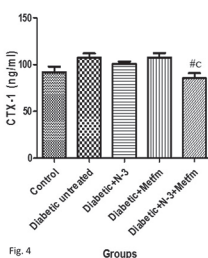


Fig. 4

Effects of omega-3 fatty acids and/or metformin on osteocalcin, total alkaline phosphatase (TAP), tartrate resistant acid phosphatase (TRAP), and c-terminal telopeptide of type 1 collagen (CTX-1) in induced diabetes mellitus in rats

Values (n=5) are expressed as mean ± SEM. *p< 0.05 is significant compared to control group; #p< 0.05 is significant compared to Diabetic untreated group; #c< 0.05 is significant – Diabetic + Metfm (metformin) vs Diabetic + N-3 + Metfm

Figure C and D with title and legends.

Adeyemi WJ & Olayaki LA (2018). *Biomedicine & Pharmacotherapy* 99, 867–875.

Masiello P et al. (1998). *Diabetes* 47, 224–229.

Wang Y et al. (2008). *Osteoarthritis & Cartilage* 16, 579–583.

The authors express their gratitude to the technical staff and students of the endocrine and reproductive unit, Department of Physiology, Faculty of Basic medical sciences, University of Ilorin, Ilorin, Nigeria.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC180

Very low-calorie diet (VLCD) improves markers of insulin sensitivity in obese individuals without reducing pre-existing elevated circulatory branched chain amino acids (BCAA)

M.H. Sayda, M. Aziz, B. Phillips, K. Hession, I. Ramzan, K. Smith, I. Idris and P. Atherton

Medicine, University of Nottingham, Nottingham, UK

Introduction: Given the burgeoning prevalence of obesity and type 2 diabetes mellitus (T2DM), predicted to increase a further ~7.7% by 2030 (Chen et al. 2011), efforts to reverse or prevent the development of T2DM are critical. One way to navigate weight-management is through the use of a very low-calorie diet (VLCD), with the primary aims of achieving weight loss and improving insulin sensitivity (Weinheimer et al. 2010). While the causes of T2DM and obesity are multi-factorial, an area of current interest is the long-standing association between elevated circulating plasma branched chain amino acids (BCAA) and insulin resistance in T2D and obesity (Newgard et al. 2009). However, whether this relationship is causal remains to be determined. Here, we investigated the effects of 6-weeks VLCD upon circulating BCAA, in overweight, non-diabetic individuals, in order to explore any associations with weight loss and improvements in markers of metabolic health. **Methods:** Overnight fasted blood samples were taken from overweight ($n=10$, BMI 32 ± 4 kg/m²), middle-aged men ($\sim 46\pm 8$ y) before and after a 6-week period of nutritional intervention, employing VLCD meal replacement diets (Lighter Life). The VLCD programme consisted of 4 meals/day, providing ~600kcal/day (subjects were allowed upto 200kcal/day extra, in the form of fruit, vegetables or meat); providing a total of 50 g of protein, 50 g carbohydrate and ~17.3 g fat, complete with 100% RDA of vitamins and minerals. Body composition (lean body mass via DXA), was measured at the start and after 6 weeks, and blood analytes such as insulin (ELISA) and glucose (YSI) for calculation of insulin sensitivity were collected throughout. Plasma BCAA were determined by gas-chromatography mass spectrometry. Data were analysed by t-tests and Pearson's correlation. **Results:** Six weeks of VLCD reduced body-weight (-11 ± 3 kg, $P<0.0001$), BMI (-3.4 ± 0.8 kg/m², $P<0.0001$) and improved HOMA-IR ($P<0.05$), while concomitantly reducing fat free mass (mainly muscle) ($P<0.05$). However, despite exhibiting elevated BCAA pre-VLCD (i.e. sum BCAA vs. age-matched controls, $P<0.018$), circulatory BCAA were unaffected by 6-weeks VLCD. There were no correlations with HOMA-IR, FFM or lean body mass

and BCAA at baseline, or following VLCD. Conclusion:VLCD was effective for rapid induction of body weight loss, fat mass loss and reducing BMI, albeit with the unwanted side-effect of lean tissue loss (e.g. due to protein deficiency, release of glucogenic/ketogenic AA). Given the role of muscle in glucose disposal, future studies should seek VLCD-adjuvant atrophy mitigating strategies. Finally, the lack of reduction of circulatory BCAA despite improved insulin sensitivity following VLCD, challenges the notion BCAA are a causal driver of insulin resistance and thus a target of therapeutic value.

Chen, L., Magliano, D.J. & Zimmet, P.Z., 2011. mellitus — present and future perspectives. *Nature Reviews Endocrinology*, 8(4), pp.228–236. Available at: <http://dx.doi.org/10.1038/nrendo.2011.183>.

Newgard, C.B. et al., 2009. A Branched-Chain Amino Acid-Related Metabolic Signature that Differentiates Obese and Lean Humans and Contributes to Insulin Resistance. *Cell Metabolism*, 9(4), pp.311–326. Available at: <http://dx.doi.org/10.1016/j.cmet.2009.02.002>.

Weinheimer, E.M., Sands, L.P. & Campbell, W.W., 2010. A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults : implications for sarcopenic obesity. *Nutrition Reviews*, 68(7), pp.375–388.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC181

Maternal fat consumption may induce oxidative challenge in the offspring with severe reproductive consequences

T.E. ADEYEMI, M. CHANNA and A. NADAR

DEPARTMENT OF PHYSIOLOGY, UNIVERSITY OF KWAZULU NATAL, DURBAN SOUTH AFRICA, Durban, South Africa

High fat diets(HFD) is associated with oxidative stress and cellular damage accompanied by changes in some metabolic functions. However, little is known about maternal fat consumption on endogenous modulation of antioxidant network and reproductive profiles of progenies. In the present study, we investigated the effects of Quercetin-3-O-rutinoside (QR) on maternal high fat diet (HFD)-induced oxidative stress and hormonal functions in offspring. The research was approved by the Animal Research Ethics Committee, University of KwaZulu-Natal.

Fifty-six female Sprague Dawley rats (180-200g) were divided into 2 groups (n=28/group), fed either normal diet (ND) or 45% HFD for 8 weeks and then mated. Post conception, rats were further divided into 4 groups each, of which 2 groups continued with ND or HFD while the remaining groups received either QR (150mg/kg) or HFD+QR. The rats were sacrificed in batches with the first set on gestation day (GD) 19, while the second group was allowed to litter naturally, and pups sacrificed at postnatal day (PND) 21, 28 and 35 respectively. We monitored changes in body weight, performed the oral glucose tolerance test and measured hormone

levels including hypothalamic GnRH, plasma LH and testosterone using ELISA. Catalase, superoxide dismutase, reduced glutathione, nitric oxide and malondialdehyde in the placenta and liver of dams and offspring were quantified. Results were expressed as mean $\pm$ standard deviation and analyzed using ANOVA while the significance level was set at $P < 0.05$.

HFD-fed rats showed increases in body weight but not sensitive to oral glucose. Our data also showed significant increases in liver MDA levels in placenta of HFD-fed dams and liver of HFD offspring at PND 21. SOD activity was decreased in the liver and placenta of all treatment groups of G19 dams, whereas HFD-treated postpartum day (PPD) 21 dams exhibited significant increases in both SOD and catalase enzymatic activities in the liver. Moreover, GSH concentration was increased in QR offspring at D21, decreased in HFD-treated groups at PND 28, and increased in all three groups at PND 35. Liver NO level was also increased in HFD GD19 dams and significant decreases in placenta and liver of HFD+QR PPD21 dams. Hormonal analysis further showed significant decrease in hypothalamic GnRH of HFD offspring at PND 21 and 28. We observed an initial rise in GnRH concentration in QR at PND 21 which later returned to low levels in the offspring throughout the study period. Also, plasma LH was decreased by either HFD or QR at PND21, as well as a significant decrease in testosterone levels in HFD offspring at PND 28 which appears reversed by QR. The results indicate that maternal nutritional drift may induce an oxidative stress challenge in the offspring, leading to hypothalamic-pituitary-gonadal axis dysregulation with a possibility of severe reproductive and metabolic consequences.

Keywords: Developmental programming, Nutrition, Quercetin-3-O-rutinoside
Zambrano, E., et al., *Fetal programming of sexual development and reproductive function*. Molecular and cellular endocrinology, 2014. 382(1): p. 538-549.

Jahan, S., et al., *Ameliorative effects of rutin against metabolic, biochemical and hormonal disturbances in polycystic ovary syndrome in rats*. Journal of ovarian research, 2016. 9(1): p. 86.

Niture, N.T., A.A. Ansari, and S.R. Naik, *Anti-hyperglycemic activity of rutin in streptozotocin-induced diabetic rats: an effect mediated through cytokines, antioxidants and lipid biomarkers*. 2014

Liang, C., K. DeCourcy, and M.R. Prater, *High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice*. Metabolism, 2010. 59(7): p. 943-950.

Charan, J. and N. Kantharia, *How to calculate sample size in animal studies?* Journal of pharmacology & pharmacotherapeutics, 2013. 4(4): p. 303.

college of Health Sciences University of Kwazulu Natal, Durban South Africa

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC182

The effect of a caffeinated beverage on supraclavicular temperature in adult humans

D. Wayne¹, J. Law¹, H. Sacks², H. Budge¹ and M. Symonds^{1,3}

¹The Early Life Research Unit, Division of Child Health, Obstetrics and Gynaecology, University of Nottingham, Nottingham, UK, ²VA Endocrinology and Diabetes Division, VA Greater Los Angeles Healthcare System, and Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, UK and ³Nottingham Digestive Disease Centre and Biomedical Research Centre School of Medicine, University of Nottingham, Nottingham, UK

Brown adipose tissue (BAT) is able to rapidly generate heat and metabolise macronutrients such as glucose and lipids through activation of mitochondrial uncoupling protein 1 (UCP1). Diet can modulate BAT function¹ but the capacity of individual nutrients to promote the abundance and activity of BAT is not well established. Caffeine consumption has been associated with loss of body weight and increased energy expenditure in rodents², but whether it can activate BAT in humans is unknown. We therefore aimed to determine whether caffeine could promote BAT thermogenesis in healthy adults.

Methods

The study was conducted according to the standards of Declaration of Helsinki following approval of the University of Nottingham Medical School Ethics Committee and with informed participant consent. Eleven healthy volunteers, aged 29 (SD 5) years, who had a normal body mass index (mean 23 (SD 3) kg/m²), were studied in a temperature-controlled room maintained at 22°C. Participants acclimated for 30 minutes prior to imaging. Infrared thermography (IRT) was undertaken using the FLIR T540 camera (FLIR Systems AB, Danderyd, Sweden). A baseline assessment of temperature within the exposed supraclavicular region was undertaken (20mins). Participants were then requested to consume either a caffeinated beverage or water (Nescafe® Original 1.8g sachet ~65mg caffeine dissolved in 200ml water at 22°C; n=9 or water alone 22°C n=9). After a 30 minute ingestion period, participants then underwent a second session of thermal imaging over a 20-minute period. Thermal images were analysed by identifying the hottest 10% of the points and then taking the median of those points for each subject (equivalent to the 95th percentile). The primary outcome being the change in temperature from pre- to post-stimulation.

Results

Drinking coffee significantly (P<0.01 Student's T test) increased the temperature of the supraclavicular region co-locating with BAT in humans, reflecting increasing supraclavicular temperature (T_{SCV}) relative to reference body surface temperature (T_{rel}) (All data presented as mean ± SEM T_{SCV}: before caffeine ingestion: 33.81 ± 0.16°C; after caffeine ingestion: 34.19 ± 0.13°C; ΔT_{rel} with caffeine ingestion: 0.41 ± 0.25°C; n=9). The same effect was not seen with consuming water alone (T_{SCV}:

before water ingestion: $34.55 \pm 0.16^{\circ}\text{C}$; after water ingestion: $34.55 \pm 0.17^{\circ}\text{C}$; ΔT_{rel} with water ingestion: $0.13 \pm 0.12^{\circ}\text{C}$; $n=9$).

Conclusion

Drinking coffee stimulated the temperature of the supraclavicular region that is indicative of enhanced thermogenesis in BAT.

1 Scotney, H. *et al.* Glucocorticoids modulate human brown adipose tissue thermogenesis in vivo. *Metabolism* 70, 125-132, doi:10.1016/j.metabol.2017.01.024 (2017).

2 Kobayashi-Hattori, K., Mogi, A., Matsumoto, Y. & Takita, T. Effect of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. *Biosci Biotechnol Biochem* 69, 2219-2223, doi:10.1271/bbb.69.2219 (2005).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC183

PERILIPIN 5, A LIPID DROPLET-BINDING PROTEIN, PROTECTS FROM NOX2-DEPENDENT OXIDATIVE STRESS ON FETAL MUSCLE FROM A MICE MODEL OF GESTATIONAL OBESITY

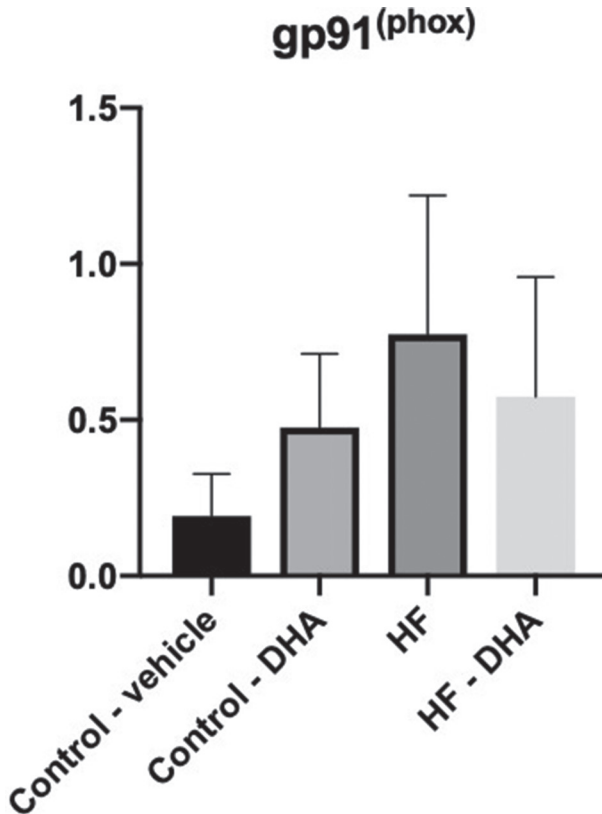
G.E. Valdebenito, D. Alvarez, M. Ortiz, A. Espinosa and M. Maliqueo

University of Chile, Concepcion, Chile

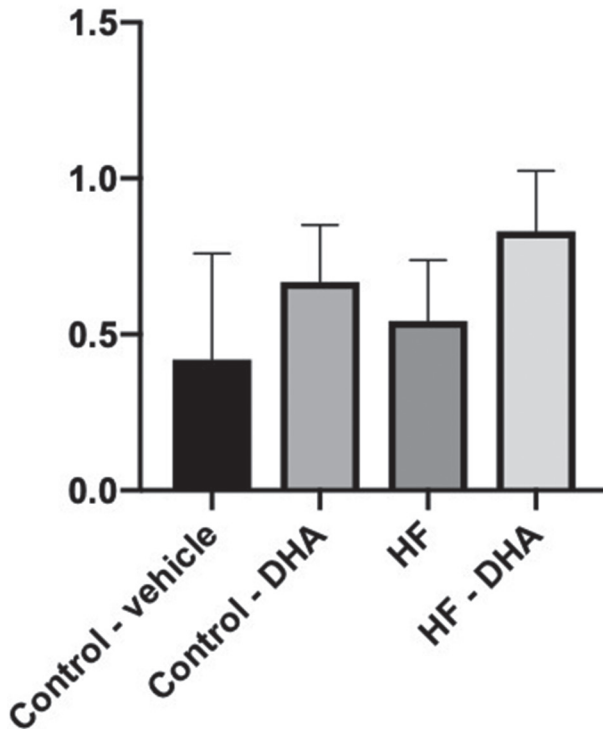
Introduction: Maternal obesity can induce an excess of fatty acid transfer to the fetus, which could alter the physiology of fetal skeletal muscle. These effects could be reverted by a supplementation with docosahexaenoic acid (DHA). In adult muscle fiber, obesity leads to elevated intramuscular lipid content that activate NOX2, by the forming of an active complex between gp91^{phox}, sarcolemma subunit with p47^{phox} (cytosolic subunit), stimulating the lipoperoxidation and disrupting the insulin signaling. In turn, perilipin 5 (PLIN5), a lipid droplet-binding protein act as a protective factor against lipotoxicity. Therefore, we aimed to test, in a mouse model of maternal obesity, if maternal obesity stimulates a higher expression of gp91^{phox}, p47^{phox} and activates NOX2 in fetal muscle, which could be prevented by the stimulation of PLIN5 expression induced by the supplementation with DHA. **Methods:** A total of 24 C57BL/6J female mice were fed with a control diet (CON; 14% Kcal of fat) or high fat diet (OB; 45% Kcal of fat) five weeks, mated and maintained with the same diet during gestation. Since gestational day (GD) 6.5 until GD 16.5, a subgroup of CON and OB were supplemented with DHA (50 mg/kg of body weight). At GD 17.5, dams were euthanatized, and fetus were collected and weighed. Legs from two fetus of each litter were obtained in order to isolate the tissues for studying the expression of PLIN5, gp91 and p47 using western blot. Assembly of gp91^{phox} and p47^{phox} was assessed by indirect immunofluorescence in isolated myoblast after 5 days of culture. Differences were evaluated by ANOVA one way following Tukey test. A p-value < 0.05 was considered as significant.

Results: Our preliminary data shows that the assembly of gp91^{phox} and p47^{phox} subunits of NOX2 is present in the sarcolemma of fetal muscle in the offspring of obese dams. We found a higher expression of gp91^{phox} in fetuses from OB dams compared to fetuses from CON ($P = 0.049$). In turn, PLIN5 showed tended to be higher in OB compared to CON, whereas DHA supplementation induced a higher expression of PLIN5 in CON+DHA and OB+DHA compared to CON and OB groups, respectively.

Conclusion: The assembly of NOX2 subunits suggests that fetal muscle is susceptible of lipoperoxidation mediated by this enzyme. It seems that gestational obesity upregulates gp91, and then activate the NOX2. DHA seems to be a stimulator of PLIN5, forming part of the lipid droplets and acting as a protection from lipid oxidation and oxidative stress in muscle of fetal mice.



PLIN5



Espinosa, A., Campos, C., Díaz-Vegas, A., Galgani, J. E., Juretic, N., Osorio-Fuentealba, C., Jaimovich, E. (2013). Insulin-dependent H₂O₂ production is higher in muscle fibers of mice fed with a high-fat diet. *Int J Mol Sci*, 14(8), 15740-15754.

Zhu, M. J., Han, B., Tong, J., Ma, C., Kimzey, J. M., Underwood, K. R., . . . Du, M. (2008). AMP-activated protein kinase signalling pathways are down regulated and skeletal muscle development impaired in fetuses of obese, over-nourished sheep. *J Physiol*, 586(10), 2651-2664.

Du, M., Tong, J., Zhao, J., Underwood, K. R., Zhu, M., Ford, S. P., & Nathanielsz, P. W. (2010). Fetal programming of skeletal muscle development in ruminant animals. *J Anim Sci*, 88(13 Suppl), E51-60.

CONICYT FONDECYT 1181798 – 1181774

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

The importance of breakfast glycemic control on systemic BDNF and dynamic cerebral autoregulation in men

H. Tsukamoto^{1,2}, A. Ishibashi³, C. Marley¹, Y. Shinohara², S. Ando⁴, D. Bailey¹, T. Hashimoto² and S. Ogoh⁵

¹University of South Wales, Ota, Gumma, Japan, ²Ritsumeikan University, Shiga, Japan, ³Japan Institute of Sports Science, Tokyo, Japan, ⁴University of Electro-Communications, Tokyo, Japan and ⁵Toyo University, Saitama, Japan

It has been established that blood glucose is an important energy substrate for the human brain at rest, and accordingly, brain cognitive function after breakfast (BF) omission isn't better than that after BF consumption with moderate-increase in blood glucose [1]. Nevertheless, although the underlying physiological mechanism remains unclear, the high-glycemic index (High GI) BF consumption with post-prandial hyperglycemia isn't more beneficial to cognitive function than when the low-glycemic index (Low GI) BF consumption [2]. Some previous studies have shown that cognitive dysfunction may be associated with both impaired brain-derived neurotrophic factor (BDNF) metabolism [3] and dynamic cerebral autoregulation (dCA) [4], thereby we hypothesized that BF glycemic control would affect systemic BDNF and dCA as shown in cognitive response. In the present study, we examined the acute impacts of BF omission, Low, and High GI BF consumptions on plasma BDNF and dCA. Ten healthy men (age: 24 ± 1 yrs) performed three trials in a randomized crossover order; BF omission, Low, and High GI BF consumption conditions. Blood samples were collected from a cephalic vein for the assessment of blood glucose (photometry), plasma insulin and BDNF (both ELISAs) and were taken before and during the 120 min post-prandial period. Blood flow velocity in the middle cerebral artery (MCA V; Transcranial doppler ultrasonography) and mean arterial blood pressure (MAP; Finger photosplethysmography) were measured for determination of dCA via transfer function analysis. The data were analyzed using one- or two-way repeated-measures analysis of variance after normal data distribution was confirmed. Specific differences were identified with a Bonferroni *post-hoc* test. Blood glucose and plasma insulin were increased after both GI BF consumptions (area under the curve (AUC) of glucose; omission vs. Low GI $P = 0.07$ and omission vs. High GI $P < 0.01$, AUC of insulin; omission vs. Low GI $P < 0.01$ and omission vs. High GI $P < 0.01$). In addition, increased blood glucose in High GI trial was higher than that in Low GI trial ($P < 0.01$), but not in terms of insulin. While plasma BDNF was decreased in omission trial (Pre vs. Post $P < 0.05$), it was preserved during both BF trials. Transfer function phase and gain were not changed in omission trial. Meanwhile, transfer function gain and normalized gain (low-frequency) were lower during both BF trials, with lower phase (very low-frequency) observed in High GI; in other words, an inadequate dCA was observed in omission and High GI trials when compared with Low GI trial. These findings highlight that BDNF metabolism is impaired by BF omission, and dCA after BF

omission and High GI BF consumption is weaker than that after Low GI BF consumption. These observations imply that BF glycemic control would be manipulation to support to prevent neurodegenerative and cerebrovascular disease in addition to metabolic dysfunction such as type 2 diabetes.

[1] Benton D, Slater O, Donohoe RT. The influence of breakfast and a snack on psychological functioning. *Physiol Behav.* 2001;74(4-5):559-571.

[2] Edefonti V, Rosato V, Parpinel M, Nebbia G, Fiorica L, Fossali E, Ferraroni M, Decarli A, Agostoni C. The effect of breakfast composition and energy contribution on cognitive and academic performance: a systematic review. *Am J Clin Nutr.* 2014;100(2):626-656.

[3] Shimada H, Makizako H, Doi T, Yoshida D, Tsutsumimoto K, Anan Y, Uemura K, Lee S, Park H, Suzuki T. A large, cross-sectional observational study of serum BDNF, cognitive function, and mild cognitive impairment in the elderly. *Front Aging Neurosci.* 2014;6:69.

[4] denAbeelen AS, Lagro J, van Beek AH, Claassen JA. Impaired cerebral autoregulation and vasomotor reactivity in sporadic Alzheimer's disease. *Curr Alzheimer Res.* 2014;11(1):11-17.

The authors appreciate the time and effort expended by the volunteer subjects. This study was supported by Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (#17H07244 to Hayato Tsukamoto). Hayato Tsukamoto is supported by a Japan Society for the Promotion of Science as an Overseas Research Fellowship (#201960215). Damian M. Bailey is supported by a Royal Society Wolfson Research Fellowship (WM 170007).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC185

ABSENCE OF THERMAL TRANSIENT RECEPTOR-POTENTIAL (TRP) CHANNELS CONTRIBUTES TO CIRCADIAN DISRUPTION IN THE LIVER

M.N. Moraes¹, L.V. de Assis² and A.D. Castrucci²

¹Anhembi Morumbi University, São Paulo, Brazil and ²Physiology, University of Sao Paulo, Sao Paulo, Brazil

Most, if not all, physiological processes, including metabolic events, are under the control of the circadian system, and thus display an oscillatory profile throughout the day, which is ensured by the presence of the clock molecular machinery. Transient receptor potential (TRP) channels are involved in thermogenesis, and energy balance control. We investigated the role of TRPA1, TRPM8 and TRPV1 channels by assessing clock genes and clock-controlled genes (CCGs) in the liver of wild type (WT) and *TrpA1*, *TrpM8* and *TrpV1* KO mice. Two to 4 month old mice were single housed at 22 ± 2°C, under 12:12 LD cycle, euthanized at ZT2 and ZT14 (2 hours after lights on and off, respectively), and the liver was collected for further quantitative PCR. Differences between time points of the same genotype or among genotypes at same time point were determined by Two-Way ANOVA followed by

Bonferroni post-test. The biological relevance of TRP channels in human liver were analyzed applying Genes Set Enrichment Analysis (GSEA) in transcriptome from Genotype-Tissue Expression (GTEx). *TrpM8* and *TrpV1* showed an oscillatory profile in the liver of C57BL/6J WT mice. Removal of TRPM8 or TRPV1 channel did not impair the circadian oscillation of *Per1*; conversely, *Bmal1* expression was markedly and partially reduced in *TrpM8* and *TrpV1* KO mice, respectively, when compared to WT mice. Surprisingly, B6;129 mice do not express *TrpV1* channel in the liver, and the expression of *TrpA1* displayed a circadian variation compared to C57BL/6J WT and *TrpV1* KO mice. Since the expression of *TrpA1* was altered in the absence of *TrpV1*, we investigated the role of TRPA1 channel in the hepatic circadian physiology. Oscillatory profiles of liver *Per1*, *Bmal1*, and *Glut2* were modified in the absence of both *TrpA1* and *TrpV1* when compared to B6;129 WT. Thus, our results reveal a new role for TRPA1/TRPV1 channels in the liver, where they participate in the regulation of clock genes and glucose transporter. Corroborating our *in vivo* mouse data, human bioinformatics demonstrated that *TRPA1* channel plays a novel role in liver physiology. In conclusion, disruption of the clock machinery is closely associated with metabolic disorders, and thus pharmacological intervention in clock genes and/or TRP channels may become a promising alternative therapeutic target to treat metabolic disorders.

ACKNOWLEDGEMENTS: This work was partially supported by the Sao Paulo Research Foundation (FAPESP, grant 2012/50214-4) and by the National Council of Technological and Scientific Development (CNPq, grants 301293/2011-2 and 303070/2015-3). M.N. Moraes and L.V.M. de Assis were fellows of FAPESP (2014/16412-9, 2013/24337-4, respectively).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC186

Age, gender and species dependency of expression of voltage-gated calcium channels in murine white fat adipose depots.

O.A. Fedorenko, M. Toledo-Rodriguez, N. Akaniro-Ejim and P.A. Smith

Life Sciences, University of Nottingham, Nottingham, UK

Extracellular Ca^{2+} influx is critical to many functions of white fat adipocytes (WFA), such as growth, lipolysis and adipokine secretion (1). Pharmacological evidence suggests that this Ca^{2+} influx is mediated in part by L-type voltage-gated calcium channels, VGCCs (2). However, due to technical constraints, electrophysiological evidence for the presence and identification of these VGCCs in WFA is still lacking. To address this problem we have utilised RT-qPCR and Western blotting to determine the effect of adipose-depot, species, age, and gender on VGCCs expression in WFA. WFA were isolated from Sprague-Dawley rats and CD-1 mice. For subcutaneous adipose depots, we used inguinal fat and for visceral, epididymal

for male and periovarian for female. In addition, 3T3-L1 cells, an established model of mouse subcutaneous WFA were used for comparison (1). Data, mean $\pm$ S.E.M., are shown as rank order of relative expression or as ratios, their statistical significance as $p < 0.05$ was determined by ANOVA with Holm-Sidak's multiple comparison test or One sample-t-test respectively.

All cells tested expressed Ca_v1.1, Ca_v1.2, Ca_v1.3 and Ca_v3.1, but not Ca_v1.4. The amount for each individual isoform in epididymal WFA was similar to that for inguinal fat.

The expression level for each VGCC was also similar between epididymal and periovarian WFA. However, expression significantly varied between isoform with a rank order of Ca_v1.2 > Ca_v1.3 $\geq$ Ca_v3.1 $\geq$ Ca_v1.1, relative expression levels to Ca_v1.2 were 0.3 $\pm$ 0.04, 0.16 $\pm$ 0.04 and 0.02 $\pm$ 0.002 respectively (n=16). We saw no difference in the expression profiles between rat and mice.

The expression of Cav1.2 was 5 times larger in juvenile (p16-p21) than adult rats, however they showed similar levels of expression for the other VGCCs studied (n=6-16).

Ca_v1.2 and Ca_v3.1 expression levels significantly decreased upon 3T3 differentiation: 1.2 $\pm$ 0.09 to 0.26 $\pm$ 0.05 and 1.2 $\pm$ 0.14 to 0.22 $\pm$ 0.05 respectively, much lower values were seen in mouse subcutaneous WFA: 0.015 $\pm$ 0.003 for Ca_v1.2 and 0.002 $\pm$ 0.0005 for Ca_v3.1 (n=4-5). Conversely, subcutaneous fat had the highest expression of Ca_v1.3; 23 $\pm$ 6.8 vs. 1.2 $\pm$ 0.25 for 3T3s. Ca_v1.1 had similar expression levels.

Western blots revealed that in WFA Ca_v1.2 has a mw >290 kD, larger than that expected, 240-250 kD(3). A fact possibly explained by extra exons at the N and C-termini as revealed by 2nd generation Deep Sequencing.

Our data shows that Cav1.2 and Cav1.3 are the dominant isoforms of VGCCs expressed in adult murine WFA, with similarity in expression levels between adipose depots, gender and species. Ca_v1.2 and Ca_v3.1 have a greater expression in juveniles or undifferentiated cells, which suggests a role for these VGCC isoforms in multipotency. Cav1.2 appears to have an isoform unique to WFA.

Bentley DC, Pulbutr P, Chan S, Smith PA. Etiology of the membrane potential of rat white fat adipocytes. *Am J Physiol - Endocrinol Metab.* 2014;307(2).

Martin BBR, Clausen T, Gliemann J. Relationships between the Exchange of Calcium and Phosphate in Isolated Fat-Cells. 1975;152:121-9.

Hofmann F, Flockerzi V, Kahl S, Wegener JW. L-Type Ca V 1.2 Calcium Channels: From In Vitro Findings to In Vivo Function. *Physiol Rev.* 2014;94(1):303-26.

This work was funded by a Leverhulme Trust Research Project Grant

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Short-term smoking cessation improves skeletal muscle structure and mitochondrial function

T. Ajime^{1,2}, J. Serre¹, G. Messa³, A. Swaminathan⁴, K. Maes¹, R. Wüst⁵, H. Degens³, T. Troosters^{2,1} and G. Gayan-Ramirez¹

¹Laboratory of Respiratory Diseases, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, Belgium, Katholieke Universiteit Leuven, Leuven, Belgium, ²Research Group for rehabilitation in internal disorders, Department of Rehabilitation sciences, KU Leuven, Belgium., Katholieke Universiteit Leuven, Leuven, Belgium, ³Research center for musculoskeletal science and sports medicine, Manchester Metropolitan University, Manchester, UK, ⁴Lithuanian Sports University, Kaunas, Lithuania and ⁵Department of Human Movement Sciences, Faculty of Behavioural and Movement Sciences, VU University Amsterdam, Amsterdam, Netherlands

Cigarette smoking is a leading cause of mortality worldwide. Various substances in cigarette smoke (CS) may either directly or indirectly, through inflammation, increase muscle proteolysis, inhibit protein synthesis, impair mitochondrial function and thereby cause skeletal muscle dysfunction. The long-term effects of CS on skeletal muscle have been widely explored, but to what extent these changes are reversible by smoking cessation is less known. To investigate this, we assessed the impact of smoking with or without 1 or 2 weeks of CS cessation on skeletal muscle structure and function in a mouse model. We hypothesized that short-term CS cessation reverses smoking-induced deterioration in muscle structure and function. Fifty-two C57Bl/6J mice were divided into 4 groups: chronic CS-exposure (for 14 weeks), 1 week cessation (13 weeks of CS and 1 week cessation), 2 weeks cessation (12 week CS and 2 weeks cessation) and controls (air-exposed for 14 weeks). CS-exposed mice were exposed via a nose-only system to mainstream CS of six 3R4F research cigarettes, twice daily. Comparison between groups was performed using ANOVA or Kruskal-Wallis tests. Animals were sacrificed with an intraperitoneal injection of an overdose of anesthesia. CS caused neutrophilic lung inflammation ($p < 0.0001$), reduced body ($p < 0.001$) and hindlimb muscle mass ($p < 0.001$), that returned to baseline after 1 ($p < 0.05$) to 2 weeks ($p < 0.001$) of smoking cessation. Dual-energy X-ray absorptiometry revealed reductions in fat mass (-26%, $p = 0.0007$) and had not returned to normal after 2 weeks of smoking cessation. Mitochondrial respiration in permeabilized soleus was assessed by high-resolution respirometry. The maximal uncoupled respiration and succinate/rotenone-stimulated respiration were lower in the CS-exposed group ($p < 0.01$) and similar to controls after 2 weeks of smoking cessation ($p < 0.01$), and total soleus protein levels of mitochondrial complexes (I, III and IV) showed a similar pattern ($P < 0.05$). Exposure of the soleus to CS extract also resulted in reduced respiration that was readily reversed after removal of the extract, indicating that substances in CS impair the function of respiratory chain complexes. Fiber sizes and capillarization were measured in the soleus, plantaris and diaphragm. While there was no

effect in the soleus and plantaris, smoking reduced the sizes of all fiber types in the diaphragm ($P < 0.001$) and without capillary loss, this resulted in an increased capillary density ($p < 0.01$). Two weeks of smoking cessation restored these fiber sizes to normal and normalized capillary density while significantly reducing the heterogeneity of capillary spacing ($p < 0.05$) that is expected to improve muscle oxygenation. In conclusion, short-term smoking cessation reverses smoking-induced decrements in mitochondrial function and diaphragm muscle atrophy, highlighting immediate beneficial effects of smoking cessation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC188

Skeletal muscle sarcoplasmic reticulum Ca^{2+} uptake preferentially use glycogenolytic derived ATP

N. Ørtenblad, M.R. Kristiansen, J. nielsen and R. Jensen

Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense M, UK

Introduction. Muscle endogenous glycogen represents a primary fuel source during exercise, and the relationship between glycogen and endurance exercise capacity is a fundamental concept in exercise physiology (Bergström et al, 1967). Studies on both rodent single fibers and humans have pointed to a modulating role of glycogen availability on SR Ca^{2+} release rate (Ørtenblad et al, 2011, 2013). However, little is known about the role of glycogenolytic derived ATP on SR Ca^{2+} uptake. **Methods.** In order to test the role of glycogenolytic derived ATP on SR Ca-ATPase function, single fibers from rat fast-twitch muscle were mechanically skinned, thereby permitting estimation of SR, under conditions with constant cytosolic [ATP] and [PCr]. The SR of the fibre was first completely emptied of its endogenous Ca^{2+} by exposure to a solution with low Mg^{2+} and high caffeine (30 mM) and 0.5 mM EGTA, with the time-integral (i.e. area) of the force response being indicative of the amount of Ca^{2+} released. The SR was then reloaded with Ca^{2+} to various levels (by exposing to a load solution at pCa 8.0 for different load times (15, 30, 60, 120 and 180 s, respectively) before being emptied again. In each fibre, responses were normalized to that observed with the longest loading time at both control situation and after treatment (i.e. GP inhibition or amylase treatment). Glycogenolytic derived ATP was selectively inhibited by using GP activity inhibitors DAB (2 mM) or CP-316,819 (10 μM), or the glycogen was removed using glucoamylase (GA). **Results.** There was a clear and pronounced treatment effect ($P < 0.0001$) of Inhibiting GP and GA treatment, decreasing maximal SR Ca^{2+} contents 46% (CP, $n=7$); 25% (DAB, $n=9$) and 24% (GA, $n=8$). With the lower maximal SR Ca^{2+} content, the time to reach half maximal content was significantly shorter for all treatments ($P < 0.01$). However, total Ca^{2+} loaded for all load times were significantly decreased ($P < 0.01$). **Conclusion.** This study provides direct evidence that

glycogenolytic derived ATP is vital for normal SR Ca^{2+} uptake rate and maximal content, even in the presence of high global muscle ATP and aerobic metabolism.

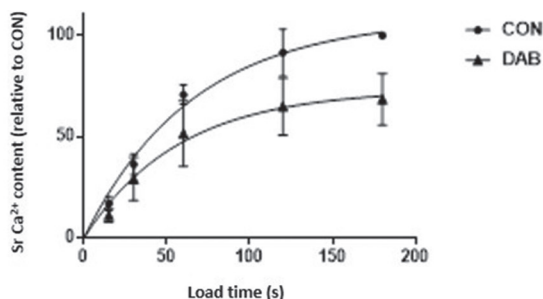


Fig.1. SR Ca^{2+} contents versus normalized area of the force response upon fully emptying the SR after loading with Ca^{2+} for the indicated time.

Bergström J, Hermansen L, Hultman E & Saltin B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71, 140-

Ørtenblad N, Nielsen J, Saltin B & Holmberg HC. (2011). Role of glycogen availability in sarcoplasmic reticulum Ca^{2+} kinetics in human skeletal muscle. *The Journal of physiology* 589, 711-725

Ørtenblad N, Westerblad H & Nielsen J. (2013). Muscle glycogen stores and fatigue. *The Journal of physiology* 591, 4405-4413.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC189

Ameliorative Effect of Resveratrol on Live Body Weight, Blood Glucose level and Serum Liver Enzymes in Cholesterol Diet Fed Rabbits

A. Jimoh¹, J.O. Ayo², Y. Tanko⁶, U. Bello³, A. Abubakar⁴ and A. Mohammed⁵

¹Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria, ²Department Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria, Zaria, Nigeria,

³Laboratory of Cell Biology and Histology, Veterinary Anatomy Department, Ahmadu Bello University, Zaria, Nigeria, Zaria, Nigeria, ⁴Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria, Zaria, Nigeria, ⁵Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria, Zaria, Nigeria and ⁶Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria, Zaria, Nigeria

Fat accumulation in the body has been implicated in the increased in body weight and blood glucose level which enhances hepatocellular damage. Increase in serum liver enzymes have been widely used as an index of liver dysfunction and that higher ALT concentrations predicted development of type 2 diabetes. Thirty rabbits

were randomly divided into six groups, each of five animals: Group 1 = control (C), Group 2 = cholesterol diet (CD) only, Group 3 = resveratrol 200 mg/kg (R200), Group 4 = resveratrol 400 mg/kg (R400), Group 5 = CD + R200 and group 6 = CD + R400. Animals were handled according to the principle guiding the use of laboratory animal in Ahmadu Bello University (ABU), Zaria, Nigeria. The preparations were administered for 8 weeks of experimental protocol. Blood glucose level and live body weight of the animals were measured on weekly bases throughout the treatment period (zero to 8th week). At the end of the study period, the rabbits were placed under light anesthesia (ketamine and diazepam) to remain unconscious. Blood sample of about 3ml was drawn via cardiac puncture for serum extraction which were evaluated for serum concentration of liver enzymes (alanine aminotransferase ALT, aspartate aminotransferase AST and alkaline phosphatase ALP). The results showed a significant increase in body weight after fifth weeks of CD intake at (1.71 ± 0.14 kg) compared to resveratrol treated groups at R200 (1.00 ± 0.03 kg), R400 (1.07 ± 0.08 kg) and resveratrol in combination with CD at CD + R200 (1.09 ± 0.05 kg), CD + R400 (1.03 ± 0.03 kg). Significant increase in the blood glucose level was also recorded in CD group with a value of (49.6 ± 7.9 mg/dL) after fifth weeks of CD consumption compared to the resveratrol groups R200 (7.2 ± 8.10 mg/dL), R400 (6.4 ± 4.45 mg/dL) and resveratrol co-administered with CD, R200+.CD (10.5 ± 4.61 mg/dL), R400 + CD (9.4 ± 5.565 mg/dL). Serum Liver enzymes activities showed significant ($P < 0.05$) decrease in ALT (CD + R200; 53.60 ± 2.09 IU/L), AST (CD + R400; 50.00 ± 2.30 IU/L) and ALP (CD + R200; 71.40 ± 1.57 IU/L) levels in groups that were administered resveratrol with CD compared with CD alone at (75.80 ± 1.98 IU/L for ALT), (67.20 ± 3.68 IU/L for AST) and (85.60 ± 1.18 IU/L). In conclusion, the observed decreased in blood glucose level, body weight and liver enzymes with resveratrol administration despite CD consumptions, elucidated the therapeutic potential of resveratrol.

A. Jimoh., Y. Tanko, J.O Ayo., A. Ahmed and A. Mohammed (2018). Resveratrol increases serum adiponectin level and decreases leptin and insulin level in an experimental model of hypercholesterolemia. *Pathophysiology*, 25(2018): 411-417.

A. Jimoh., Y. Tanko, A. Ahmed., A. Mohammed and J.O. Ayo (2018). Resveratrol prevents high-fat diet-induced obesity and oxidative stress in rabbits. *Pathophysiology*, 25(2018): 359-364

Youn, J. Y., Siu, K. L., Lob, H. E., Itani, H., Harrison, D. G. and Cai, H. (2014). Role of vascular oxidative stress in obesity and metabolic syndrome. *Diabetes*, 63: 2344-2355.

Szkudelska, K. and Szkudelski, T. (2010). Resveratrol, obesity and diabetes. *European journal of Pharmacology*, 635: 1-8.

Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., Prabhu, V. V., Allard, J. S., Lopez-Lluch, G., Lewis, K., Pistell, P. J., Poosala, S., Becker, K. G., Boss, O., Gwinn, D., Wang, M. Y., Ramaswamy, S., Fishbein, K. W., Spencer, R. G., Lakatta, E. G., Le Couteur, D., Shaw, R. J., Navas, P., Puigserver, P., Ingram, D. K., de Cabo, R. and Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 444 (7117): 337-342.

The authors wish to thank Mr Bamidele Adetigba of Human Anatomy Department, Faculty of Basic Medical Sciences and Mr Olu Ayebusi of Chemical Pathology Department, Faculty of Basic Clinical Sciences, Ahmadu Bello University, Zaria, Nigeria for their assistance throughout the period of this work

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC190

Protective Effect of Combined Administration of Lycopene and Zinc Supplements on Oxidative Stress and Lipid Peroxidation Biomarkers in Alloxan Induced Diabetic Wistar Rats

K.M. Ahmed

Human Physiology Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria

Diabetes mellitus is a chronic metabolic disorder and its incidence has been increasing all around the world. Oxidative stress and lipid peroxidation are central factors in the metabolic dysfunctions and pathologies associated with diabetes. Reports have shown that antioxidants could be useful in preventing or attenuating the adverse effects of chronic hyperglycemia. Lycopene is one of the most powerful antioxidants among dietary carotenoids found in foods. It has gained increased attention for its health giving properties. The present study was designed to investigate the protective effect of combined administration of zinc and lycopene supplements on oxidative stress and lipid peroxidation biomarkers in alloxan induced diabetic wistar rats. Thirty (30) adult male Wistar rats weighing (180 – 200) grams were randomly divided into five groups of five rats each ($n = 5$). Group 1 normal control receive 0.5mg/kg of olive oil, Group 2 diabetic control were given 0.5mg/kg of olive oil, Group 3 diabetic were given 10mg/kg lycopene dissolve in 1ml olive oil, Group 4 diabetic were given 20mg/kg of zinc supplements, Group 5 diabetic were given 10mg/kg of lycopene + 20mg/kg zinc and Group 6 diabetic were given 2mg/kg of glibenclamide respectively all treatments were administered via oral route, at the end of the fourth week rats were euthanized and blood samples were drawn from the heart by cardiac puncture and used to estimate oxidative stress biomarkers. Superoxide dismutase (SOD) Catalase (CAT) Glutathione peroxidase (GPx) and lipid peroxidation biomarkers Malondealdehyde (MDA). Analysis of variance (ANOVA) and Turkey's post hoc test were used to analyse the data obtained. For the oxidative stress biomarkers assessed, the results showed that there was significant increase ($P < 0.05$) in the SOD, CAT and GPx (41.50 ± 3.64 , 9.96 ± 0.92 and 77.50 ± 2.12) level of the groups administered with 10mg/kg lycopene when compared with the diabetic control group (5.01 ± 0.30 , 1.47 ± 0.02 and 19.01 ± 0.84) only. The result also showed a significant ($P < 0.05$) increase in the serum CAT, SOD and GPx levels for the group treated with 20mg/kg of Zinc supplement as (42.78 ± 1.87 , 4.90 ± 0.50 and 48.68 ± 2.57) compared to the diabetic control group. Furthermore the group that was co-administered with lycopene and zinc supplement had a significant ($P < 0.05$) increase in the SOD and GPx levels (30.50 ± 2.58 and 66.6 ± 1.42) when compared to the diabetic control group. The result of (MDA) concentrations, showed significant ($P < 0.05$) decrease in the groups

treated with lycopene, zinc and the combined regimen (16.42 ± 1.47 , 19.64 ± 1.27 and 14.24 ± 1.29) respectively when compared to the diabetic control group (189.84 ± 3.28). The present study reveals that oral administration of combined lycopene and zinc supplements elevates oxidative stress biomarkers and lowers malondialdehyde level in alloxan induced diabetic wistar rats to a greater extent than the use of individual regimens.

Omayma, A.E., Mohamed, A.E and Gamil, E.I. (2013). Lycopene and Lutein; A review for their Chemistry and Medicinal Uses. *Journal of Pharmacognosy and Phytochemistry*. 2(1):245-254.

Al- Rubeaan, K., Al Derwish, M., Ouizi, S., Youssef, M. A., Subhani, S. N., Ibrahim, H. M. and Alamri, B. N. (2015). Diabetic Foot Complication and Their Risk Factors from a Large Retrospective Cohort Study. *Open Access Journal*, 10(5): 1-17

Maritim, A.C., Sanders, R.A and Watkins, J.B. (2003). Effect of alpha lipoic acid on biomarkers of oxidative stress in streptozotocin induced diabetic rats. *Journal of Nutrition and Biochemistry*. 14: 288-94

Wang, X., Li, H., Fan, Z. and Liu, Y. (2012). Effect of zinc supplementation on type 2 diabetes parameters and liver metallothionein expressions in Wistar rats. *Journal of Physiology and Biochemistry*, 68(4): 563-572.

Arab, L and Steck, S. (2000). Lycopene and cardiovascular disease. *American Journal of Clinical Nutrition*. 71(6):1691-1695.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC191

Comparison of Salivary Cortisol Levels in Normal and Severely Depressed Patients

Q.U. Khan

Physiology, CMH Medical and Dental College, Lahore, Pakistan

Introduction: Depression is defined as a mood disorder marked especially by sadness, inactivity, difficulty with thinking and concentration, a significant increase or decrease in appetite feeling of dejection, hopelessness, and suicidal ideation. Cortisol known as a stress hormone is found to be raised in the cases of severe depression and its higher levels were detected in saliva early morning sample.

Aims and Objectives: To compare the salivary cortisol levels of normal and severely depressed patients.

Materials and Methods: This study was cross-sectional, comparative and was conducted in Department of Physiology, Shaikh Zayed FPGMI Lahore in collaboration with Punjab Institute of Mental Health Lahore, after getting permission from ERB. A total 60 participants were included in this study divided equally into two groups; each group having equal number of normal and depressed individuals respectively. Patients diagnosed as having major depression based on outdoor assessment, clinically and confirmed by ICD-10 Depression Diagnostic criteria and

DSM4. All known cases of hyperaldosteronism, Cushings syndrome or disease were excluded. Data was collected in the form of a questionnaire based on Becks Inventory. Saliva samples were taken, processed and assessed for cortisol levels using ELISA.

Results: We found higher salivary cortisol levels in severely depressed patients. Moreover, BMI was found to be associated with depression. Cortisol levels were also found to be elevated in those subjects who had positive family history of depression as compared to normal individuals. (p-value 0.001)

Conclusion: Salivary cortisol (p-value 0.03) may be considered and explored as a biomarker for diagnosis and prognosis of depression.

Diagnostic and statistical manual of mental disorders. 4th Text Revision ed. Washington, DC: American Psychiatric Association; 2000.

Owens M, Herbert J, Jones PB, Sahakians BJ, Wilkinson PO, Dunn VJ, et al. Elevated morning cortisol is a stratified population-level biomarker for major depression in boys only with high depressive symptoms. *Proceeding National Academy Sci* 2014; 111 (9):3638-43.

Kurina LM, Schneider B, Waite LJ. Strees, symtoms of depression and anxiety, and cortisol patterens in working parents. *Stress and Health*, 2004; 20(2): 53-63.

Robinson LA, Berman JS, Neimeyer RA. Psychotherapy for the treatment of depression: Acomprehensive review of controlled outcome research. *Psychological bulletin* 1990; 108 (1): 30-49

World Health Organization. The World Health Report 2001: Mental Health: New Understanding, New Hope. WHO, 2001.

Prof Dr Sibgha Zulfiqar

Prof Dr Shahid Hasan

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC192

17 β -Estradiol protects sequestosome 1 deficient mice from hyperphagia and obesity

E. Warabi, A. Hakamata, J. Uwayama, T. Yanagawa and T. Ishii

University of Tsukuba, Tsukuba, Japan

Leptin, a metabolic hormone released from adipose tissue, targets the hypothalamus and plays a key role in the suppression of food intake and body weight gain. We previously reported that a signal modulator sequestosome 1 (SQSTM1), also termed p62/ZIP/A170, positively regulates leptin signaling in mouse brain [1]. An apparent phenotype of SQSTM1 deficient mice is late-onset obesity during feeding a regular chow diet. We concluded that obesity in SQSTM1-KO mice is a consequence of hyperphagia due to defects in brain leptin signaling including STAT3 activation in pro-opiomelanocortin neurons [1].

In this study, we examined differences in the kinetics of age-dependent body weight (BW) gain between male and female SQSTM1-KO C57BL mice during feeding a standard chow diet. All experiments were conducted according to the guidelines of the Animal Experiment Committee in the University of Tsukuba. Female mice, unlike male SQSTM1-KO mice, exhibited a delayed increase in BW with a sharp rise after 25-30 weeks of age and their mean BWs compared to wild type female mice at 30 and 50 weeks were respectively 117 and 207% (n=5). Food intake in female SQSTM1-KO mice also gradually increased after 25-30 weeks. These results suggest that estrogen may protect against the deficits in leptin signaling in young female SQSTM1-KO mice, as estrogen is associated with anorectic effects similar to leptin [2]. This hypothesis is confirmed by the following results: (i) ovariectomy of SQSTM1-KO mice at 10 weeks of age induces about 20% increase in food intake and greater BW gain relative to control KO mice with BW changes in over the next 10 weeks increasing respectively from 18.9 ± 2.7 to 28.9 ± 2.7 and 19.3 ± 1.6 to 24.0 ± 3.3 (g, n=7, $P < 0.01$, Student t-test) for ovariectomized and control sham operated mice, and (ii) implanting 17 β -estradiol (E2) releasing tablets to maintain physiological levels of E2 for several weeks in male SQSTM1-KO mice at 12 weeks of age induces suppression of food intake by about 20% and largely inhibited BW gain, with BW gain over the next 6 weeks increasing respectively from 30.1 ± 2.1 to 30.6 ± 2.3 and 30.6 ± 1.6 to 34.8 ± 3.8 (g, n=6) for the E2 tablet and placebo treated male SQSTM1-KO mice. These results suggest that E2 compensates for deficits in leptin signaling in both male and female SQSTM1-KO mice.

Interestingly, Gao et al. [2] reported that E2 induces STAT3 activation in POMC neurons in the arcuate nucleus in both leptin-deficient and leptin receptor-deficient mice. Their study and our results suggest that SQSTM1 plays a role in sensitising the leptin-receptor signaling while estrogen-mediated signaling bypasses the leptin-receptor pathway allowing activation of leptin-like effects including STAT3 activation via different signaling pathways. Further studies are required to uncover the mechanisms underlying SQSTM1 involvement in leptin signaling including STAT3 activation in the brain.

Harada H, Warabi E, Matsuki T, Yanagawa T, Okada K, Uwayama J, Ikeda A, Nakaso K, Kirii K, Noguchi N, Bukawa H, Siow RC, Mann GE, Shoda J, Ishii T, Sakurai T. Deficiency of p62/Sequestosome 1 causes hyperphagia due to leptin resistance in the brain. *J Neurosci*. 2013;33:14767-14677.

Gao Q, Mezei G, Nie Y, Choi CS, Roberts JL, Gao XB, Mobbs C, Shulman GI, Diano S, Horvath TL. Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. *Nat Med*. 2007;13:89-94.

Supported by JSPS and Great Britain Sasakawa Foundation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC193

An emerging role for the regulatory factor ZFH3 in growth and energy balance.

R. Dumbell¹, A.G. Wilcox¹, G. Banks¹, N. Bourbia^{1,2}, L. Bentley¹, M. Parsons¹, P. Barrett³ and P.M. Nolan¹

¹Mammalian Genetic Unit, MRC Harwell Institute, Didcot, UK, ²Public Health England, Harwell Campus, Didcot, UK and ³The Rowett Institute, University of Aberdeen, Aberdeen, UK

We previously described a mouse point mutation in the *Zfhx3* gene which encodes zinc-finger homeobox 3, named short circuit (*Sci*), during an *N*-ethyl-*N*-nitrosourea mutagenesis screen (1). The mutation is homozygous lethal while *Zfhx3*^{*Sci*/+} mice have a short circadian period, driven in the suprachiasmatic nucleus by decreased activation of an AT motif bound by ZFH3. We have also demonstrated a role for *Zfhx3* in the hypothalamus of adult mice (2). RNA-seq comparison of *Zfhx3*^{*Sci*/+} (*Sci*) and *Zfhx3*^{+/+} (WT) mice indicated differential expression of genes implicated in energy balance in the hypothalamus (1). The role of ZFH3 in energy balance is supported by a recently identified protein-altering variant of ZFH3 in a human genome-wide association study, associated with decreased body mass index (3). We aim to investigate the metabolic phenotype of *Sci* mice.

Here we show reduced body mass in two cohorts of male and female *Sci* mice at 6 and 12 months, with shorter body length at 12 months (all data in table 1). At 6 months, *Sci* mice had reduced fat mass % and bone mineral density. At 12 months, lean mass and fat mass were both reduced in proportion to body mass and mesenteric fat pad mass was reduced in *Sci* mice. Overnight fasted hormone measurements in these mice revealed reduced circulating insulin and insulin-like growth factor-1 (IGF1) in *Sci* mice and leptin concentration was not altered in mutants when corrected for fat mass. In addition, preliminary data from 3 month old female mice indicates that *Sci* mice have reduced dark phase food intake (24 h single housed, TSE Phenomaster). These data together indicate a role for *Zfhx3* in the regulation of growth and energy balance.

A further cohort of mice (n=12 / group) will undergo calorimetric measurements at 3 months to confirm preliminary observations. Brains from these mice will be collected in order to measure hypothalamic gene expression and localisation of candidate genes by *in situ* hybridisation to investigate potential mechanisms of ZFH3 action to alter appetite, growth and/or energy expenditure pathways.

Comparison	Cohort	Female-Sci	Female-WT	Male-Sci	Male-WT	Significant P-Values
Body Mass	6 months	n=4 32.06±2.32g	n=5 40.62±1.68g	n=5 38.77±2.67g	n=5 46.07±2.82g	Genotype: P<0.01
Body Mass	12 months	n=11 38.76±2.03g	n=12 48.71±1.13g	n=8 38.89±0.88g	n=12 48.54±0.87g	Genotype: P<0.0001 Sex: P<0.05
Body Length	12 months	n=11 9.54±0.09cm	n=12 10.46±0.07cm	n=8 9.75±0.07cm	n=12 10.57±0.07cm	Genotype: P<0.0001 Sex: P<0.05
Fat Mass % (DEXA)	6 months	n=4 34.31±3.04%	n=5 44.62±1.01%	n=5 30.65±3.40%	n=5 34.84±3.62%	Genotype: P<0.05 Sex: P<0.05
Bone mineral density (DEXA)	6 months	n=4 0.057±0.001g/cm2	n=5 0.066±0.002g/cm2	n=5 0.062±0.004g/cm2	n=5 0.064±0.001g/cm2	Genotype: P<0.05
Lean mass (EchoMRI)	12 months	n=11 23.24±1.15g	n=12 38.39±0.96g	n=8 31.55±0.85g	n=12 29.86±0.86g	Genotype: P<0.0001 Sex: P<0.0001
Lean mass % (EchoMRI)	12 months	n=11 55.72±1.47%	n=12 56.40±1.62%	n=8 72.54±1.12%	n=12 71.85±1.30%	Sex: P<0.0001
Fat mass (EchoMRI)	12 months	n=11 20.29±1.54g	n=12 25.24±1.20g	n=8 13.13±0.67g	n=12 16.64±0.91g	Genotype: P<0.001 Sex: P<0.0001
Fat mass % (EchoMRI)	12 months	n=11 48.58±1.70%	n=12 47.4±1.70%	n=8 30.61±1.28%	n=12 31.09±1.52%	Sex: P<0.0001
Mesenteric fat mass	12 months	n=11 1.327±0.181g	n=12 1.705±0.103g	n=8 0.781±0.039g	n=12 1.167±0.113g	Genotype: P<0.001 Sex: P<0.01
Fasted insulin	12 months	n=11 0.70±0.2ng/ml	n=11 2.04±0.37ng/ml	n=8 1.39±0.40ng/ml	n=11 3.04±0.61ng/ml	Genotype: P<0.01
Fasted IGF1	12 months	n=10 542.69±36.61pg/ml	n=11 666.93±44.91pg/ml	n=8 611.22±35.24pg/ml	n=11 802.87±37.80pg/ml	Genotype: P<0.001 Sex: P<0.05
Fasted leptin / fat mass	12 months	n=10 2.06±0.18ng/ml/g	n=10 2.13±0.15ng/ml/g	n=8 1.26±0.12ng/ml/g	n=10 1.35±0.19ng/ml/g	Sex: P<0.0001
Dark phase food intake / 12 h	3 months	n=4 0.055±0.04g	n=4 0.085±0.010g	-	-	Genotype: P<0.05

Data and statistics, mean±SEM, food intake 2 tailed t-test; all others 2-way ANOVA; DEXA: dual energy X-ray absorptiometry, IGF1: insulin-like growth factor-1

1. Parsons MJ, Brancaccio M, Sethi S, Maywood ES, Satija R, Edwards JK, et al. The Regulatory Factor ZFHX3 Modifies Circadian Function in SCN via an AT Motif-Driven Axis. *Cell*. 2015;162(3):607-21.

2. Wilcox AG, Vizor L, Parsons MJ, Banks G, Nolan PM. Inducible Knockout of Mouse Zfhx3 Emphasizes Its Key Role in Setting the Pace and Amplitude of the Adult Circadian Clock. *Journal of biological rhythms*. 2017;32(5):433-43.

3. Turcot V, Lu Y, Highland HM, Schurmann C, Justice AE, Fine RS, et al. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. *Nature genetics*. 2018;50(1):26-41.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC194

Melatonin Mitigates Hormonal Toxicity in Cannabis-Treated Female Wistar Rats: Involvement of Cannabinoid Receptor Blockers

L.A. Olayaki¹ and A. Oluwanisola^{1,2}

¹Physiology, University of Ilorin, Ilorin, Nigeria and ²Department of Medical Laboratory Sciences, Al-Hikmah University, Ilorin, Nigeria

Cannabis sativa (CS) has been identified as critical signals of the intricate network that control male and female reproduction, at different stages, with direct effects on gonads, and having target on both the hypothalamus and the pituitary (Schuel and Burkman, 2005). After tobacco and alcohol, marijuana is the most commonly abused substance by women of childbearing age (NSDUH, 2013). Cannabis ingestion has been associate with low birth weight, intrauterine growth retardation, presence of congenital abnormalities, prenatal death and delayed the time of commencement of respiration (Gibson *et al.*, 1983; Sherwood *et al.*, 1999). It has been reported that cannabinoid receptors (CBRs) 1 and 2 are well expressed in the mammalian ovaries (EL-Talatini *et al.*, 2009). However, no study has investigated the influence of melatonin on cannabinoid-induced female reproductive hormones in human or animal models.

Fifty five female wistar rats were randomised to 11 groups (n=5 rats each) as follows: Groups I-XI (n=5 rats each) received normal saline (1 ml), ethanolic extract of cannabis sativa (EECS) (2.00mg/kg), EECS+melatonin (4.00 mg/kg), melatonin, cannabinoid receptor blocker 1 (CBRB₁) (rimonabant hydrochloride) (2.00mg/kg), cannabinoid receptor blocker 2 (CBRB₂) (am630) (2.00mg/kg), CBRB₁ +EECS, CBRB₂ +EECS, CBRB₁ +CBRB₂ +EECS, CBRB₁ +EECS+melatonin, CBRB₂ + EECS +melatonin respectively orally for 2 weeks. Gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E), progesterone and prolactin were measured using standard methods. Values were expressed as means $\pm$ S.E.M., compared by ANOVA.

Cannabis sativa significantly ($p<0.05$) decreased GnRH, FSH, LH, E, progesterone and prolactin levels respectively. However, cannabinoid receptors 1 and 2 when blocked separately, significantly ($p<0.05$) increased the levels of all these reproductive hormones compared to CS-treated rats but decreased significantly ($p<0.05$) when compared to the control, though that of former is more than the latter. All these effects were ameliorated by melatonin when the cannabinoid receptors (CBRs) were stimulated and blocked.

This study showed that the gonadotoxic effects of CS could be mediated by endocrine disruption through the use of both cannabinoid receptors 1 and 2, though that of CBRB₂ is more than that of CBRB₁. However, the gonadotoxic effects of CS could be ameliorated by melatonin.

El-Talatini, M.R., Taylor, A.H., Elson, J.C., Brown, L., Davidson, A.C. and Konje J.C. (2009). Localisation and function of the endocannabinoid system in the human ovary. *PLoS One*, 4, e4579.

Gibson, G.T., Baghurst, P.A. and Colley, D. P. (1983). Maternal alcohol, tobacco and cannabis consumption and the outcome of pregnancy, *American Journal of Obstetrician and Gynaecology*, 23, 15–19.

National Survey on Drug Use and Health (NSDUH) (2013). Substance Abuse and Mental Health Services Administration. <http://www.samhsa.gov/data/sites/default/files/NSDUH>.

Schuel, H. and Burkman, L.J. (2005). A tale of two cells: endocannabinoid-signaling regulates functions of neurons and sperm. *Biological Reproduction*, 73, 1078–1086

Sherwood, R.A., Keating, J., Kavvadia, V., Greenough, A. and Peters, T.J. (1999). Substance misuse in early pregnancy and relationship to fetal outcome, *European Journal of Pediatric*, 158, 488–492.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC195

Comparative Evaluation of Anti-hyperglycaemic Activity of Different Fractions of Methanolic Root Extract of *Sansevieria liberica*.

O.S. AMAO¹, O.M. Sofidiya² and A.W. Olusanya¹

¹Pharmacology, Therapeutics, and Toxicology, College of Medicine, University of Lagos, Surulere, Nigeria and ²Pharmacognosy, Faculty of Pharmacy, University of Lagos, Surulere, Nigeria

Diabetes is a global health problem because of its effects on mortality and morbidity. Despite the variety of available conventional treatment options, many patients with diabetes fail to achieve glycaemic control. The objective of this study was to investigate the effects of different solvent fractions of methanolic root extract of *Sansevieria liberica* (*S. liberica*) in alloxan-induced diabetic rats. *S. liberica* (100g) was extracted in methanol. For the different fractions, *S. liberica* (150g) was successively extracted with solvent of different polarity (hexane, chloroform, ethyl-acetate and butanol) using a separating funnel. Alloxan model was used to induce hyperglycaemia and administered intraperitoneally in male albino rats (100-130g). Rats were randomly divided into 8 groups (n=7); I: control (DMSO), II: alloxan (150 mg/kg), III: *S. liberica* (400 mg/kg), IV-VII: hexane, chloroform, ethyl-acetate and butanol fractions (100 mg/kg), VIII: glibenclamide (5 mg/kg). Different fractions were administered to group of rats for 21 days. Blood samples were collected by tail tipping and glucose levels were measured using a glucometer. At the end of the 21-day treatment, blood samples were collected by ocular puncture. Haemoglobin and glycosylated haemoglobin were estimated in whole blood, liver glycogen was estimated using a spectrophotometer and serum was analyzed for urea and creatinine using diagnostic reagent kits. Results were analysed using one-way variance (ANOVA) and represented as mean $\pm$ SEM (n = 5).

Out of all the treatment groups, the peak hypoglycaemic effect was achieved in chloroform group (62.60 ± 5.12 mg/dl, $P < 0.001$) with a percentage reduction of

86.7%. There was also a significant increase in the haemoglobin ($12.03 \pm 0.54\text{g/dl}$, $P < 0.001$) but reduced glycosylated haemoglobin ($5.66 \pm 0.08\%$) in the chloroform group. Additionally, the liver glycogen level of the chloroform group was significantly increased (4.37 ± 0.22 , $P < 0.001$). The kidney enzymes of all the treated groups were significantly ($p < 0.05$) brought to normal level when compared to alloxan (diabetic control) group.

In conclusion, our data suggests that *S. liberica* and its fractions (hexane, chloroform, ethylacetate, and butanol) produce potential anti-hyperglycaemic activity. The most active fraction was the chloroform fraction, this is probably due to high concentration of phytochemical constituents responsible for the anti-hyperglycaemic activity.

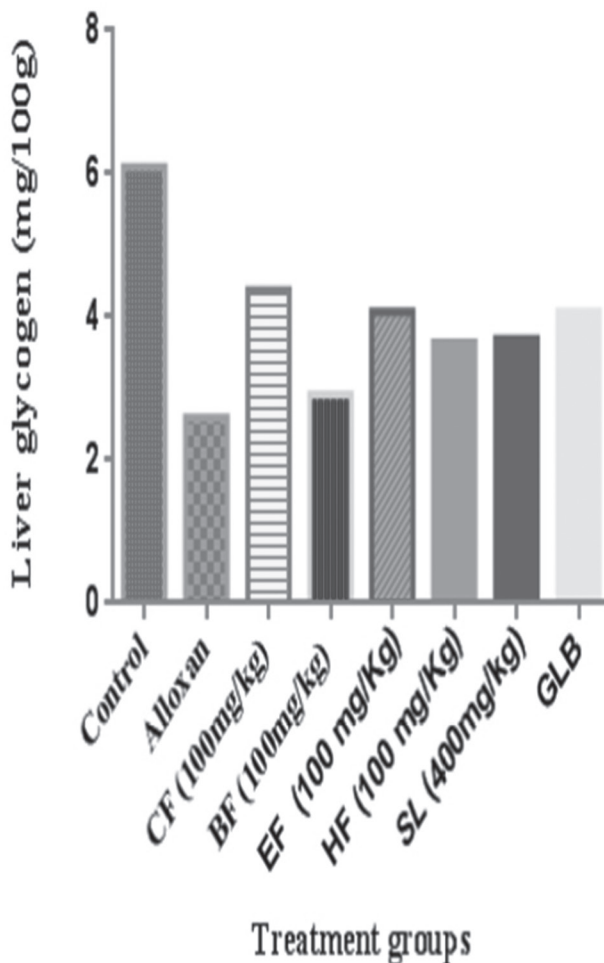
Table 1: Blood glucose levels (mg/dl) after treatment with SL and its fractions.

Groups	Doses (mg/kg)	Before	After	% Reduction
Control	-	101.40 $\pm$ 3.17	93.60 $\pm$ 3.08	7.7%
Alloxan	150	437.80 $\pm$ 85.53	184.50 $\pm$ 32.21c	57.9%
Hexane	100	434.00 $\pm$ 30.20	106.00 $\pm$ 9.00 β	75.6%
Chloroform	100	471.20 $\pm$ 49.18	62.60 $\pm$ 5.12a, γ	86.7%
Ethyl-acetate	100	472.60 $\pm$ 71.12	89.66 $\pm$ 17.03 γ	81%
Butanol	100	292.00 $\pm$ 58.21	126.75 $\pm$ 34.71a, α	56.6%
S.liberica	400	409.60 $\pm$ 71.19	109.25 $\pm$ 4.48 β	73.3%
GLB	5	399.40 $\pm$ 21.19	92.50 $\pm$ 4.03	76.8%

Table 8: Lipid profiles after 21 days of daily oral administration of *Sansevieria liberica* (SL) methanol root extract and its fractions in alloxan-induced diabetic rats

Lipid Profiles (mg/dl)				
Treatment groups	HDL	LDL	CHOL	TG
Control	3.5 $\pm$ 0.06	0.3 $\pm$ 0.02	2.0 $\pm$ 0.06	1.0 $\pm$ 0.16
Alloxan	3.2 $\pm$ 0.02	1.0 $\pm$ 0.09	2.8 $\pm$ 1.79	1.8 $\pm$ 0.62
Hexane	3.0 $\pm$ 0.02	0.4 $\pm$ 0.07 γ	2.4 $\pm$ 0.03	0.6 $\pm$ 0.22
Chloroform	3.2 $\pm$ 0.09	0.2 $\pm$ 0.01 γ	1.9 $\pm$ 0.01	0.6 $\pm$ 0.02 β
Ethyl-acetate	2.8 $\pm$ 0.06 ^{b} , ^{a}	0.4 $\pm$ 0.06 γ	2.2 $\pm$ 0.21	0.6 $\pm$ 0.01
Butanol	3.0 $\pm$ 0.02	0.5 $\pm$ 0.01 γ	2.5 $\pm$ 1.08	1.1 $\pm$ 0.30
<i>S. liberica</i>	2.8 $\pm$ 0.00 ^{b} , ^{a}	0.2 $\pm$ 0.01 γ	2.4 $\pm$ 0.21	0.7 $\pm$ 0.01 ^{a}
Glibenclamide	3.1 $\pm$ 0.04	0.2 $\pm$ 0.06 γ	1.2 $\pm$ 0.02 ^{b} , ^{a}	1.3 $\pm$ 0.22

Groups	Haemoglobin (Hb) g/dl	Glycosylated haemoglobin (HbA1c)%
Control	13.56 $\pm$ 0.29	3.73 $\pm$ 0.14
Alloxan	9.16 $\pm$ 0.16	7.13 $\pm$ 0.08
Hexane F	10.73 $\pm$ 0.14 γ	6.43 $\pm$ 0.70 ^{b}
Chloroform	12.03 $\pm$ 0.54 γ	5.66 $\pm$ 0.08 ^{b}
Ethyl- acetate	11.03 $\pm$ 0.54 γ	5.93 $\pm$ 0.26 ^{b}
Butanol	9.73 $\pm$ 0.14 γ	6.56 $\pm$ 0.72 ^{b}
<i>S. liberica</i>	10.93 $\pm$ 0.23 γ	6.36 $\pm$ 0.64 ^{b}
Glibenclamide	11.00 $\pm$ 0.12 γ	5.86 $\pm$ 0.04 ^{b}



Adeneye, A.A., Adeyemi, O.O., Agbaje, E.O., Sofidiya, M.O (2012). The Novel Anti-hyperglycaemic Action of *Hunteria Umbellata* Seed Fractions Mediated Via Intestinal Glucose Uptake Inhibition. *African Journal of Traditional, Complementary, and Alternative Medicines*, 9(1): 17–24.

Amao, O.S., Nwaiwu, O.O., Olusanya, A.W, and Arikawe, A.P (2018). Prescription Pattern of Anti-diabetic Drugs in a Private Medical Facility in Lagos. *Nigerian Medical Practitioner*, 73:5-6

Mashi, J.A., Atiku, M.K., Shehu, D., Idris, R.I., Sa'id, A.M., Dangambo, M.A., Babagana, K., Ya'u, M, and Babandi, A (2019). Comparative Study of Different Solvents Extract of *Persea americana* Leaf on Alloxan Induced Hyperglycemic Rats. *Asian Journal of Biological Sciences*, 12 (1): 67-72.

Singh, M.P, and Pathak, K (2015). Animal models for biological screening of anti-diabetic drugs: An overview. *European Journal of Experimental Biology*, 5(5):37-48.

This work was fully supported by TETFUND (Tertiary Education Trust Fund).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC196

Impact of carotid sinus nerve resection on metabolic dysfunction induced by ageing and by long-term hypercaloric diet consumption in rats

J.F. Sacramento, C.C. Batista, B.F. Melo, C.S. Prego and S. Conde

CEDOC, NOVA Medical School | Faculdade Ciências Médicas, Universidade Nova de Lisboa, Lisboa, Portugal

Ageing is associated with deregulation of glucose homeostasis and with morphological and functional alterations in the carotid body (CB) (1). The CB is an oxygen sensor being also involved in energy and glucose homeostasis. Resection of carotid sinus nerve (CSN), the CB sensitive nerve, has shown to prevent and reverse insulin resistance and glucose intolerance induced by hypercaloric diets (2-4). Herein, we evaluated the effect of CSN resection on metabolic dysfunction induced by ageing and by long-term hypercaloric diet consumption in rats. Also, since animal models of dysmetabolism and metabolic syndrome patients exhibit increased CB chemosensitivity (2,5), we tested CB function in hypercaloric diet-old animals.

Male Wistar rats (8-10 weeks) were used: a control (CTL, n=12) group that fed a chow diet and a high-fat high-sucrose (HFHSu, n=15) group that fed a 60% lipid-rich-35% sucrose diet during 44 weeks. Groups were randomly divided, anesthetized (medetomidine 0.5 mg/kg; ketamine 75 mg/kg) and submitted to CSN resection or to a sham surgery, with 9 weeks follow-up. Insulin sensitivity and glucose tolerance were evaluated as well as fasting glycemia, insulinemia, C-peptide and ventilatory responses to hypoxia. At a terminal experiment, fat depots were analyzed. CB dysfunction was evaluated through the morphometric analysis of CBs for tyrosine hydroxylase (TH), GFAP and nestin-positive cells. One- and two-way Anova tests were used.

Ageing and long-term HFHSu consumption decreased insulin sensitivity by 59.11 ($p<0.0001$) and 58.11% ($p<0.0001$), respectively (K_{ITT} CTL= 4.50 ± 0.36 glucose/min), an effect restored by CSN resection and maintained until 9 weeks after surgery. Ageing did not alter glucose tolerance in CTL animals, but HFHSu diet induced glucose intolerance (AUC HFHSu before diet= 21778 ± 686 mg/dl*min; AUC HFHSu 44weeks diet = 28112 ± 2035 mg/dl*min, $p<0.05$), an effect decreased by CSN resection (AUC HFHSu 9weeks after surgery = 24392 ± 558 mg/dl*min). HFHSu diet did not exacerbate ageing-induced hyperinsulinemia (CTL_{OLD}= 6.22 ± 0.08 mg/l; HFHSu_{OLD}= 6.33 ± 0.14 mg/l), an effect unaltered by CSN resection (HFHSu 9weeks after surgery = 6.32 ± 0.53 mg/l). HFHSu diet increased significantly epididymal, perienteric and perinephric fat, an effect that was not modified by CSN resection. Ageing decreased TH in the CB by 49.05% ($p<0.01$), an effect that was not modified

by the CSN resection. Ageing and long-term HFHSu diet consumption did not alter GFAP expression in the CB.

In conclusion, long-term hypercaloric consumption in old animals did not modify ageing-induced insulin resistance, while exacerbates glucose intolerance, effects that were restored and ameliorated by CSN resection, respectively. We suggest that modulation of CB activity can be important in ageing-induced insulin resistance as well as in ageing-induced metabolic dysfunction exacerbated by diet.

Conde SV, Obeso A, Rigual R, Monteiro EC, Gonzalez C. Function of the rat carotid body chemoreceptors in ageing. *J Neurochem*. 2006;99(3):711-23.

Ribeiro MJ, Sacramento JF, Gonzalez C, Guarino MP, Monteiro EC, Conde SV. Carotid body denervation prevents the development of insulin resistance and hypertension induced by hypercaloric diets. *Diabetes*. 2013; 62(8):2905-16.

Sacramento JF, Ribeiro MJ, Rodrigues T, Olea E, Melo BF, Guarino MP, Fonseca-Pinto R, Ferreira CR, Coelho J, Obeso A, Seica R, Matafome P, Conde SV. Functional abolishment of carotid body activity restores insulin action and glucose homeostasis: key roles for visceral adipose tissue and the liver. *Diabetologia*. 2017; 60(1):158-68.

Sacramento JF*, Chew DJ*, Melo BF, Donegá M, Dopson W, Guarino MP, Robison A, Prieto-Lloret J, Patel S, Holinski BJ, Ramnarain N, Famm K, Conde SV. Bioelectronic modulation of carotid sinus nerve activity in the rat: a potential therapeutic approach for type 2 diabetes. *Diabetologia*. 2018; 61(3):700-10.

Conde SV, Cunha-Guimaraes JP, Timoteo AT, Caires I, Sacramento JF, Ribeiro MJ, Selas M, Guarino MP, Santiago J, Mota-Carmo M. Carotid body chemosensitivity correlates with insulinemia and insulin resistance but not with blood pressure in metabolic syndrome patients. 2018; *Proc Physiol Soc*. 41: PCA202.

J.F. Sacramento and B.F. Melo were supported by the Portuguese Foundation for Science and Technology grants PD/BD/105890/2014 and PD/BD/128336/2017, respectively.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC197

Inhibition of chemerin/CMKLR1 axis in the hypothalamus reduces body weight and food intake

G. Helfer¹, S. Kantamneni², K. Pors² and Q. Wu³

¹School of Chemistry and Biosciences, University of Bradford, Bradford, UK, ²School of Pharmacy and Medical Sciences, University of Bradford, Bradford, UK and ³Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

Accumulating evidence has revealed critical roles of peripheral signals, such as adipokines, in regulating appetite and energy metabolism in the hypothalamus, a brain area closely associated with homeostatic body weight regulation. Chemerin is a newly discovered adipokine and a natural ligand for the G protein coupled

receptors CMKLR1 and GPR1. The chemerin/CMKLR1 axis is involved in inflammation and energy metabolism and has been hypothesised as a possible link between obesity and the development of type 2 diabetes (1). In support, local chemerin gene expression and circulating levels are elevated in humans affected by obesity. In animal studies, chemerin has been shown to modulate hypothalamic neuropeptides that control feeding and energy homeostasis (2). Here, we used a combination of *in vivo* and *in vitro* studies to examine signalling pathways activated by chemerin and how this links to the control of body weight and feeding. Using single-cell RNA sequencing, we found cell-specific expression of chemerin and CMKLR1 in mouse hypothalamic tanycytes, neurons and microglia. Using fluorescence *in situ* hybridization probing for chemerin followed by immunostaining for vimentin, we confirmed that chemerin mRNA is expressed by tanycytes in male Sprague Dawley rats. To show that chemerin activates hypothalamic cells, we investigated ERK and Akt signalling *in vitro* and *in vivo*. In hypothalamic neural cells, chemerin treatment (10nM) induces ERK1/2 phosphorylation peaking at 11 min (n=4/group). Akt phosphorylation occurred more quickly and peaked at 6 minutes (n=4/group). Likewise, chemerin (100nM) stimulated ERK1/2 phosphorylation within 15 min of intranasal administration in male C57BL/6 mice (n=5/group). Interestingly, chemerin treatment resulted in an increased expression of the pro-inflammatory markers IL-6 and TNF α (t-test: p=0.003, fold change vs vehicle control (FC)=2.69 and p=0.004, FC=2.96, respectively; n=4/group), thus it may play a role in acute hypothalamic inflammation. In line with our *in vivo* data (2), chemerin treatment (10nM) upregulated the appetite regulatory genes AgRP (FC=4.21) and NPY (FC=4.49) (t-test: p<0.05 vs vehicle control, n=4/group), suggesting that it contributes to the neuroendocrine control of appetite. To further test this, we used the small molecule CMKLR1 antagonist 2-(α -naphthoyl) ethyl-trimethylammonium iodide (α -NETA) to block chemerin/CMKLR1 signalling in the hypothalamus. A single injection of 5 μ M α -NETA into the third ventricle of the hypothalamus of male Sprague Dawley rats significantly reduced body weight over 24 hours (t-test, p<0.05, body weight change: 3.20g $\pm$ 0.65 vs -6.43g $\pm$ 3.96; n=5/group). This was accompanied by a reduction in food intake (24.3g $\pm$ 0.86 vs 20.39g $\pm$ 2.6, n.s.). This study demonstrates the potential of the chemerin/CMKLR1 axis as a possible therapeutic target for anti-obesity treatment.

Helper G and Wu QF (2018). *J Endocrinol* 238, R79-R94.

Helper G et al. (2016) *Sci Rep* 6: 26830.

This work was funded by the British Society for Neuroendocrinology and the University of Bradford.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC198

Phoenixin stimulates insulin secretion in INS-1E beta cells and rat pancreatic islets via cAMP/Epac pathway

M. Billert, M. Skrzypski, T. Wojciechowicz, M. Jasaszwili and K.W. Nowak

Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, Poznan, Poland

Phoenixin (PNX), a newly discovered pleiotropic neuropeptide is produced by proteolytic cleavage of Smim20 protein and exerts its biological effects by Gpr173 receptor [1]. Despite important functions in controlling reproduction system and memory processes, PNX is centrally involved in food intake and its systemic levels positively correlate with BMI [2–4]. Notably, the peptide is also peripherally expressed in adipose tissue [5]. Although recent data suggest that PNX is involved in energy homeostasis, its role in modulating glucose control and metabolism remains unknown.

The aim of this study was to investigate the effect of PNX on insulin secretion in INS-1E beta cells and isolated rat pancreatic islets. Furthermore, we studied the mechanism by which PNX modulates insulin exocytosis. Insulin producing INS-1E beta cell line and pancreatic islets isolated from Wistar rats (male, 300-350 g) were used. The presence of PNX peptide in INS-1E cells and pancreas slides was studied by immunofluorescence. The mRNA expression was measured by real-time PCR. Insulin secretion was evaluated by RIA Rat Insulin assay. cAMP synthesis was measured by ELISA kit. Values are means $\pm$ S.E.M., compared by ANOVA.

We found that *Smim20* and *Gpr173* mRNA are expressed in INS-1E cells and rat pancreatic islets. PNX peptide is present in INS-1E and rat pancreatic alpha and beta cells. We demonstrated that PNX is secreted in isolated pancreatic islets in a glucose-dependent fashion, more robustly under high (16.7 mmol/l) than under low (2.8 mmol/l) glucose concentrations (0.0615 ± 0.01 vs. 0.1320 ± 0.021 ng/ml; $n=6$; $p<0.05$ respectively). Moreover, PNX increased glucose-stimulated insulin secretion in INS-1E cells and isolated pancreatic islets ($n=6$; $p<0.05$). In addition, we found that PNX (100 nmol/l) increased cAMP level in INS-1E cells (21.72 ± 0.275 pmol/ml vs. 25.573 ± 1.687 PNX treated; $n=6$; $p<0.05$). Pharmacological blockade of cAMP downstream target protein Epac by ESI-09 prevented PNX from stimulating insulin secretion. These results provide strong evidence that PNX modulates insulin secretion via cAMP/Epac pathway.

Stein LM, Haddock CJ, Samson WK, Kolar GR & Yosten GLC (2018) The phoenixins: From discovery of the hormone to identification of the receptor and potential physiologic actions. *Peptides* 106, 45–48.

Ullah K, ur Rahman T, Wu D-D, Lin X-H, Liu Y, Guo X-Y, Leung PCK, Zhang R-J, Huang H-F & Sheng J-Z (2017) Phoenixin-14 concentrations are increased in association with luteinizing hormone and nesfatin-1 concentrations in women with polycystic ovary syndrome. *Clin. Chim. Acta* 471, 243–247.

Jiang JH, He Z, Peng YL, Jin WD, Wang Z, Mu LY, Chang M & Wang R (2015) Phoenixin-14 enhances memory and mitigates memory impairment induced by A β 1-42 and scopolamine in mice. *Brain Res.* 1629, 298–308.

Schalla M, Prinz P, Friedrich T, Scharner S, Kobelt P, Goebel-Stengel M, Rose M & Stengel A (2017) Phoenixin-14 injected intracerebroventricularly but not intraperitoneally stimulates food intake in rats. *Peptides* 96, 53–60.

Billert M, Wojciechowicz T, Jasaszwilli M, Szczepankiewicz D, Wasko J, Kazmierczak S, Strowski MZ, Nowak KW & Skrzypski M (2018) Phoenixin-14 stimulates differentiation of 3T3-L1 preadipocytes via cAMP/Epac-dependent mechanism. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* 1863, 1449–1457.

This work was supported by the National Science Centre of Poland (grant number 2017/25/N/NZ4/02760).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC199

Neuropeptide B increases proliferation and differentiation of white and brown rat preadipocytes in vitro

T. Wojciechowicz¹, M. Billert¹, S. Kazmierczak², M. Jasaszwilli¹, M. Skrzypski¹ and K.W. Nowak¹

¹Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, Poznan, Poland and ²Institute of Zoology, Poznan University of Life Sciences, Poznan, Poland

Neuropeptide B (NPB) regulates appetite, reduces obesity and promotes energy expenditure by activating NPBW1 and NPBW2 receptors. Humans express both types of receptors, while rodents NPBW1, only. Studies using genetically engineered mice depleting NPBW1 and neuropeptide B-deficient mice developed mild adult onset obesity. These data suggested that NPB may be involved in controlling adipose tissue functions.

Nevertheless, the role of NPB in controlling of preadipocytes biology and adipogenesis is unknown. Thus, we evaluated the effects of NPB on brown and white preadipocytes proliferation and differentiation.

The role of NPB in adipogenesis was evaluated in vitro using isolated rat white and brown preadipocytes. All procedures concerning welfare of animals were accorded with the ethical standards by the National Ethics Commission for Investigations on Animals in Poland. Cells were isolated and cultured in DMEM/F12 with 10% FBS. Proliferation was measured using BrdU ELISA kit (1, 10 and 100 nM NPB and control group; 24 h). Experiments assessing the role of NPB in differentiation were performed using differentiating medium containing T3, insulin and/or dexamethasone with or without NPB. Expression of adipogenic genes were analysed in 1, 3 and 6th days using real-time PCR. Intracellular lipid accumulation was analysed using

ORO staining and microphotographic documentation. Glycerol released into the medium was measured using Free Glycerol Reagent. Analysis of variance followed by the Bonferroni post hoc test was used to determine statistical significance. Values are means $\pm$ S.E.M.

We found *Npb* and *Npw1* mRNA expression in rat white and brown preadipocytes. Our findings indicates that 10 and 100 nM NPB stimulates brown ($0,388 \pm 0,040$ and $0,410 \pm 0,024$ vs. control $0,270 \pm 0,021$ OD450nm; $n=8$, $p<0,05$), but not white, preadipocytes proliferation. Next, we found that NPB increased triacylglycerol accumulation induced by differentiation medium in rat brown ($0,176 \pm 0,013$ OD520nm vs. $0,191 \pm 0,011$ and $0,192 \pm 0,017$ for 10 and 100 nM NPB treated) and white ($0,200 \pm 0,005$ OD520nm vs. $0,235 \pm 0,008$ and $0,234 \pm 0,072$ for 10 and 100 nM NPB treated) adipocytes evaluated at 1th day ($n=6$, $p<0,05$). Moreover, NPB increases glycerol release in brown adipocytes ($0,2309 \pm 0,005$ and $0,2249 \pm 0,006$ nmol/l for 10 and 100 nM NPB vs. control $0,2016 \pm 0,006$ at 1th; $0,2275 \pm 0,005$ nmol/l vs. $0,2673 \pm 0,008$ NPB 100 nM treated at 3th day of differentiation; $n=7$, $p<0,05$). NPB at 100 nM increased *Ucp-1* and *Prdm16* mRNA expression in brown adipocytes at 1, 3 and 6th day of differentiation ($n=5$, $p<0,01$). Expression of *Ppar γ* and *C/ebp β* were increased by 100 nM of NPB in white adipocytes differentiated for 1 day ($n=5$, $p,0,05$).

In summary, we found that NPB stimulates proliferation and differentiation of rat white and/or brown preadipocytes into mature adipocytes. These results suggest that NPB may contribute to energy homeostasis regulation by controlling brown and white adipogenesis.

The study was supported by the National Science Centre, Poland 2016/21/B/NZ9/00943 OPUS grant to Tatiana Wojciechowicz

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC200

Adropin modulates proliferation and differentiation of 3T3-L1 cells and rat primary preadipocytes

M. Jasaszwili, T. Wojciechowicz, M. Billert, K. Nowak and M. Skrzypski

Department of Animal Physiology and Biochemistry, Poznan University of Life Sciences, Poznan, Poland

Adropin is a secreted protein encoded by Energy Homeostasis Associated (Enho) gene. Enho mRNA is abundantly expressed in the liver and brain (1). There is evidence indicating that biological effects of adropin are mediated through GPR19 receptor (2). It was shown that in mice adropin deficiency leads to increased adiposity, impaired glucose production and dyslipidaemia (3). Adropin overexpression or adropin administration attenuates insulin resistance, reduces lipogenic genes expression and protects from body weight gain (1). These data suggested

that adropin is involved in lipid metabolism and body weight regulation. However, the role of adropin in modulation of adipose tissue biology including adipogenesis remains unknown. In the present work we studied the effects of adropin on proliferation and differentiation of white preadipocytes into adipocytes. The influence of adropin on proliferation and differentiation of preadipocytes was studied using primary preadipocytes isolated from Wistar male rats (weighing 80-100 g) and murine 3T3-L1 cells. Cell proliferation was assessed by BrdU assay. Gene mRNA expression and protein phosphorylation were determined by Real Time PCR and Western blot. Triacylglycerol level was evaluated by Oil Red O staining. Statistical analysis was performed using ANOVA followed by the Bonferroni post hoc test. Data are shown as mean $\pm$ SEM. Enho and Gpr19 mRNA expression was detected in the rat primary preadipocytes and 3T3-L1 cells. Adropin (100 nmol/l) increased proliferation of rat preadipocytes (0.45 ± 0.02 vs. 0.54 ± 0.01 OD 450–690 nm) and 3T3-L1 cells (0.47 ± 0.02 vs. 0.58 ± 0.02 OD 450–690 nm, $p < 0.05$). Furthermore, adropin (100 nmol/l) stimulated AKT (1.0 ± 0.42 vs. 3.68 ± 0.71 arb. u., $p < 0.05$) and ERK1/2 (1.0 ± 0.02 vs. 1.82 ± 0.20 arb. u., $p < 0.05$) phosphorylation in 3T3-L1 cells. Adropin failed to induce cell proliferation in the presence of AKT and ERK1/2 inhibitors. In addition, adropin (100 nmol/l) decreased mRNA expression of adipogenic genes in rat preadipocytes (Ppar γ 4.96 ± 0.17 vs. 3.67 ± 0.29 arb. u., Fabp4 6.43 ± 0.28 vs. 4.46 ± 0.38 arb. u. and Cebpa 1.84 ± 0.23 vs. 1.23 ± 0.59 arb. u., $p < 0.05$). Similar effect was also observed in 3T3-L1 adipocytes (Ppar γ 19.69 ± 2.33 vs. 13.26 ± 1.31 arb. u., Fabp4 1499 ± 167.2 vs. 803 ± 158.7 arb. u., $p < 0.05$). Furthermore, lower level of intracellular triacylglycerol was found in rat preadipocytes (0.43 ± 0.01 vs. 0.35 ± 0.008 OD 520 nm, $p < 0.05$) and 3T3-L1 cells (0.22 ± 0.003 vs. 0.18 ± 0.002 OD 520 nm, $p < 0.05$) differentiated for 7 days in the presence of adropin (100 nmol/l). These results showed that adropin stimulates preadipocytes proliferation via ERK1/2- and AKT-dependent mechanisms. Furthermore, we provide evidence that adropin suppresses differentiation of preadipocytes into mature adipocytes. These data suggest that adropin may contribute to controlling energy homeostasis by modulation of white adipogenesis.

Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell Metab.* 2008;8(6):468-81.

Stein LM, Yosten GL, Samson WK. Adropin acts in brain to inhibit water drinking: potential interaction with the orphan G protein-coupled receptor, GPR19. *American journal of physiology Regulatory, integrative and comparative physiology.* 2016;310(6):R476-80.

Ganesh Kumar K, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. *Obesity (Silver Spring).* 2012;20(7):1394-402.

This research was supported by grant from the National Science Centre of Poland (2016/23/D/NZ4/03557).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Human placental DNA methylation is associated with infant size at birth and at three months, but is not altered by maternal metformin therapy.

L. Yang¹, J. Manning², M. Aldhous³, F. Denison³, J. Norman³, A. Drake¹ and R. Reynolds¹

¹University/British Heart Foundation Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK, ²Edinburgh Genomics, University of Edinburgh, Edinburgh, UK and ³MRC Centre for Reproductive Health, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

Background: Metformin is widely used for treatment of gestational diabetes mellitus, despite crossing the placenta. The limited available data reporting long-term effects for offspring of exposure to metformin in utero show risk of increased adiposity in childhood but mechanisms are unknown. As epigenetic modifications have been proposed as a candidate pathway linking early-life development to later-life obesity, we hypothesised that alterations in placental DNA methylation would be associated with increased adiposity in infants exposed to metformin in utero. **Methods:** Placental samples were collected at delivery from obese pregnant women (n=104) who participated in the EMPOWaR trial and were randomised to metformin (up to 2.5g daily) vs. placebo from 16 weeks' gestation until delivery. DNA methylation arrays (Illumina® Infinium HumanMethylation450 BeadChips, USA) were performed on bisulphite-converted DNA (EZ DNA Methylation kit, Zymo Research, UK) extracted from the placenta samples (n=89, n=43 (48.3%) Male, n=45 (50.6%) metformin treated). We tested the association of DNA methylation and metformin treatment with infant size at three months using R programming (CpGassoc package) and adjusted for baby sex and treatment with Holm-Bonferroni adjustment for significance. Two candidate genes (ACADS, acyl-CoA dehydrogenase short chain, a key enzyme in mitochondrial fatty acid beta-oxidation and CYP11A1, Cytochrome P450 Family 11 Subfamily A Member 1, involved in drug metabolism and synthesis of cholesterol, steroids and lipids) were chosen from the significant (Holm.sig= TRUE) top picked CpG sites from Holm-Bonferroni analysis for validation. PCR primers were designed using PyroMark Assay Design Software 2.0 for pyrosequencing. Associations of DNA methylation (%) data were tested with infant size at birth and at three months with adjustment for infant sex.

Results:

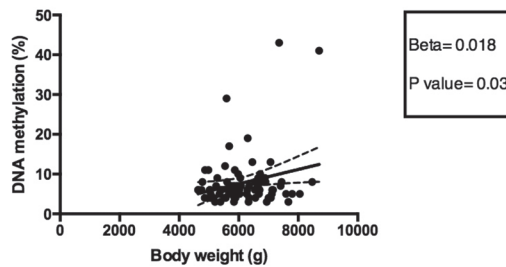
Analysis of array data showed increased placental DNA methylation of ACADS was associated with higher infant weight (cg27246729, $p=3.81E^{-08}$) and ponderal index (PI) (cg27246729, $p=1.42E^{-11}$) at three months of age. DNA methylation at ACADS was further analysed by pyrosequencing: increased DNA methylation at a neighbouring CpG site was positively associated with infant weight (cg10600917, $\text{Beta}=0.018$; $p=0.03$) at three months and methylation at cg27246729 associated

with increased PI at birth (Beta=0.31, $p=0.004$). Pyrosequencing of CYP11A1 failed to replicate the array findings. Metformin treatment was not associated with placental DNA methylation or infant adiposity.

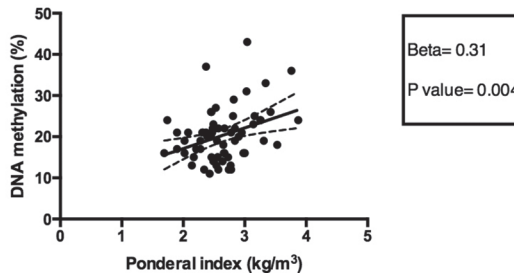
Conclusion

Our findings suggest placental DNA methylation of ACADS, a gene involved in key metabolic pathways, is linked to infant size at birth and at three months. Further investigation is needed to understand the relevance of these findings. Metformin exposure was not associated with placental DNA methylation or infant adiposity in this small sample, adding to the literature indicating it is safe to use in pregnancy.

Placental DNA methylation of ACADS vs body weight at 3 months



Placental DNA methylation of ACADS vs ponderal index at birth



Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC202

Human placental metabolism of vitamin D and transfer to maternal and fetal circulationsB. Ashley¹, F. Hey², E. Lofthouse¹, R. Lewis¹, K. Jones², N. Harvey¹ and J. Cleal¹¹*Institute of Developmental Sciences, The University of Southampton, Southampton, UK and* ²*MRC Epidemiology Unit, University of Cambridge, Cambridge, UK*

Insufficient 25-hydroxyvitamin D [25(OH)D] levels are common amongst pregnant women. Poor maternal 25(OH)D levels are associated with reduced fetal growth, reduced offspring bone density and bone mineral content, and childhood obesity. This suggests that poor maternal vitamin D levels may set the offspring on a pathway of poor health. 25(OH)D is the primary circulating form of vitamin D, however it is converted to a more active form, 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] within the body. This study investigates whether the human placenta is a site of 25(OH)D metabolism, and what vitamin D metabolites are transferred from the placenta to the maternal and fetal circulations.

Term human placentas (n=5) were collected within 30 min of delivery with ethical approval and informed consent. The metabolism of ^{13}C -25(OH)D₃ within placental tissue and transfer across the placenta was investigated using the whole placental cotelydon perfusion open loop methodology as adapted in our laboratory. Maternal and fetal circulation was re-established, and the maternal circulation was perfused with 30 nmol/l ^{13}C -25(OH)D₃ for a period of 5h. Perfusate was collected periodically from both the maternal and fetal vein. The presence of vitamin D metabolites in perfusate samples was identified and quantified using liquid chromatography mass spectroscopy.

Following 5h of perfusion there is transfer of 24.03% maternal ^{13}C -25(OH)D from the maternal circulation into the placenta and 36.72% of this (8.82% of maternal stock) is transferred into the fetal circulation. There is evidence of placental metabolism of ^{13}C -25(OH)D, with transfer of ^{13}C -24,25-dihydroxyvitamin D [$24,25(\text{OH})_2\text{D}$] but not ^{13}C -epi25(OH)D into the maternal and fetal circulations. qPCR measurement of the vitamin D responsive gene *CYP24A1* indicates increased expression in the placental tissue following 5h perfusion of ^{13}C -25(OH)D₃. This indicates placental metabolism of ^{13}C -25(OH)D₃ into the transcriptionally active $1,25(\text{OH})_2\text{D}$.

This study provides evidence that once within the placenta the 25(OH)D can be metabolised and the downstream products could have effects within the placenta itself or be transferred to both the fetal and maternal circulation. Insufficient placental transport and metabolism of maternal 25(OH)D may therefore limit the fetal supply of 25(OH)D and $1,25(\text{OH})_2\text{D}$, impacting fetal development. Placental transfer of 25(OH)D metabolites to the maternal circulation may contribute to the altered vitamin D status that occurs to help adapt the maternal physiology to support pregnancy.

The Rank Prize Fund

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC203

The physiological conundrum of inorganic phosphate stimulation of urinary ammonium excretion in the acidotic rabbit

P.A. Walsh¹ and D.J. O'Donovan²

¹Physiology, School of Medicine, RCSI Bahrain, Manama, Bahrain and ²Physiology, National University of Ireland Galway, Galway, Ireland

An increase in urinary ammonium excretion is an established renal compensatory mechanism to the induction of acidosis in man and a number of experimental animals, including rodents and the dog. In contrast, the poor renal ammoniagenic capacity and the ensuing low urinary buffer capacity is known to contribute to the particular susceptibility of herbivores to a relatively low systemic acid dose. Some 40 years ago, however, it was shown, in experiments involving isotonic sodium phosphate loading of acidotic rabbits, that renal ammoniagenesis could be enhanced resulting in a stimulation of urinary ammonium excretion in this species, thus increasing their capability to unload acid at the renal level, and consequentially improve their acid tolerance. With the view of securing a plausible physiological explanation for this rabbit-unique phenomenon, sodium phosphate loading studies were repeated on anaesthetized, acidotic rabbits (1). It was observed that while the animals were being rendered hyperphosphataemic (7.9 ± 0.1 mmol.Litre⁻¹ (N=5) vs 2.1 ± 0.1 mmol.Litre⁻¹ (N=10) pre-infusion, ($p < 0.001$)) and hyperphosphaturic (72 ± 3.8 mmol.Litre⁻¹ vs 5 ± 1 mmol.Litre⁻¹ (N=9), pre-infusion, $p < 0.001$) over an 80 minute isotonic sodium phosphate infusion period, two unrelated sets of urinary ammonium excretion data were obtained. When assayed via an enzymatic validated Berthelot reaction, urinary ammonium excretion was found to increase 1.6 fold (0.08 ± 0.02 μ mol.min⁻¹ vs 0.05 ± 0.01 μ mol.min⁻¹ (N=9) pre-infusion, $p < 0.05$). In contrast, a formol-titrimetric method of urinary ammonium analysis reported a higher baseline of urinary ammonium output, in addition to a 2.6 fold increase in ammonium excretion (2.33 ± 0.19 μ mol.min⁻¹ vs 0.9 ± 0.09 μ mol.min⁻¹ (N=11) pre-infusion, $p \leq 0.001$). Values are means $\pm$ S.D., significant difference being determined by student t-test. An investigation of the discrepancy between the two assays revealed a particular susceptibility of the formol titration procedure of urinary ammonium analysis to phosphate interference (2), thus yielding exaggerated estimations of urinary ammonium, and casting doubt on the relative significance of phosphate as an in vivo stimulant of ammonium output in the acidotic, hyperphosphataemic rabbit. Rather it would appear from additional measurements done in this laboratory (3) that phosphate infusion increases acid unloading in the acidotic rabbit primarily through the mechanism of increased

titratable acid excretion. The physiological relevance of the minuscule though significant increase in urinary ammonium excretion, that was observed following phosphate infusion in the acidotic rabbit, must be of minimal significance, considering that pharmacological quantities of phosphate were used. Furthermore, the use of phosphate infusion as a protocol to treat acidosis in humans is limited, because of the toxic consequences of elevated plasma phosphate levels following sodium phosphate infusion.

Yu HL, Giammarco R, Goldstein MB, Stinebaugh DJ, Halperin ML. Stimulation of ammonia production and excretion in the rabbit by inorganic phosphate. Study of control mechanisms. *J Clin Invest.* 1976;58(3):557-64.

Walsh P, O'Donovan DJ. Potential errors in the determination of urinary ammonium by formol titration. *Clinical chemistry and laboratory medicine* [Internet]. 2016 Oct 1; 54(10):[e293-5 pp.].

Walsh Patrick A, O'Donovan DJ. An appraisal of the in vivo role of phosphate as a modulator of urinary ammonium and titratable acid excretion in the acidotic rabbit. *Journal of Animal Physiology & Animal Nutrition.* 2019. (In Press).

The authors gratefully acknowledge the financial support received for this research from the Medical Research Council of Ireland, the Department of Education, and the National University of Ireland Galway (Postgraduate Fellowship). We would like to thank Dr. Bindhu Nair for helping with the compiling of the references.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC204

Endogenous stem cell mobilization versus exogenous stem cell therapy in rat model of chronic kidney disease

Y.M. ELWAZIR, S. Morsy, M. Farouk and M. Abdo

Medical Physiology, Suez Canal University, Ismailia, Egypt

Endogenous stem cell mobilization versus exogenous stem cell therapy in rat model of chronic kidney disease

Chronic kidney disease (CKD) affects 14% of the world population and is still a major health problem despite advances in renal transplantation. Adipose-derived mesenchymal stem cells (AD-MSCs) can restore damaged renal cells, while granulocyte colony-stimulating factor (G-CSF) is known to mobilize hematopoietic stem cells from bone marrow to peripheral circulation. The aim of this study is to compare the effect of endogenous CD34⁺ cell mobilization with exogenous AD-MSCs administration in the treatment of a rat model of CKD, induced by a single IV injection of ADR at a dose of 5 mg/kg. Forty eight male albino Sprague Dawley rats were divided into 4 equal groups. GI: control negative, GII: control positive, GIII: rats received 2×10^6 iron-labeled AD-MSCs IV on day 5 after ADR injection, and GIV received SC injection of G-CSF for 5 consecutive days at a dose

of 70 µg/kg. In each group, 6 rats were sacrificed after 28 days and the other 6 after 84 days. Renal functions were assessed by measuring serum creatinine, albumin, urea and 24-hour urinary protein level at days 0, 5, 28 and 84. HB level was measured at days 0 & 84. Oxidative stress markers malondialdehyde (MDA) and total anti-oxidant (TAO) were measured, in kidney tissue, at day 28. CD 34⁺ cells % was measured in peripheral blood at day 9 using flowcytometry and showed a marked increase in GIV compared to other groups. After scarification, kidneys were removed for histopathological assessment. Data are expressed as means + SD and compared by ANOVA. Results revealed that both ADSCs and G-CSF significantly and similarly improved the following parameters of renal function at day 84 ($p < 0.05$): serum creatinine was 0.48 ± 0.08 & 0.48 ± 0.08 mg/dl in GIII & GIV respectively compared to 0.74 ± 0.09 mg/dl in GII, albumin was 3.84 ± 0.25 & 3.98 ± 0.29 gm/dl in GIII & GIV respectively compared to 2.2 ± 0.2 gm/dl in GII, urea was 38.4 ± 4.10 , 41.0 ± 6.04 mg/dl in GIII & GIV respectively compared to 80.40 ± 6.11 mg/dl in GII, and 24-hour urinary protein level was 982 ± 77.85 & 815.80 ± 81.11 mg in GIII & GIV respectively compared to 2841.20 ± 461.85 mg in GII. HB was 14.10 ± 0.55 , 14.90 ± 0.74 gm/dl in GIII, GIV respectively compared to 11.50 ± 0.5 gm/dl in GII. Comparing GIII & GIV, using post-hoc tukey test, G-CSF treated group had less 24-hour urinary protein level and higher serum albumin ($p < 0.05$). Homing of iron labeled AD-MSCs was microscopically detected in kidney sections. MDA level were less in groups GIII 1.56 ± 0.21 & GIV 1.48 ± 0.19 compared to GII 2.28 ± 0.08 U/mg kidney tissue ($P < 0.05$). TAO level was significantly higher in GIII & GIV (48.40 ± 8.62 & 40.63 ± 11.09 U/mg kidney tissue respectively) compared to GII (18.20 ± 1.68 U/mg kidney tissue) ($P < 0.05$). We concluded that both stimulating endogenous hematopoietic stem cells by G-CSF is at least as effective therapeutic line as injecting exogenous ADSCs in CKD.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC205

The protective role of Sestrin2 in high fat diet-induced nephropathy

D.Y. Lee², Y. Gorin², A. Eid³, K. Sharma² and A. Agouni¹

¹College of Pharmacy, Qatar University, Doha, Qatar, ²Center for Renal Precision Medicine, Division of Nephrology, Department of Medicine, School of Medicine, University of Texas Health Sciences Center at San Antonio, San Antonio, TX, USA and

³Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Obesity is associated with a high risk of type-2 diabetes predisposing patients to diabetic nephropathy (DN), the leading cause of end-stage renal failure worldwide. Glomerular injury is a prominent pathological feature of DN. Sestrin 2 (Sesn2) is a stress-induced protein, but its role in DN has not been investigated. Therefore, we have determined the impact of Sesn2 deletion in a mouse model of obesity-induced nephropathy.

We examined the effects of *Sesn2*-deficiency in a long-term (22 weeks) mouse model of high fat diet (HFD)-induced obesity. The severity of renal pathology in wild type (*Sesn2*^{+/+}) mice (fed HFD or chow diets) was compared to that in *Sesn2*-deficient mice (*Sesn2*^{-/-}) fed HFD or chow diets (n = 3 to 4 mice per group). All animal work was carried out under approved protocol by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas health science center (San Antonio) and followed the principles of laboratory animal care (NIH).

Data showed that *Sesn2* ablation exacerbated HFD-induced glomerular fibrotic injury as evidenced by mesangial matrix hypertrophy (*Sesn2*^{+/+}: chow 182.4±29 vs 321.7±37.5 HFD, p<0.001; *Sesn2*^{-/-}: chow 270±24.8 vs 343.2±65 HFD) and accumulation of both fibronectin (*Sesn2*^{+/+}: chow 7.37±0.89 vs 11.83±0.89 HFD, p<0.05; *Sesn2*^{-/-}: chow 12.44±2.72 vs 13.18±0.81 HFD), and collagen IV (*Sesn2*^{+/+}: chow 2.98±0.63 vs 7.35±0.74 HFD, p<0.01; *Sesn2*^{-/-}: chow 6.26±1.47 vs 6.51±0.76 HFD). Western blot analysis revealed that *Sesn2*^{-/-} mice fed HFD or chow diets exhibited an upregulation of protein expression of key lipogenic enzymes, fatty acid translocase CD36 (an indicator of lipid uptake) (*Sesn2*^{+/+}: chow 0.1±0.01 vs 0.4±0.05 HFD, p<0.01; *Sesn2*^{-/-}: chow 0.45±0.08 vs 0.92±0.09 HFD, p<0.01), fatty acid synthase (*Sesn2*^{+/+}: chow 0.15±0.02 vs 0.55±0.02 HFD, p<0.01; *Sesn2*^{-/-}: chow 0.56±0.07 vs 1.1±0.05 HFD, p<0.01) and ATP citrate lyase (ACLY) (*Sesn2*^{+/+}: chow 0.11±0.01 vs 0.36±0.1 HFD, p<0.01; *Sesn2*^{-/-}: chow 0.53±0.06 vs 1.32±0.14 HFD, p<0.01). *Sesn2*-deficiency in obese mice resulted in podocyte loss as indicated by reduced expression of synaptopodin (*Sesn2*^{+/+}: chow 2.98±0.63 vs 7.35±0.74 HFD, p<0.01; *Sesn2*^{-/-}: chow 6.26±1.47 vs 6.51±0.76 HFD). Interestingly, glomerular lesions similar to those observed in HFD-fed wild-type mice were detected in *Sesn2*^{-/-} mice fed a chow diet, indicating that the basal deletion of *Sesn2* is deleterious by itself.

We provide the first evidence that *Sesn2* is renoprotective in obesity-induced nephropathy by diminishing lipid accumulation and blocking excessive lipid uptake and *de novo* lipid synthesis. Understanding the protective of *Sesn2* should yield novel therapeutic interventions to effectively preserve glomerular function in obesity and diabetes.

This work was supported by a NPRP award (NPRP8-1750-3-360) from Qatar National Research Fund (a member of Qatar Foundation). The findings achieved herein are solely the responsibility of the authors.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC206

Planarian Flatworms: an emerging animal model in metabolic physiology

M. Lewallen and W.W. Burggren

Biology, University of North Texas, Flower Mound, TX, USA

Measuring metabolic rate quantifies energetic investment and impact of environment, reproduction, genetics, and diet, providing a greater understanding of whole animal physiology. Very simple animal models can offer insight into factors affecting metabolic rate more easily than complex models. Planaria, free-living aquatic flatworms, are studied for their remarkable ability to regenerate completely when cut into fragments, due to abundant pluripotent stem cells, longevity due to cellular renewal, and ability to remodel tissues with food availability. They may reproduce asexually, sexually, or both, allowing metabolic study of reproductive mode. Information in modern literature on planarian metabolic rates is lacking, with a need to establish consistent measurement techniques using current methodologies in commonly studied species. High throughput optode closed respirometry was used to measure oxygen consumption in asexual and sexual *S. mediterranea* and *G. dorotocephala*, as a function of temperature, taxon, stressors, reproductive mode, age, regeneration, and specific dynamic action. Values were compared by ANOVA and are presented as means $\pm$ SE. Oxygen consumption in *S. mediterranea* was 1.5-2.1X greater in the sexual strain over the asexual strain at 18°, 23°, and 28°C (26.6 ± 1.7 , 21.8 ± 2.5 , and 29.4 ± 2.5 O₂/g/h vs. 12.7 ± 1.8 , 11.9 ± 2.5 , and 18.9 ± 2.5 μ L O₂/g/h, $p < 0.05$ for all). Within the sexual strain, oxygen consumption at 18°C (control) in sexually mature adults was 1.8X greater over hatchlings (22.6 ± 4.0 vs. 12.5 ± 1.4 O₂/g/h, $p = 0.025$) and 2.2X greater over sexually immature juveniles (22.6 ± 4.0 vs. 10.3 ± 2.1 O₂/g/h, $p = 0.006$). Differences in sexual morphology between these groups were visualized with DAPI staining. 100% of the higher oxygen consumers (sexually mature *S. mediterranea*) exhibited sexual structures, such as testes, seminal vesicles, and copulatory apparatus (11/11). In contrast, lower oxygen consumers (asexuals, hatchlings, and juveniles) were devoid of these sexual structures. These results support a theory of higher metabolic costs associated with sexual maturity in *S. mediterranea*. Therefore, this study has established sexual and asexual *S. mediterranea* as simple, yet attractive models for investigating energetic costs of reproductive mode. This research also provided quantitative values for specific dynamic action in planaria, with a maximum increase in oxygen consumption of 160% induced by feeding (13.7 ± 4.8 O₂/g/h at 24 h prior to feeding vs. 35.7 ± 8.8 O₂/g/h at 24 h post-feeding, $p < 0.05$), as well as metabolic relationships in planaria involving temperature (range 13-28°C), age, and regeneration state. This research has contributed to the overall knowledge of the basic physiology in this animal, providing the framework for future metabolic studies in planaria involving environmental factors, reproduction, regeneration, development, and aging.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC207

Neurotransmission in mouse intrinsic cardiac ganglia *in situ*

A.A. Harper^{1,2} and D.J. Adams²

¹Life Sciences, University of Dundee, Dundee, UK and ²Illawarra Health & Medical Research Institute (IHMRI), University of Wollongong, Wollongong, NSW, Australia

There is accruing interest in the cardiac nervous system, particularly its role in the generation of cardiac arrhythmias and sudden cardiac death (Fukuda et al., 2015). Intracardiac ganglia (ICG) form the final common pathway for the cardiac nervous system. Further understanding of how ICG neurones integrate information is likely to contribute to improved or novel therapeutic strategies for the prevention of arrhythmias.

Although there are several reports characterising murine ICG neurones (e.g. Brown et al., 2018; Hoard et al., 2007), to our knowledge there are no studies on ganglionic transmission. Using intracellular microelectrode recording and nerve stimulation, we describe the intrinsic electrical characteristics and synaptic transmission in intracardiac ganglia of the adult mouse heart *in situ*. Young (6 week) male adult c57BL/6NJAusb mice (Australian BioResources, NSW) were terminally anaesthetized with Fluothane as approved by the University of Wollongong Animal Ethics Committee (AE16/10). The heart and lungs quickly excised and the right atrial ganglion plexus and underlying myocardium isolated from the dorsal surface of the atria. Experiments were performed at 36°C using a bicarbonate buffered physiological salt solution, and atrial contractions were suppressed using blebbistatin (5 µM, Cayman Chemical, Ann Arbor, MI USA).

The mean cell capacitance of mouse ICG neurones was 26.7 ± 8.9 pF and the values for the resting membrane potential and input resistance in mouse ICG neurones were -49.2 ± 4.8 mV and 201 ± 80 MΩ (SD, n=20) respectively. Somatic action potentials (AP) were evoked by short current pulses (2 ms). The AP after-hyperpolarization amplitude and time to 50% recovery being 9.9 ± 2.6 mV and 37.8 ± 25.3 ms (n=15), respectively.

Synaptic responses to single stimuli of the vagus or interganglionic nerves were divided into three groups with the following relative frequency: *strong* 15/20, *secure* 4/20 and *weak* 1/20 (see Figure 1). The efficacy of synaptic transmission as a function of stimulation frequency (5, 10, 20, 50 and 100 Hz) was studied in neurones with strong synaptic responses. Trains of stimuli (20), were applied and AP discharge monitored in the postganglionic neuronal soma. Synaptic efficacy was determined as the percentage of postsynaptic APs as a function of nerve stimulation. The number of successful APs, 100% at 5-50 Hz decreased to $92 \pm 13\%$ SD (n=10) at 100 Hz stimulation. This framework will now be used to organize the characterization of the ICG in specific genetically modified animals.

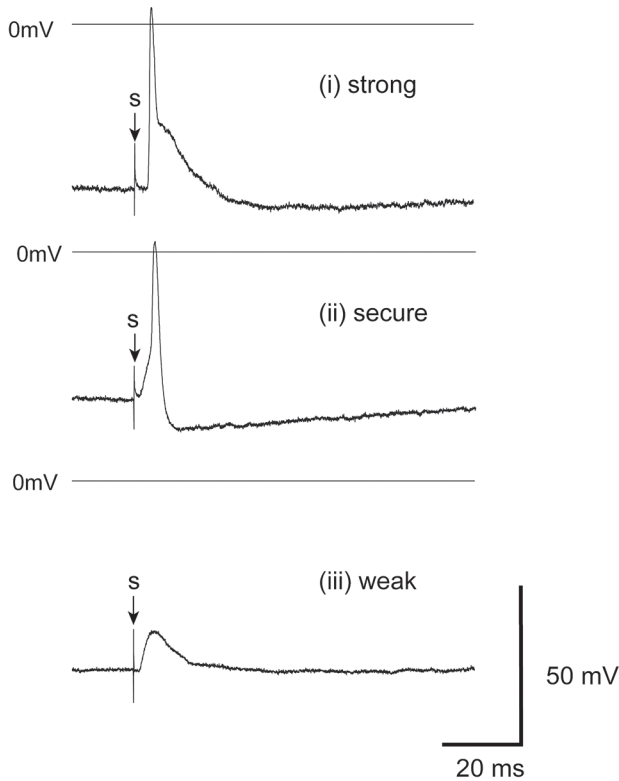


Figure 1. Representative synaptically-evoked postsynaptic responses recorded from mouse ICG neurones in response to vagal nerve stimulation. Postsynaptic responses were classified as:(i) strong where the AP arises early during the EPSP, (ii) secure where the AP arises late in the EPSP, when present, or without an obvious EPSP, and (iii) weak where nerve stimulation (s) evokes a subthreshold EPSP.

Brown TC, Bond CE, Hoover DB. (2018) *Auton Neurosci*. 210:44-54.

Fukuda K, Kanazawa H, Aizawa Y, Ardell JL, Shivkumar K. (2015) *Circ Res*. 116: 2005-2019.

Hoard JL, Hoover DB, Wondergem R. (2007) *Am J Physiol Cell Physiol*. 293(6):C1875-83

This work was supported by grants from the National Health & Medical Research Council of Australia (DJA). AAH thanks The Physiological Society for a Travel Grant. We thank AR Bony and M Sadeghi for technical assistance.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Hypothalamic and mesencephalic regions involved in the control of laryngeal activity and subglottic pressure in spontaneously breathing anaesthetized rats

M. Gonzalez-Garcia^{1,3}, M. Lopez-Gonzalez^{1,3}, L. Carrillo-Franco², C. Carrillo-Franco² and M. Dawid-Milner^{1,3}

¹*Departamento de Fisiología Humana, Universidad de Malaga, Malaga, Spain,*

²*Facultad de Psicología y Logopedia, Universidad de Malaga, Malaga, Spain and*

³*Unidad de Neurofisiología del Sistema Nervioso Autónomo (CIMES), Universidad de Malaga, Malaga, Spain*

Background

Abduction and adduction of the larynx allow changes in airflow necessary for the vibration of the vocal folds and emission of voice. It is known that stimulation of the Periaqueductal Gray matter (PAG) and nucleus retroambiguus (nRA) produces vocalization (1) and lesions in PAG cause mutism in animals and humans (2). The nRA is the perfect target to turn passive into active expiration modifying the activity of laryngeal motoneurons located in the nucleus ambiguus (3). We have shown that rostral and ventral pontine structures are involved in changes of laryngeal caliber (4). It has been also demonstrated a high expression of FOXP2 protein at mesencephalic and pontine regions (PAG, Parabrachial complex and A5 Region) involved in cardiorespiratory control. FOXP2 is a transcription factor required for brain and lung development and it is closely related to vocalization (5).

Objectives

The aim of this study was to characterize the relations between hypothalamic and mesencephalic regions involved in cardiorespiratory control and their possible role in modulating laryngeal activity and their effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=7), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2 mg/ kg, i.v., as necessary). A double tracheal cannulation to develop the classical technique of the "glottis isolated in situ" and for the recording of respiratory airflow was carried out. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, $\pm 0,1$ psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70 ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Thus, at constant air flow, changes in pressure indicate changes in laryngeal resistance.

Bilateral parietostomy allowed access to the Dorsomedial Hypothalamic Nucleus and Perifornical area (DMH-PeF), dIPAG and Cuneiform Nucleus (CnF). Electrical stimulations of these regions using concentric bipolar electrodes (1 ms pulses, 20-40 μ A, 100 Hz for 5 s) was performed. Respiratory flow, pleural pressure, blood pressure, heart rate and ECG activity were also recorded.

Results

DMH-PeF, dIPAG, and CnF stimulations evoked a decrease of laryngeal resistance (subglottal pressure) ($p < 0.001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p < 0.001$), together with a pressor ($p < 0.001$) and tachycardic response ($p < 0.001$).

Conclusions

The results of our study contribute with new data on the role of the hypothalamic-mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Holstege G. Anatomical study of the final common pathway for vocalization in the cat. *J Comp Neurol.* 1989; 284, 242-252.

Esposito A, Demeurisse G, Alberti B, Fabbro F. Complete mutism after midbrain periaqueductal gray lesion, *Neuroreport.* 1999; 10: 681– 685.

Subramanian HH, Holstege G. The nucleus retroambiguus control of respiration, *J Neurosci.* 2009; 29:3824-3832.

Lara JP, Dawid-Milner MS, Lopez MV, Montes C, Spyer KM, Gonzalez-Baron S. Laryngeal effects of stimulation of rostral and ventral pons in the anaesthetized rat, *Brain Res.* 2002; 934(2): 97-106.

Stanic D, Dhingra R and Dutchmann M. Expression of the transcription factor FOXP2 in brain-stem respiratory circuits of adult rat is restricted to upper airway premotor areas, *Respir Physiol Neurobiol.* 2018; 250: 14-18.

The study was supported by a program grant Junta de Andalucía, Grupo Consolidado code CTS156, Spain. Part of the final study was supported with a grant from the University of Málaga.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC209

Characterizing diurnal rhythms of gene expression in sympathetic preganglionic neurones

C. Nathan¹, J. Deuchars¹, S. Deuchars¹ and J. Aspden²

¹Faculty of Biological Sciences, School of Biomedical Sciences, University of Leeds, Leeds, UK and ²School of Molecular and Cellular Biology, University of Leeds, Leeds, UK

Rhythmic gene expression has been correlated to circadian rhythms observed in physiological processes such as liver metabolism, stomach gastric activity and cardiovascular activity. Blood pressure exhibits circadian rhythmicity, dipping during the evening and increasing during the morning, and is controlled by sympathetic nervous system activity. As sympathetic preganglionic neurones (SPNs) are the final common pathway for central nervous system influence on blood pressure, this project aims to determine if SPN function could be regulated by diurnal expression of genes.

The diurnal expression of genes encoding proteins involved in determining neuronal activity was investigated in the spinal cord and from micro-punches that included the IML, obtained at 7:30 AM and 7:30 PM. C57/Bl6 mice (N= 10) were terminally anaesthetised, spinal cords removed and RNA extracted. qPCR revealed mRNA levels of Bmal1 and Per2 varied with time of day in the IML; Bmal1 mRNA (0.16 ± 0.03 relative fluorescent units, rfu) was higher at 7:30 PM while Per2 mRNA (0.10 ± 0.02 rfu) was higher in IML at 7:30 AM. To investigate a broader sample of genes, RNAseq was performed on micropunches obtained as above. Diurnal rhythm of expression was observed in potassium channel subunits (e.g. Potassium voltage-gated channel, Shaw-related subfamily, member 3) and glutamate subunits (e.g. Glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1) consistent with increased neuronal activity. Diurnal rhythm of Bmal1 and Per2 protein levels in SPNs were then examined using immunofluorescence. C57/Bl6 mice (N= 5 animals n=15 sections) were terminally anaesthetised at 7:30 AM and 7:30 PM and had their spinal cords removed and sectioned. Bmal1 and Per2 protein levels within the SPN nucleus vary with time of day with Bmal1 levels being higher in the 7:30 AM (103.5 ± 4.3 relative units). Single-cell RNA-seq was conducted on patch electrode-aspirated cells from the IML region. C57/Bl6 mice (N= 12) were terminally anaesthetised and had their spinal cords removed and sectioned, cells (N=26) were aspirated from the sections. RNA was extracted from the cells using a kit. SPNs were identified based on expression of choline acetyltransferase gene. Analyses of RNA-seq data is currently ongoing. This study is the first to attempt to characterize the SPN expression profile and diurnal rhythmicity in these cell types.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC210

Glutamate receptor and transducer channel modulation of baroreceptor firing in a rat isolated aorta/aortic depressor nerve preparation.

C. Giuraniuc¹, R.W. Banks², J.F. Paton³ and G.S. Bewick¹

¹*Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK,* ²*Department of Biosciences, University of Durham, Durham, UK and* ³*Department of Physiology, University of Auckland, Auckland, New Zealand*

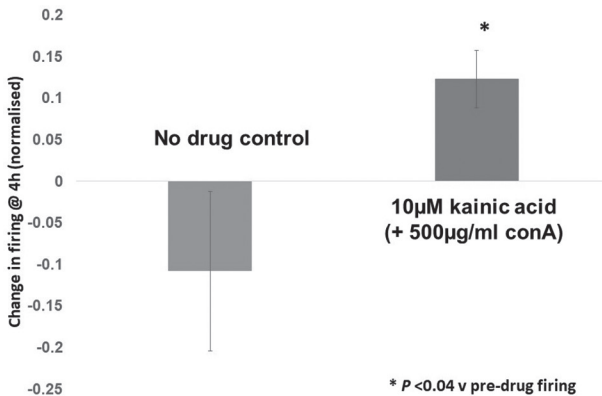
We are targeting baroreceptors as a promising new drug target for treating high blood pressure (hypertension). Hypertension is the world's most common killer disease (WHO), due to its contribution to cardiovascular disease, stroke and kidney failure, which account for >7m deaths p.a.. Current treatments are inadequate: most patients need multiple drugs either to treat the initial condition or the resulting side-effects. Even then >10% of patients are unresponsive and there have been no better drugs against existing targets for over 20 years. Thus, new

drugs and therapeutic targets are needed. Baroreceptors are such a novel and highly effective target. Electrical stimulation of the aortic depressor nerve (ADN) in drug-resistant patients reduces blood pressure by >20 mmHg, and can even replace medication. However, the surgery is invasive, expensive, challenging and only 50% of patients are anatomically suitable. We propose a more inclusive pharmaceutical approach, by targeting a system we discovered that modulates mechanosensory terminal sensitivity via an atypical glutamate receptor linked to PLD activation^{1,2}, which we recently reported as GluK2^{3,4}. We are pharmacologically characterising the efficacy of targeting this receptor in isolated aorta-ADN preparations as a translational step towards clinical application.

ADN-aorta preparations from humanely killed (ASPA, 1986) adult Sprague-Dawley rats (200-400 g) were maintained in a bath perfused with oxygenated artificial CSF. The aorta was inflated to just below firing threshold then pulsed above it at 10 min intervals or continuously at ~1 Hz. The effects of glutamate receptor and candidate transducer channel ligands on firing responses were analysed automatically using custom written Matlab codes. Data are presented as normalised mean $\pm$ SEM, with significance threshold for differences being $P = 0.05$, using a paired t -test.

Kainic acid (10 μ M) robustly increased firing in the presence of concanavalin A (500 μ g/ml) to block glutamate receptor desensitisation. The enhancement has slow onset (initiates 90 min after addition) and persists >1 h after wash-out. Normalised to the pre-drug control level, kainic acid increased firing $+14.5 \pm 3.5\%$ ($P < 0.04$, $n=4$) while controls showed a non-significant decrease ($-12.1 \pm 9.8\%$, $P > 0.31$, $n=6$), a difference of >25%. The TRP channel blocker 2-APB strongly inhibited pressure-evoked firing at 100 μ M ($-76.9 \pm 10.7\%$ of control, $P < 0.02$, $n=3$) while 10 μ M had no effect. Similarly, the DEG/ENaC channel blocker amiloride (1 mM) markedly decreased firing ($-84.1\% \pm 17.5\%$ of control at 3 h, $P < 0.05$, $n=3$).

These data show baroreceptor firing can be pharmacologically controlled and the GluR-mediated enhancement offers a novel approach to raising baroreceptor firing, and hence lowering blood pressure, which may have important clinical implications. The effects are slow onset (~90 min) but long lasting. Further experiments are planned to fully characterise this response.



10 μ M kainate (with concanavalin A to block receptor desensitisation) significantly increased baroreceptor firing relative to pre-drug levels ($P < 0.04$, $n=4$), while no drug control preparations underwent an overall, but non-significant, decrease ($P=0.31$, $n=6$).

Bewick GS *et al.* (2005) *J Physiol* 562, 381-394.

Bewick GS & Banks RW (2015) *Pflügers Arch.* 467, 175-190.

Thompson KJ *et al.* (2016) *Proc Physiol Soc* 37, SA010.

See Banks *et al.* (2019) Physiology 2019 - poster at this meeting.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC211

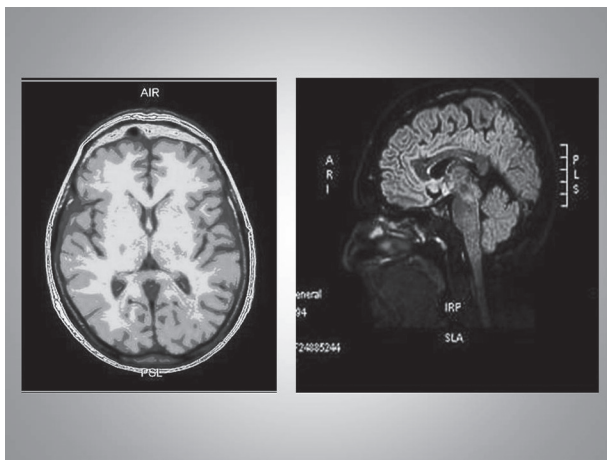
The first presentation of dementia with psychosis after a systemic inflammation: a neurophysiological explanation

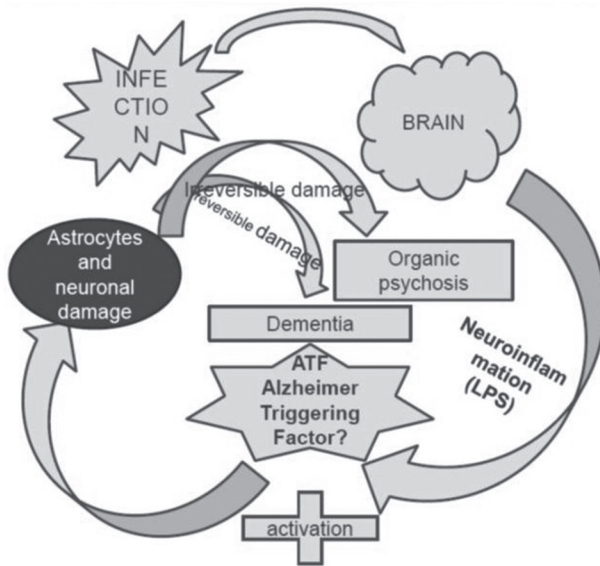
C. Lazzari, T. Rajanna, A. Nusair and T. Rance

Psychiatry, South-West Yorkshire NHS Trust, Wakefield, UK

Research suggests that neuroinflammation of the brain has a role in dementia by activating glial cells and then releasing proinflammatory agents and lipopolysaccharide (LPS) responsible for cognitive deficits [1-3]. Other authors suggest that there is no clear evidence if neuroinflammation after a generalised infection is a cause, agent, or consequence of Alzheimer's Disease (AD) with bacterial LPS acting on neurones and microglia [4,5]. The current case report refers to a 64-year old lady who developed symptoms of dementia and psychosis a month after she was operated for a duodenal perforation resulting in pneumoperitoneum and generalised bacterial sepsis. Past psychiatric history was negative, and former cognitive deficits were non-existent before the systemic inflammation. At the assessment, brain Magnetic Resonance Image presented mild to moderate supratentorial small

vessel disease and mild cortical and subcortical atrophy (Figure 1). Addenbrooke's ACE-III total score was 74/100 (more affected were memory, fluency and visuo-spatial) also after the underlying depression was stabilised. The patient became doubly incontinent and started misplacing objects in her kitchen, confusing the use of familiar objects, and having difficulties in word finding. The Bristol Activity of Daily Living was 10/60. The comprehensive neurocognitive assessment resulted in good episodic memory, poor attention, misplacing objects, social phobia, and subjective memory problems with the inability to complete routine tasks. The psychiatric presentation included believing that her food was poisoned, that one of her daughters was a fake, deeming that people on the road were making derogatory comments about her and passing her intrusive thoughts, and thought block. Passivity experiences consisted of thinking that passers-by were able to interfere with her mind or command her to harm herself. She also had auditory-commanding hallucinations to harm herself, believing deriving from people passing by her house. Other auditory hallucinations included hearing people walking on the stair of her home, hearing someone knocking on the door of her bedroom when no one was there, and tactile hallucinations as if someone was tapping on her shoulders when she was alone in her room. We posed the diagnosis of AD and psychosis. After several months, the presentation remains unchanged. In conclusion, the authors of the current research speculate that in the case described, systemic inflammation enduringly unlocked dementia, cognitive deficits, and psychosis [6]. The mediator of the unlocking process could be a hypothetical Alzheimer Triggering Factor linked to the microglia activated by LPS which causes a cascade of processes leading to irreversible dementia (Figure 2).





[1] Bevan-Jones WR, Surendranathan A, Passamonti L, Patricia Vázquez Rodríguez P, Arnold R, et al. Neuroimaging of inflammation in memory and related other disorders (NIMROD) study protocol: a deep phenotyping cohort study of the role of brain inflammation in dementia, depression and other neurological illnesses. *BMJ Open* 2017; 7: e013187.

[2] Reale M, Brenner T, Greig NH, Inestrosa N, and Paleacu D. Neuroinflammation, AD, and Dementia. *Int J Alzheimers Dis* 2010; 2010: 1-2.

[3] Cunningham C, Campion S, Lunnon K, Murray CL, Woods JFC, Deacon RMJ, Rawlins JNP, Perry VH. Systemic inflammation induces acute behavioural and cognitive changes and accelerates neurodegenerative disease. *Biol Psychiatry* 2009; 65: 304-312.

[4] Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's diseases. *Lancet Neurol* 2015; 14: 388-405.

[5] Enciu A-M, Popescu BO. Is there a causal link between inflammation and dementia? *Biomed Research International* 2013; Article ID 316495.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Schwann cell glycogen: a potential role in supporting A fibre conduction during high frequency stimulation in sciatic nerve

L.R. Rich and A.M. Brown

School of Life Sciences, University of Nottingham, Nottingham, UK

Astrocytes supply glycogen-derived lactate to central nervous system axons during simulated aglycaemia, a feature shared with Schwann cells that myelinate sciatic nerve A fibres, a peripheral nerve (Brown et al., 2003, 2005, 2012). However, only astrocytes, to date, are known to supply axons with glycogen-derived lactate during increased neuronal activity (Brown et al., 2003, 2005). We report on-going investigations into the putative supply of glycogen-derived lactate during high frequency stimulation (HFS) in peripheral nerve, providing further insight into a possible universal theory of axon-glia metabolic interactions in the central and peripheral nervous system.

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 under appropriate authority of establishment, project and personal licenses. Adult male CD-1 mice were killed by cervical dislocation and decapitated. Sciatic nerves were dissected, placed in a perfusion chamber, superfused with aerated aCSF containing 10mM glucose. The compound action potential (CAP) was evoked with supra-maximal stimuli at a baseline frequency of 1Hz, with its amplitude monitored as a measure of conduction. HFS was defined as 100Hz.

The maintenance of A fibre conduction during HFS was assessed in the presence of 10mM (n=9) or 30mM (n=9) glucose, revealing an energy demand versus supply relationship. Lactate uptake into axons is prevented by 200µM cinnamate; its addition to 30mM glucose during HFS had no effect (n=5), but when supplied with 10mM glucose conduction failed (n=4), suggesting a requirement for lactate under normoglycemic conditions. Since the source of this lactate is unclear, nerves were exposed to HFS for increasing durations in 10mM or 30mM glucose before removal of substrate supply. In substrate free conditions glycogen is the sole source of energy therefore glycogen content determines latency to failure. Increasing the duration of exposure to 10mM glucose and HFS reduced the latency to failure in subsequent substrate free conditions (58.24.9mins after 2hrs (n=4), 34.76.5mins after 4hrs (n=5) and 38.311.5mins after 6hrs (n=4) compared with 94.315.1mins after baseline incubation in 10mM glucose at 1Hz (n=14)), implying a role of glycogen during both HFS and normoglycemic conditions. In contrast, latency to failure after extended exposure to 30mM glucose and HFS was increased (101.735.1mins after 2hrs (n=4) and 132.818.0mins after 4hrs (n=4) compared with 94.315.1mins after baseline incubation (n=14)), suggesting glucose is supplied surplus to demand; excess glucose stored as glycogen in Schwann cells.

During increased neuronal activity, sciatic nerve A fibres require increased energy substrate in order to maintain conduction, as expected. This can be achieved by increased supply of glucose; alternatively, Schwann cell lactate, potentially glycogen derived, may provide support.

Brown AM, Evans RD, Black J & Ransom BR (2012). Schwann cell glycogen selectively supports myelinated axon function. *Ann Neurol* 72, 406–418.

Brown AM, Sickmann HM, Fosgerau K, Lund TM, Schousboe A, Waagepetersen HS & Ransom BR (2005). Astrocyte glycogen metabolism is required for neural activity during aglycemia or intense stimulation in mouse white matter. *J Neurosci Res* 79, 74–80.

Brown AM, Tekk  k SB & Ransom BR (2003). Glycogen regulation and functional role in mouse white matter. *J Physiol* 549, 501–512.

This work was supported by the BBSRC grant number BB/M008770/1

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC213

Microchamber arrays for delivery of bioactive compounds: functional testing of cargo release on-demand.

O. Kopach¹, O.A. Sindeeva^{2,3}, K. Zheng¹, G.B. Sukhorukov² and D.A. Rusakov¹

¹Department of Clinical and Experimental Epilepsy, Queen Square Institute of Neurology, University College London, London, UK, ²School of Engineering and Materials Science, Queen Mary University of London, London, UK and ³Remote Controlled Theranostic Systems Laboratory, Saratov State University, Saratov, Russian Federation

Over the years, a number of nano-engineered cargo systems have been explored for their capability to encapsulate, deliver on-target, and release bioactive compounds (1). Whilst the widely used microcapsule drug encapsulation has successfully combined several functionalities (2), uncontrollable capsule spreading within the target tissue remains a serious issue. Recent advances in the fabrication of patterned nanostructured films appear to overcome the scattering issue, by providing arrays of micro-wells (microchambers) with a predetermined size and spatial patterns. This in turn enables release of loaded bioactive compounds, controlled in space and time. There have been, however, only a few attempts to functionally test such delivery systems in live cells (3-5). We, therefore, carried out a physiological study of the fabricated polylactic acid (PLA)-based microchamber arrays in human cells of neuronal phenotype, Neuro2A cell line, in order to examine the efficiency of cargo delivery for various bioactive compounds.

First, we documented the biocompatibility of the fabricated microchamber arrays by setting human neuroblastoma cells to grow on the surface of the microchamber arrays. There were no signs of excitotoxicity in differentiating Neuro2A cells on either empty PLA-based arrays (no payload) or those loaded with a fluorescent indicator or principal excitatory neurotransmitter glutamate, for up to ten days *in vitro*. Scanning electron microscopy confirmed the effective cargo packing inside individual microchambers when loading i) soluble substances of low molecular weight (glutamic acid or its analogue, L-glutamic acid hydrochloride) or ii) peptides (nerve growth factor, NGF). Next, we demonstrated the site-specific cargo release

on-demand by the laser-triggered opening of individual microchamber(s). This resulted in physiological cellular responses in local cells. For the laser-triggered glutamate release from PLA-based microchambers, we monitored intracellular Ca^{2+} dynamics in differentiated Neuro2A cells using two-photon excitation imaging and detected a robust intracellular Ca^{2+} rise in local cells in response to opening microchambers loaded with glutamate (n=102 cells tested, $p < 0.001$ compared to group of the cells on empty microchamber arrays, n=33 cells); the Ca^{2+} rise was repeatable, reproducible, and within physiological range. For the laser-triggered opening of microchambers loaded with NGF, we observed directed neurite outgrowth of the local cells toward the opening sites (n=5 microchamber array samples). In summary, our results indicate that microchamber arrays can provide a versatile nanostructured drug delivery system, which is biologically safe and efficient in packaging bioactive compounds for geometry-constrained action in the target tissue.

Stuart MA, Huck WT, Genzer J, Muller M, Ober C, Stamm M, et al. Emerging applications of stimuli-responsive polymer materials. *Nat Mater.* 2010;9(2):101-13.

Kopach O, Zheng K, Dong L, Sapelkin A, Voitenko N, Sukhorukov GB, et al. Nano-engineered microcapsules boost the treatment of persistent pain. *Drug delivery.* 2018;25(1):435-47.

Sindeeva OA, Gusliakova OI, Inozemtseva O, Abdurashitov AS, Brodovskaya EP, Gai M, et al. The effect of a controlled release of Epinephrine hydrochloride from PLGA microchamber array: in vivo studies. *ACS applied materials & interfaces.* 2018;10(44):37855-37864.

Gai M, Frueh J, Kudryavtseva VL, Mao R, Kiryukhin MV, Sukhorukov GB. Patterned Microstructure Fabrication: Polyelectrolyte Complexes vs Polyelectrolyte Multilayers. *Scientific reports.* 2016;6:37000.

Kopach O, Zheng K, Sindeeva OA, Gai M, Sukhorukov GB, Rusakov DA. Polymer microchamber arrays for geometry-controlled drug release: a functional study in human cells of neuronal phenotype. *Biomaterials Science* 2019; Mar 27. doi: 10.1039/c8bm01499j.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC214

Disruption of the actions of exogenous glutamate on the stretch-evoked responses of muscle spindles in GluK2-deficient mice

R.W. Banks³, C. Mulle² and G.S. Bewick¹

¹*Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK,* ²*The Neuroscience Institute at Bordeaux, Universite de Bordeaux, Bordeaux, France* and ³*Department of Biosciences, University of Durham, Durham, UK*

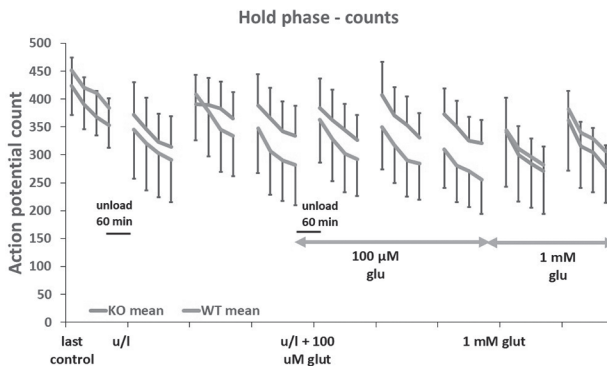
Mechanosensory nerve terminals, including those of the muscle spindle, possess an autogenic glutamatergic modulatory mechanism, mediated by the non-canonical metabotropic action of a phospholipase D-linked receptor¹. We have previously reported to the Society our evidence that the receptor is the kainate receptor,

GluK2². We have now compared the responses of muscle spindles from GluK2-disrupted (GluK2-D) and wild type littermate (WT) mice, each exposed to a repeated battery of stretch profiles, to test the effects of periods of complete unloading to minimise endogenous glutamate release and exogenous glutamate application (Fig 1). Here we report the actions of exogenous glutamate on responses during the hold phase of ramp stretches.

Experiments were performed under relevant national animal experimentation licences, and local ethical committee approvals. Adult GluK2-D mice had the *Grik2* gene disrupted by insertion of a Neo-cassette into MD2 (from Prof Christophe Mulle, Univ. of Bordeaux)³. Soleus nerve-muscle preparations were removed after humane killing (Schedule 1, Animals (Scientific Procedures) Act, 1986). Afferent firing in response to stretches was recorded as the electroneurogram using a suction electrode. The numbers of action potentials (APs) fired during the hold phases of the series of stretch-hold-release (ramp) profiles were counted and compared between genotypes in the absence and then the presence of glutamate added exogenously at 100 μ M and 1 mM. Data are expressed as mean $\pm$ SEM, from 'n' muscles and 'm' mice. Statistical analysis was carried out by repeated measures ANOVA.

When 100 μ M glutamate was increased to 1 mM, GluK2-D mouse spindles displayed a very modest reduction in firing (342 ± 3 vs 299 ± 3 APs; $P < 0.001$; $n = 5$, $m = 9$). In contrast, spindles in WT mice showed one of two responses to the change: either a marked increase in firing (440 ± 5 vs 501 ± 6 APs; $P < 0.001$; $n = 4$, $m = 2$), or a very marked decrease in firing (139 ± 3 vs 52 ± 2 APs; $P < 0.001$; $n = 3$, $m = 2$). At present we can only speculate about the mechanisms of decreased firing in some WT mice. We suggest it may reflect receptor desensitisation, since kainare receptors are very prone to desensitisation⁴, or excitotoxicity from prolonged exposure to high concentrations of glutamate.

We conclude that the absence of an excitatory action of glutamate in the GluK2-D mice is due to the functional disruption of GluK2 receptors, indicating an important role for GluK2 in spindle peripheral terminal modulation.



Muscle spindle action potential counts during hold phase of ramp stretch in GluK2-D and WT mice during series of unloading and exogenous glutamate applications. Increasing glutamate from 0.1 to 1 mM caused mild inhibition of firing in GluK2-D mice, but increased mean firing in WT. Mean $\pm$ SEM, $n = 5$ (GluK2-D) and 7 (WT).

Bewick GS & Banks RW (2015) *Pflügers Arch* 467, 175-190.

Thompson KJ *et al.* (2016) *Proc Physiol Soc.* 37, SA010.

Mulle C *et al.* (1998) *Nature* 392, 601-605.

Pinheiro P & Mulle C (2006) *Cell Tissue Res* 326, 457-482.

We thank Noelle Grosjean & Dr Pierre Costet, University of Bordeaux & Prof Gernot Riedel, University of Aberdeen for arranging supply of GluK2-D mice, plus Prof Mirela Delibegovic for facilitating data collection.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC215

Neuroprotective Potentials of *Carica papaya* Leaves on Lipopolysaccharide-induced Neuroinflammation in Wistar Rats

G.S. Olayinka, O.O. Akande, G.F. Ibironke and A.S. Onasanwo

Department of Physiology, University of Ibadan, Ibadan, Nigeria

Introduction: *Carica papaya* leaf extract has been shown to have anti-inflammatory effects in peripheral inflammation. The aim of this study was to investigate the anti-inflammatory potentials of *Carica papaya* leaf extract (CPLE) on neuroinflammation induced by systemic lipopolysaccharide (LPS) administration.

Materials and Methods: Twenty-five male Wistar rats (150g-180g) were randomly divided into five groups of six animals each. All groups except the control group were given LPS (0.33mg/kg) 24 hours after seven days of administration of their various treatment substances. Group 1 (control) was administered distilled water, group 2 – LPS only, group 3 – indomethacin (4mg/kg), group 4 – CPLE (100mg/kg), and group 5 – CPLE (200mg/kg). The animals were taken through Morris water maze training trials for three days and water maze test 4 hours after LPS administration to assess probable memory dysfunction. Nitric Oxide (NO), Malondialdehyde (MDA), Superoxide Dismutase (SOD), and catalase levels were estimated from whole brain of the animals. Data were reported as Mean $\pm$ S.E.M. and compared using student's t-test. P<0.05 was considered significant.

Results: There was no significant difference in the behavioural test indices. Lipopolysaccharide only treated rats had significantly increased MDA level (32.24 $\pm$ 1.239 μ moles/mg protein), NO level (0.49 $\pm$ 0.004 mM Nitrite) and SOD level (0.85 $\pm$ 0.046 unit/mg protein) compared with control (MDA - 24.66 $\pm$ 0.71 μ moles/mg protein; NO - 0.39 $\pm$ 0.018 mM Nitrite; SOD - 0.61 $\pm$ 0.030 unit/mg protein). 100mg/kg and 200mg/kg CPLE reduced MDA level (100 mg/kg - 27.71 $\pm$ 2.199 μ moles/mg protein; 200mg/kg - 24.53 $\pm$ 3.168 μ moles/mg protein) and SOD level (100mg/kg - 0.58 $\pm$ 0.012 unit/mg protein; 200mg/kg - 0.52 $\pm$ 0.025 unit/mg protein) almost to that of control while only 100mg/kg CPLE was observed to reduce NO level (0.41 $\pm$ 0.008 mM Nitrite). Lipopolysaccharide-treated rats had significantly decreased catalase level (19.76 $\pm$ 0.783 unit/mg protein) compared

with control (34.00 ± 2.409 unit/mgprotein). 200mg/kg CPLE increased catalase level to almost the control level (34.17 ± 0.776 unit/mgprotein).

Conclusion: This research work showed no memory impairment in neuroinflammation induced by single administration of 0.33mg/kg lipopolysaccharide, and that ethanol extract of *Carica papaya* leaf possesses anti-inflammatory and neuro-protective potential.

Owoyele BV et al., (2008). Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. *Inflammopharmacology* 16, 168–173

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC216

Progress towards making neuromuscular junctions in microfluidic devices using human induced pluripotent stem cells

C.D. Hetherington¹, M. Kruth², C. Giuraniuc¹, S. Chandran³, G. Miles⁴ and G.S. Bewick¹

¹Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK, ²Cornell University, New York, NY, USA, ³University of Edinburgh, Edinburgh, UK and ⁴University of St Andrews, St Andrews, UK

iPSC-derived motor neurons (iPSC-MNs) from MND patients display disease phenotype in-vitro¹, but represent only one part of the neuromuscular partnership in the disease process. In-vitro neuromuscular junction (NMJ) interaction and pathology using human iPSC-MNs and muscle is a more clinically relevant platform to research the disease² but NMJs are difficult to reproduce in standard co-culture systems. We hypothesise this reflects different cell-type media requirements. We are therefore developing microfluidic culture systems which allow precise compartmentalized environmental control, modelling the in-vivo segregation of neurons and muscle cell microenvironments³, and facilitating high resolution studies of disease processes and screening of potential therapeutics. Microfluidic chips ($40 \pm 5 \times 5 \times 20 \pm 5$ mm), were fabricated from moulded PDMS applied to glass coverslips (22×60 mm)^{4,5}, providing two parallel chambers ($300 \times 400 \times 10$ μ m), with fine connecting cross-channels ($400 \times 4 \times 4$ μ m). Human iPSC-MNs from healthy and MND patient donors (C9ORF72 repeat expansion), plus isogenic gene-corrected controls were cultured in neuronal chambers, and axons grew through cross-channels into a parallel muscle chamber. iPSC-MN marker expression was probed by immunofluorescent staining for HB9 and ChAT, quantified using mean fluorescence intensity. All data is mean $\pm$ SEM. Neurons were visualised by immunofluorescence for Neurofilament-heavy (axons), MAP2 (dendrites) and Hoechst 33341 (nuclei), and C2C12 myoblasts with MitoTracker Red (mitochondria), α -bungarotoxin (acetylcholine receptors, AChRs), myosin (cytoskeletal). All iPSC-MN lines were cultured for 120 days and expressed mature MN markers. Axon length per cell increased over time in all chips (e.g. in healthy iPSC-MNs from

481.9±83.6 µm day 15 to 916.5±368.1 µm on day 120), with no significant difference between healthy, ALS or controls (n=3, p=0.683, two way ANOVA). In other experiments, C2C12 myoblasts were successfully differentiated to myotubes in-chip, with mean fluorescence intensity and myotube length calculated to assess differentiation (e.g. length of C2C12s at day 11, 119.0±60.6 µm; n=3). Finally, iPSCs-MNs and C2C12 myocytes were cultured in separate parallel compartments linked by micro-channels. iPSC-MN axon terminals grew towards C2C12 myotubes. While the percentage of AChR clusters associated with axon terminals in ALS was less (28.3±9.7%) than gene corrected controls (51.2±4.4%), this failed to reach significance (n=7 chips, p=0.0539, unpaired t-test). We have demonstrated successful culture and differentiation of human iPSC-MNs and an accessible muscle cell line in a novel design of microfluidic chips, as well as viable co-cultures that expressed markers of NMJ formation. This provides the basis of further motor neuron-muscle co-cultures to undertake high resolution studies of the physiology and pathology of MND in the different cellular components: MNs, muscle and the NMJ.

Devlin, A.-C., Burr, K., Boroah, S., Foster, J.D., Cleary, E. M., Geti, I., ... Miles, G.B. (2015). Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. *Nature Communications*, 6, 5999. <http://doi.org/10.1038/ncomms6999>

Ionescu, A., Zahavi, E.E., Gradus, T., Ben-Yaakov, K., & Perlson, E. (2016). Compartmental microfluidic system for studying muscle-neuron communication and neuromuscular junction maintenance. *European Journal of Cell Biology*, 95(2), 69–88. <http://doi.org/10.1016/j.ejcb.2015.11.004>

Guo, X., Das, M., Rumsey, J., Gonzalez, M., Stancescu, M., & Hickman, J. (2010). Neuromuscular junction formation between human stem-cell-derived motoneurons and rat skeletal muscle in a defined system. *Tissue Engineering. Part C, Methods*, 16(6), 1347–55. <http://doi.org/10.1089/ten.TEC.2010.0040>

Saka, Y., MacPherson, M., & Giuraniuc, C.V. (2017). Generation and precise control of dynamic biochemical gradients for cellular assays. *Physica A: Statistical Mechanics and Its Applications*, 470, 132–145. <http://doi.org/10.1016/j.physa.2016.11.134>

Phatnani, H., Maniatis, S., Pandit, K. (2017). Photolithographic mask manufacture. Unpublished, New York Genome Centre, New York, NY.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC217

Accelerated Maturation of Human iPSC-derived Cortical and Sensory Neurons

S.D. Broadbent, S. Gillotin, D. Rock and S. Prime

Axol Bioscience Ltd, Cambridge, UK

The development of neurons from induced pluripotent stem cells (iPSCs) provides research models which offer the potential of being more representative of human cells than existing animal and cell-line based models. The potential of iPSC-derived cells is currently undermined by two major drawbacks; the length of time required

to differentiate and culture them and an often-immature phenotype. The ability to produce more phenotypically relevant cells in a shorter time-frame would be a major boon and would allow iPSC-derived cells to truly achieve their potential as a game-changing research platform.

Here we present data obtained from Axol's Human iPSCs-derived cortical and sensory neurons demonstrating a more rapid maturation and more physiological-relevant phenotype than produced via conventional cell-culturing protocols. The maturation and functionality of the neurones was assessed from their morphology, PCR, immunocytochemistry (ICC) and electrophysiology using calcium imaging and multielectrode (MEA) arrays.

For the sensory neurons, Axol's Human iPSC-derived sensory neuron progenitors (ax0055) were thawed and plated. After Mitomycin C, treatment the cells were placed in an Axol media (ax0058) to mimic the *in vivo* environment during development and were cultured up to Days In Vivo 32 (DIV32). Morphological changes were apparent in cells treated with a sensitisation cocktail by DIV8 $\pm$ 1.5 (SEM, n=4) and the improved maturity and functionality was confirmed by PCR, ICC, MEA recording and calcium imaging compared to control cells by DIV20 compared to 4-7 weeks using conventional tissue culture.

For the cortical neurones, Axol's Human iPSC-derived neuronal stem cells (ax0016) were thawed, plated and differentiated into cerebral cortical neurones. Maturation was accelerated through changing media composition, co-culture and the transfection of transcription factors. The maturation and functionality of the neurones was assessed from their morphology, ICC and electrophysiology using calcium imaging and MEA arrays. Morphological differences were apparent from DIV9.2 $\pm$ 1.1 (SEM, n=5). PCR confirmed an increase in expression levels of several key cortical markers by DIV3 (1.74 - 2.69-fold increase in TUJ1, MAP2, TBR1 & PAX6, n=3). Accelerated functional maturity was confirmed by the earlier appearance of spontaneous spike firing (DIV16 vs DIV34; 178.3 $\pm$ 6.3 vs 57.6 $\pm$ 7.5 spikes/min), burst firing (DIV16 vs DIV34; 8.1 $\pm$ 0.9 vs 0.90 $\pm$ 0.48 bursts/min) and synchronised burst firing (DIV35 vs DIV64+), compared to control conditions.

With the methods described here Axol Bioscience demonstrate the ability to produce more functionally relevant cortical and sensory neurones in a shorter time-frame than currently typical. These findings are should be translatable to other cell-types and this will greatly improve the utility of iPSC-derived cells to research and industry.

Thanks to the staff of Axol Bioscience Ltd and the MESO-Brain Consortium for all their technical assistance and Alpha MED Scientific for the use of the MED64 Presto amplifier.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC218

Metabolic Switch of Nerve Cells During Secondary Response to Axotomy of Neighbouring Neurons

R.I. Ozturk^{3,2}, S. Kablan², S. Bay² and G. Ozturk^{1,2}

¹International School of Medicine, Physiology Department, Istanbul Medipol University, Istanbul, Turkey, ²Regenerative and Restorative Medicine Research Center (REMER), Istanbul Medipol University, Istanbul, Turkey and ³Nutrition and Dietetics, Istanbul Medipol University, School of Health Sciences, Istanbul, Turkey

Following an injury, uninjured neighbouring neurons may die by secondary mechanisms; which underlies expanding of tissue damage in many neurodegenerative diseases and traumas. There are various mechanisms suggested to be responsible for this transneuronal degeneration from excitotoxicity to neuroinflammation. The aim of this study was to analyse metabolic changes in these indirectly affected nerve cells upon axotomy of a small population of neighbouring neurons in culture. Adult Primary sensory neurons of dorsal root ganglia were isolated from adult BALB/c mice, which were anesthetised by an IP injection of ketamin (100mg/kg) and killed by cervical transection. After enzymatic and mechanical dissociation, neurons were seeded in 96-well plates at 10000 cells / well density. After 24 hrs in incubation, outgrown axons of 50 neurons in each well was cut with a UV laser. As a sham procedure, in control wells laser beam was fired near neurons without damaging any extensions. Following another 24 hrs of incubation, preparations were transferred to a cell metabolism analyser and processed according to manufacturer's instructions for a metabolic dependency assay. In the injury wells the dependency of cultured nerve cells to glutamine as an energy source rose from 6,8 to 39,01 % ($p<0,05$) and to glucose from 25,3 to 70 % ($p<0,05$). Fatty acid utilization also increased from 12,4 to 18,8%, albeit not significantly.

The study has demonstrated that even when a small fraction of nerve cells was injured, a large population of neighbouring cells are affected secondarily. These findings are important in understanding secondary neural degeneration in nervous system pathologies.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Anti-neuroinflammatory potentials of *Waltheria americana* leaf in experimental animalsI.O. OWEMIDU^{1,2}, A.S. Onasanwo² and A. Ajayi³¹Physiology, Kogi State University, Anyigba, Nigeria, ²Physiology, University of Ibadan, Ibadan, Nigeria and ³Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria

Neuroinflammation in the Central Nervous System (CNS) is associated with the development of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Neuronal death in many neurodegenerative diseases is due to the release of cytokines and other inflammatory mediators by the glial cells (microglia and astrocytes). Preventing the release of pro-inflammatory mediators during neuroinflammation, will be an effective strategy in the management of neurodegenerative diseases associated with neuroinflammation. *Waltheria americana* is used in folkloric medicine for the treatment of pain and inflammation. This study therefore evaluated the anti-neuroinflammatory properties of the methanol extract of *Waltheria americana* leaf (MEWA) in experimental animals.

Leaves of *W. americana* were collected at the College of Agriculture, Kabba, Kogi State, and authenticated at the Herbarium, Forest Research Institute of Nigeria (FRIN), Ibadan (FHI:111064). The leaves were extracted by maceration in methanol. The study had four groups (n=5) of rats: Normal Control and groups that received vehicle (Veh) (10 mL/kg p.o), MEWA (200 mg/kg p.o) and Quercetin (50 mg/kg p.o) for 30 days after a single intra-peritoneal administration of 2 mg/kg Lipopolysaccharide (LPS). Memory was assessed using Y maze test and Morris water maze test. At termination, Striatum (STR), Prefrontal cortex (PFC) and Hippocampus (HPC) were sectioned. ELISA method was used for IL-6 and TNF- α while iNOS and β -amyloid (A β) were quantified using Immunohistochemistry. Golgi and Nissl staining was used for neuronal morphology. Data were analysed using ANOVA at $\alpha_{0.05}$.

The MEWA (200 mg/kg) significantly increased percentage alternation (82.88 ± 3.0 vs 54.26 ± 4.85) in the Y maze test and time spent in quadrant area in the probe trial (26.50 ± 0.76 s) compared to Veh (17.67 ± 0.71 s) in Morris water maze test. The MEWA 200 mg/kg significantly decreased IL-6 and TNF- α (pg/g tissue) in STR (48.07 ± 3.42 , 2.31 ± 0.33), PFC (44.01 ± 1.03 , 2.27 ± 0.16), HPC (76.41 ± 0.78 , 7.49 ± 0.19), compared to Veh STR (86.09 ± 4.19 , 4.47 ± 0.10), PFC (79.74 ± 1.16 , 3.51 ± 0.04) and HPC (101.90 ± 3.42 , 9.58 ± 0.38) respectively. The MEWA 200 mg/kg decreased the expression of iNOS and A β in STR PFC and HPC compared to Veh group. The MEWA 200 mg/kg reduced dendritic degeneration compared to Veh group.

Waltheria americana leaf extract ameliorated neuroinflammation in striatum, prefrontal cortex and hippocampus by reducing the level of pro-inflammatory mediators and preventing neuronal loss.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC220

Protective effect of co-administration of vitamins C and E on reserpine-induced motor and cognitive impairments and oxidative stress in mice

T. Danboyi¹, A. Alhassan³, A. Jimoh³ and E. Hassan-Danboyi²

¹Human Physiology, Kaduna State University, Kaduna, Nigeria, ²Human Physiology, Ahmadu Bello University, Zaria, Zaria, Nigeria and ³Human Physiology, Ahmadu Bello University, Zaria, Zaria, Nigeria

Introduction: Given the central role oxidative stress (OS) played in the pathogenesis of Parkinson's disease, several antioxidants have been explored. **Aim:** Combining two vitamins (C and E) is aimed at conferring greater neuroprotection against reserpine-induced OS and motor and cognitive impairments in mice. **Methods:** Twenty-five mice were randomly assigned into 5 groups of 5 animals each. Group I received distilled water only. Groups II-V received reserpine 0.1 mg/kg *intraperitoneally* on alternate days. In addition, Group III received vitamin E 200 mg/kg/day orally, group IV, vitamin C 250 mg/kg/day orally and group V, combined vitamin E 200 mg/kg/day and vitamin C 250 mg/kg/day orally. All vitamins were given concurrently one hour before reserpine injection for 28 days. Neurobehavioral assessment using novel object recognition test (NORT), Y-maze, beam walking and open field test (OFT) was carried out. Thereafter, the mice were humanely sacrificed and brain homogenate made. Values at $p < 0.05$ were considered significant. **Results:** The negative discrimination index observed in group II (-0.35 ± 0.23) was significantly ameliorated by the co-administration of both vitamins (0.59 ± 0.12). In the y-maze, a significant increase in percentage alternation was recorded in group V ($66.7 \pm 9.25\%$) compared to the other groups ($p = 0.003$). In the beam walk, there was a significant decrease in number of foot slips (0.3 ± 0.25) as well as the time to reach the safe box ($3.00 \pm 0.41s$) in group V compared to other groups. In the OFT, the transfer latency was significantly decreased ($10.3 \pm 1.45s$) while the number of lines crossed was significantly increased (56.0 ± 13.53) in group V compared to the other groups. The malondialdehyde concentrations was significantly decreased in all vitamin-treated groups compared to reserpine-only group (42.2 ± 0.28 Umol/L). A significant increase was seen in superoxide dismutase and catalase levels with a non-significant decrease in GSH level across all the vitamin-treated groups compared to the reserpine-only group. **Conclusion:** The co-administration of vitamins C and E confers a significant neuroprotection against motor and cognitive impairments and OS induced by reserpine in mice.

Dawud, F. A., Mabrouk, M. A. & Umar, I. A. (2014). Effects of vitamins C and E on aspirin-induced gastric mucosal damage and oxidative stress. *J. Biol. Sci.* 6(1): 36-41.

Eftimov, M., Dobрева, C., Velkova, D. & Valcheva-Kuzmanova, S. (2014). Effect of *Aronia melanocarpa* fruit juice on reserpine-induced hypokinesia and oxidative stress in rats. *Scripta Scientifica Pharmaceutica*, 1(2): 29-34.

Hsiang-Chien, T., Mao-Hsien, W., Hung-Sheng, S., Yi, C., and Kuo-Chi, C. (2015). Epigallocatechin-3-gallate prevents the reserpine-induced impairment in short-term social memory in rats. *Behav. Pharmacol.*, 26(8): 741-747.

None external source of funding

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC221

A method for increasing experimental efficiency whilst maintaining statistical power: application to electrophysiological recordings from rodent optic nerve

A.M. Brown and L.R. Rich

School of Life Sciences, University of Nottingham, Nottingham, UK

The compound action potential (CAP) recorded from *ex vivo* mouse optic nerve is a popular model used to study nervous system metabolism. A recent advance in this technique has been recording from both optic nerves simultaneously using paired sets of stimulating and recording electrodes to acquire CAPs from both nerves simultaneously. The method has advantages in studies where one nerve can act as the control for its treated partner. We report on recordings from pairs of sciatic nerve from which we derived a mathematically based rationale that demonstrates decreased experimental variability whilst maintaining statistical power and reducing animal use.

All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986 under appropriate authority of project and personal licenses. Adult male CD-1 mice were killed by cervical dislocation and decapitated. Sciatic nerves were dissected, placed in a superfusion chamber, with aerated aCSF containing 10 mM glucose. The stimulus evoked A fibre CAP was evoked with supra-maximal stimuli.

We employed two separate recording set ups, a conventional one in which single sciatic nerve CAPs were recorded, and a set up where pairs of sciatic nerve were recorded simultaneously. The protocol involved stimulating nerves continuously at a rate of 1 Hz during the baseline period, followed by a period of aglycaemia, during which nerves were stimulated at either 1 Hz or 50 Hz. Increasing the stimulus frequency to 50 Hz accelerated A fibre CAP failure during aglycaemia, which was assessed as the latency for the CAP to fall to 95% of its baseline value. In recordings from single nerves stimulated at 1 Hz, the mean latency to CAP failure was 99.4 ± 28.0 minutes ($n = 12$) versus 79.7 ± 24.9 minutes ($n = 12$) for nerves stimulated at 50 Hz, a significant difference ($p = 0.040$: two sample unequal variance t-test). In recordings from pairs of nerves the equivalent values were

96.4 ± 17.2 minutes (n = 12) versus 80.1 ± 18.9 minutes (n = 12), p = 0.019. The relative standard deviation (RSD), which is the coefficient of variation expressed as a percentage showed a statistically significant decrease from pairs of nerves compared to single nerve recordings (p = 0.015, n = 12, unpaired t-test).

Recordings from pairs of nerves simultaneously has allowed us to reduce the number of mice used in experiments designed to test the role of glycogen in supporting axonal conduction during periods of aglycaemia in sciatic nerve. Mathematical analysis of our data showed that for equivalent number of nerves, when recordings derived from paired recordings the p value of comparisons was lower and power was higher meaning that under these conditions the risk of making Type I and Type II error was reduced compared to recordings from single nerves.

Funded by the BBSRC grant number BB/M008770/1.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC222

Understanding the role of HACD enzymes and very long chain fatty acids in zebrafish muscle development and disease

R. Morgan¹, I. McConnell², R. Piercy², M. Peffers¹, R. Barrett-Jolley¹ and G. Walmsley¹

¹*Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK and*

²*Department of Clinical Sciences & Services, Royal Veterinary College, London, UK*

3-hydroxyacyl-coA dehydratase (HACD) enzymes are required for the elongation of fatty acids with very long carbon chain lengths (VLCFA). There are four HACD enzymes in mammals (HACD1-4), each with a distinct tissue distribution: HACD1 is specifically expressed in developing and mature striated muscles. Mutations in HACD1 cause myopathies in humans, dogs and mice, however the specific functions of this enzyme and VLCFA in muscle physiology are poorly understood. Centronuclear myopathy in Labrador dogs is the best characterised of these conditions and results in progressive disorganisation of tubuloreticular membranes and mislocalised triads. We aimed to evaluate the suitability of zebrafish for studying the functions of HACD enzymes and the role of HACD1 in muscle development and disease.

Zebrafish homologues of HACD enzyme genes were identified and by conventional and quantitative RT-PCR and *in situ* hybridisation we characterised the expression of HACD enzymes during embryonic development and in adult tissues of AB wildtype zebrafish. Like mammals, zebrafish express two main isoforms of the HACD1 homologue of which the full length, active isoform was specifically expressed in developing and mature striated muscles. Full length *hacd2* was ubiquitously expressed in adult zebrafish tissues and upregulated early in embryonic development with strong expression in the eyes and brain. *hacd3* was also widely expressed, however both *hacd2* and *hacd3* transcripts had relatively low abundance in skeletal muscle.

We documented putative zebrafish *hacd4* at low levels in several tissues. CRISPR/Cas9 genome editing was used to introduce mutations into *hacd1* and a muscle phenotype was observed in F₀ embryos including abnormal tail morphology, motor function and disorganised myofibres with abnormal RYR1 immunostaining. In conclusion, the zebrafish homologues of the four HACD enzymes have expression and tissue distribution similar to that seen of mammals; in particular *hacd1* shows strong, specific expression in developing and mature striated muscles. Mutations in *hacd1* result in skeletal muscle abnormalities and reproduce key features of HACD1-CNM. This suggests that zebrafish are a good model for studying HACD enzyme functions in muscle including the pathophysiology of HACD1-deficient myopathies.

Ikeda, M. *et al.* 2008. Characterization of four mammalian 3-hydroxyacyl-CoA dehydratases involved in very long-chain fatty acid synthesis. *FEBS Letters*.

Pelé, M. *et al.* 2005. SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. *Hum Mol Genet*.

Muhammad, E. *et al.* 2013. Congenital myopathy is caused by mutation of HACD1. *Hum Mol Genet*.

Toscano, A. *et al.* 2017. Pseudo-dominant inheritance of a novel homozygous HACD1 mutation associated with congenital myopathy: The first caucasian family. *Neuromuscular Disorders*.

Maurer, M. *et al.* 2010. The PTPLA gene, mutated in a dog model of centronuclear myopathy, is prone to a physiological tissue specific alternative splicing that produces isoforms with putatively distinct structural and functional properties. *Proceedings of the XIIth International Congress on Neuromuscular diseases*.

The authors would like to thank the Institute of Ageing and Chronic Disease and the Wellcome Trust for funding this research.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC223

Kolaviron protects rats from cognitive decline induced by lipopolysaccharide in rats

A.S. Onasanwo

Physiology, University of Ibadan, Ibadan, Nigeria

Kolaviron is a mixture of biflavonoids of seed *Garcinia kola*, and has been previously shown to exhibit Nrf2 antioxidant-mediated inhibition of neuroinflammation in LPS-activated BV2 microglia. In this study we investigated neuroprotective effects of kolaviron in LPS-induced memory impairment in rats.

Wistar rats (225–250) g was used for this study. Memory impairment was induced with the systematic administration of 250µg/mg (i.p.) lipopolysaccharide (LPS).

The effect of kolaviron (50, 100 and 200 mg/kg, p.o.) on the cognition and learning processes were assessed using the behavioral responses in the Morris water maze model. Effects of LPS injections on the physiological activities were assessed by biochemical assays before and after treatment. Animals were handled in accordance to the guidelines of National Institute of Health (NIH) for laboratory animal care and use, and approved by the University of Ibadan Animal Ethics Review Committee.

Peripheral administration of LPS was showed to reduce cognitive and locomotor process. It also led to reductions in superoxide dismutase (SOD), and catalase levels, with an increase in Membrane lipid-peroxidation (MDA), intracellular glutathione (GSH) and nitric oxide (NO₂). These pro-inflammatory mediators produced in response to LPS are hypothesized to affect cognition, and kolaviron was able to ameliorate the effect by significantly improving the cognitive and learning processes, revealed in the reduction of escape latency and path-length during the probe trial and increase in time spent within the quadrant during retrieval using Morris water maze. Similarly, LPS at 250µg/kg induced a hypothermic effect in the treated animals. Kolaviron significantly was able to ameliorate the level of SOD and CAT by causing a significant increase while it caused a significant reduction in the level of NO₂, GSH, and MDA.

Kolaviron has significant anti-inflammatory potentials, reducing lipopolysaccharide activation of macrophages. The memory-enhancing activity of kolaviron was comparable to sulindac sulfide (a non-steroidal anti-inflammatory drug).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC224

Brain activity during choice reaction task in military servicemembers with mild traumatic brain injury and posttraumatic stress disorder

V. Vozniuk¹, N. Filimonova¹, M. Makarchuk¹, I. Zyma¹, O. Horbunov² and V. Kalnysh³

¹Human and animal physiology, ESC Institute of Biology and Medicine of Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, ²Computer science and cybernetics, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine and ³Kundiiev Institute of Occupational Health of the NAMS of Ukraine, Kyiv, Ukraine

Traumatic brain injury (TBI) and posttraumatic stress disorder (PTSD) are the most common impairments among military servicemembers who took part in armed conflicts. TBI and PTSD cause long-term cognitive impairments and respectively alterations in information processing and decision making (1,2). Choice reaction (CR) task is the simplest way to model decision making in the study.

Participants. The study involved 31 volunteers (male 18-28 y.o.) without health complaints or reported PTSD and TBI (control group); 13 volunteers (male, 20-54 y.o.), servicemembers of Armed Forces of Ukraine with combat-related PTSD, and

17 volunteers (male, 22-50 y.o.), combat-injured servicemembers of Armed Forces of Ukraine with mild TBI (mTBI). All participants were right-handed and took part in the study after providing informed consent consistent with the Declaration of Helsinki.

Procedure and Methods. CR computer task with two types of visual stimuli was used to test participants. CR time (CRT) of responses was detected. EEG was recorded at sampling rate 500 Hz during whole testing procedure. 19 electrodes were placed according to the international 10-20 system. Localization of regions of brain activity was identified with neuroimaging LORETA software (3). To display differences between groups statistical non-parametric mapping was conducted in LORETA (4). Functional connectivity was assessed with EEG coherence between all possible coupled pairs of electrodes 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 (20-35 Hz). Significant level of coherence value was established equal or greater than 0,7 (5).

Results. CRT of mTBI group was significantly longer compared to control group (471 [446; 579] ms vs. 410 [392; 430] ms ($U=116$; $p=0,002$)), while there was no difference between PTSD patients and control group (434 [405; 485] ms vs. 410 [392; 430] ms ($U=135$; $p=0,087$)). In control group there was revealed activation of the fronto-parieto-occipital and temporo-occipital regions as well as connectivity between respective regions (Table 1). PTSD group demonstrated activation of the right fronto-parieto-temporal regions and the right insula in comparison vs. zero. There was no statistically significant difference revealed in comparison with control group ($p=0,493$). In PTSD group coherences were revealed between following pairs of electrodes demonstrated in (Table 1). mTBI group had statistically significantly greater activation of the left temporo-occipital regions, the left insula and posterior cingulate gyrus during CR task compared to control group ($p=0,018$; $F(0,05)=0,574$)) (Table 2). Revealed coherences between pairs of electrodes in mTBI patients also are shown in (Table 1).

Conclusions. In general PTSD group demonstrated similarity to control group in brain activity and functional connectivity that provided efficient CR, while mTBI group had slower CR, strong left lateralized brain activity and altered weak functional connectivity.

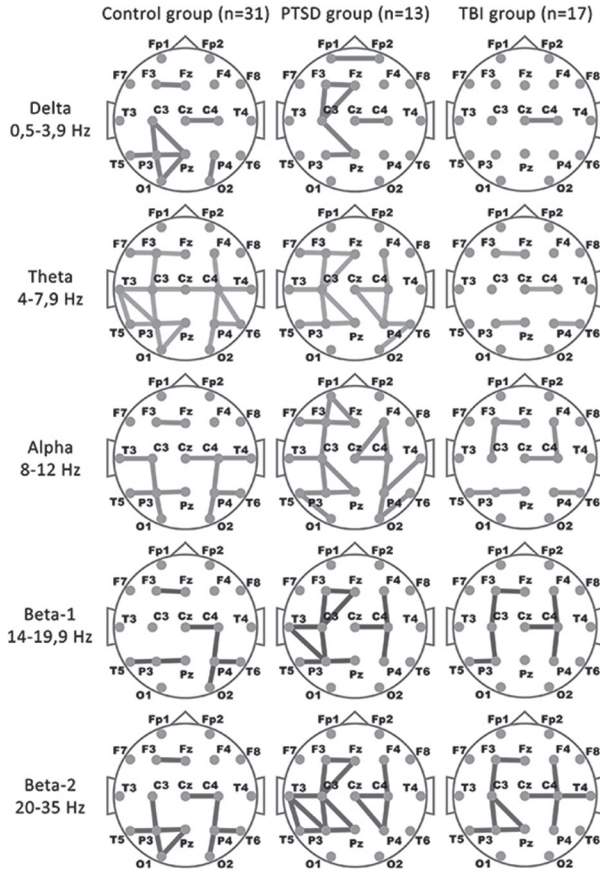


Figure 1. Functional connectivity in control (n=31), PTSD (n=13) and mTBI (n=17) groups estimated as EEG coherence between respective pairs of electrodes in delta (0.5-3.9 Hz), theta (4-7.9 Hz), alpha (8-12 Hz), beta-1 (14-19.9 Hz) and beta-2 (20-35 Hz) bands.

X(MNI)	Y(MNI)	Z(MNI)	Voxel Value	Brodmann area	Lobe, Side	Structure
-30	-45	-5	0,647	19	Limbic Lobe, L	Parahippocampal Gyrus
-30	-40	-10	0,638	37	Limbic Lobe, L	Parahippocampal Gyrus
-35	-40	-10	0,632	37	Temporal Lobe, L	Fusiform Gyrus
-35	-40	-20	0,621	20	Temporal Lobe, L	Fusiform Gyrus
-25	-55	0	0,619	30	Limbic Lobe, L	Parahippocampal Gyrus
-25	-40	-10	0,618	36	Limbic Lobe, L	Parahippocampal Gyrus
-25	-30	-5	0,608	27	Limbic Lobe, L	Parahippocampal Gyrus
-35	-35	10	0,604	41	Temporal Lobe, L	Superior Temporal Gyrus
-45	-45	-20	0,596	37	Temporal Lobe, L	Inferior Temporal Gyrus
-40	-35	5	0,596	41	Temporal Lobe, L	Superior Temporal Gyrus
-45	-40	-30	0,593	36	Temporal Lobe, L	Fusiform Gyrus
-45	-45	-15	0,593	37	Temporal Lobe, L	Sub-Gyral
-20	-55	0	0,592	18	Occipital Lobe, L	Lingual Gyrus
-25	-55	-15	0,588	19	Occipital Lobe, L	Fusiform Gyrus
-35	-25	5	0,583	13	Sub-Lobar, L	Insula
-50	-50	-20	0,575	20	Temporal Lobe, L	Inferior Temporal Gyrus
-20	-60	5	0,574	30	Limbic Lobe, L	Posterior Cingulate Cortex

Table 1. mTBI (n=17) vs. Control group (n=31). There was revealed significant difference between mTBI and Control group in One-Tailed test mTBI>Control ($p=0,018$; $F(0,05)=0,574$) and Two-Tailed test mTBI<>Control ($p=0,036$; $F(0,05)=0,627$).

Dikmen SS et al. (2009). Cognitive outcome following traumatic brain injury. J Head Trauma Rehabil 24(6), 430-438.

Scott JC et al. (2015) A quantitative meta-analysis of neurocognitive functioning in posttraumatic stress disorder. Psychol Bull 141(1), 105-140.

Pascual-Marqui RD (2002). Standardized low resolution brain electromagnetic tomography (sLORETA): technical details. Methods Find Exp Clin Pharmacol 24, 5-12.

Nichols TE and Holmes AP (2002). Nonparametric permutation tests for functional neuroimaging: A primer with examples. Hum Brain Mapp 15(1), 1-25.

Kulaichev AP (2011). The Informativeness of Coherence Analysis in EEG Studies. Neurosci Behav Physiol 41(3), 321-328.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC225

Honey offers neuroprotective benefit against Paraquat-induced experimental Parkinson's Disease in Wistar rats.

R.O. Oladejo and A. Onasanwo

Physiology, University of Ibadan, Ibadan, Nigeria

Parkinson Disease (PD) is a neurologic disorder involving progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (Spc) and motor imbalance. Previous studies have linked neurotoxic pollutant (e.g. Paraquat) exposure to incidence of PD. Here, we investigate the protective effect of Honey – a

polyphenol-rich natural food with proven antioxidant potential – against Paraquat induced experimental PD in Wistar rats.

Twenty animals weighing between 200 and 250g were randomly divided into four groups with five animals per group. Group I animals were the vehicle-treated group, group II animals were exclusively injected (intraperitoneally) with paraquat (10mg/kg). Groups III and IV received oral administration of bromocriptine (2.5mg/kg) and honey (250mg/kg) respectively in addition to paraquat injection. Catalepsy and locomotor activity were assessed with bar test and computerized actophotometer respectively. Oxidative stress biomarkers such as SOD, GSH, lipid peroxidation and nitrate were estimated in homogenized brain samples. Mid brain region structural integrity was also examined with histological sectioning and analysis.

Chronic Co-administration of honey with paraquat injection significantly ($p < 0.01$) improved the catalepsy and locomotor inactivity of the animals. Lipid peroxidation and nitrite generation were significantly suppressed ($p < 0.01$) in honey co-treated animals compared to exclusive paraquat treated. Superoxide dismutase (SOD) and Glutathione (GSH) were significantly spared as well ($p < 0.01$). Histological assessment also revealed that honey co-treatment ameliorates the morphological disruption and nissle granules disintegrations in the midbrain region. Honey administration attenuates the behavioral and oxidative stress adversities in Paraquat-Parkinsonian-like rats' brain. Hence, honey offers a significant neuroprotective benefits in Paraquat-induced experimental PD.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC226

Detection of Ion Channel Events with “Artificial Intelligence” (AI) Deep Learning

N. Celik¹, F. O'Brien¹, Y. Zheng³, F. Coenen² and R. Barrett-Jolley¹

¹Musculoskeletal Biology, University of Liverpool, Liverpool, UK, ²Computer Science, University of Liverpool, Liverpool, UK and ³Eye and Vision Science, University of Liverpool, Liverpool, UK

Single channel recordings obtained with the patch-clamp technique have made an enormous impact to our understanding of ion channel function, allowing researchers to obtain detailed and precise information about the kinetic behaviour of ion channels. A major limitation in the current systems used for analysis is that automatic analysis is unreliable and manual analysis is extremely laborious. We present here an “artificial intelligence” (AI) deep learning approach to automatically process large collections of ion channel data. Recently, deep learning, a machine learning development, has begun to revolutionise automatic analysis of data. Here, we have generated semi-synthetic single-channel data and developed deep learning models to detect ion channel current events re-construct idealised records and estimate open probability (P_o).

Methods: Ion channel events were simulated from published stochastic gating models by the Gillespie (1977) method, passed through a HEKA patch-clamp EPC7 amplifier (List-Medical, Darmstadt, Germany) and recorded back to file with Signal (CED, Cambridge UK) via an Axon (Molecular Devices, CA, USA) electronic “model cell”. Two different stochastic gating models were used to generate single channel and multi-channel records (up to 5 channels). To validate the proposed DL model, 6 different datasets were used in total throughout the experiments: 3 datasets for single and 3 datasets for multi-channel recordings. Two different models were also compared, with recurrent neural network (LSTM) and convolutional layers. During training, a stochastic gradient descent optimizer was used with 80/20% training/testing split. To reduce overfitting, a dropout layer (0.2) was applied to each layer after batch normalization. Due to a class-imbalance in the datasets, a class weighting scheme was used and loss measured with categorical cross entropy.

Results: Our best models correctly detected channel events with 0.98 ± 0.04 accuracy (98%) when a single channel was present in the dataset and 0.91 ± 0.06 accuracy (91%) in datasets with a few single channels present. Open probability (P_o) was $99 \pm 3\%$ accurate compared to the ground truth/fiducial input. When compared to a traditional method of single channel analysis using QuB software, mean P_o was remarkably similar; 0.044 ± 0.017 with traditional software (QuB), vs 0.0436 ± 0.018 was achieved with AI detection.

Early models are highly promising. This model will further be adapted with different network architectures and compared the detection power and generalisability. Furthermore, the developed models will be validated to robustly detect transition events in both single and multiple ion channels in a range of biological and biosynthetic datasets without need for re-training.

Gillespie DT (1977) Exact stochastic simulation of coupled chemical reactions. *J Phys Chem* 81:2340-2361.

Funded by the BBSRC.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC227

Disruption of neurovascular coupling in a rodent model of vascular dementia – impact on cognition and rescue by nitrate supplementation

C.F. Lourenço Marques^{1,2}, J. Gonçalves^{1,2} and J. Laranjinha^{1,2}

¹Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal and ²Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

The functional and structural integrity of the brain rely on a prompt delivery of metabolic substrates (via local changes in cerebral blood flow) matching the increase in neuronal activity. The dysfunction of this process - neurovascular coupling – is suggested to critically contribute to neurodegeneration occurring

during neuropathological conditions, such as vascular dementia. Nitric oxide (NO) is a key messenger in neurovascular coupling by signaling from active neurons to blood vessels. Altered NO signaling and ensued impairment of cerebral blood flow (CBF) have a prominent role in the dysfunctional cascade leading to neurodegeneration. Nitrate and nitrite are increasingly recognized as relevant regulatory molecules acting as metabolic precursors of NO . It has been suggested that sodium nitrate supplementation may improve cerebral blood perfusion and improve NVC, thereby mitigating age-related cognitive impairment. Yet, a conclusive proof of concept for this hypothesis and the mechanisms involved are still to be defined.

In this study, we aimed to investigate the functionality of neurovascular coupling, in connection with neuronal-derived NO signaling, in a rodent model of vascular dementia and to test whether nitrate is able to circumventing the altered NO bioactivity thereby maintaining the normal behavioral function. To achieve these goals, the dynamics of NO and CBF were monitored in response to neuronal activation in urethane-anesthetized rats submitted to bilateral common carotid occlusion (2VO), either acutely (2-4 h after occlusion) and chronically (6-8 weeks after occlusion). In the latter, the functionality of neurovascular coupling was associated to cognitive performance, evaluated in terms of working and reference spatial memory (Y-maze and Barnes maze). The effect of nitrate was accessed acutely via a bolus intraperitoneal injection of a sodium nitrate solution or chronically via nitrate orally administered for 6 weeks (in water ad libitum).

Preliminary data supports a dramatic impairment in the neurovascular coupling immediately after 2VO and a modulatory action of nitrate improving the cerebrovascular response to neuronal activation. Also, the results point towards a sustained derailment of neurovascular coupling weeks after 2VO, matching the compromised cognitive function. These observations support a connection of impairment of neurovascular response with cognitive decline and suggest nitrate supplementation may configure an attractive strategy to rescue cognitive function in vascular dementia.

(Work funded by FEDER through COMPETE and FCT - POCI-01-0145-FEDER-007440/POCI-01-0145-FEDER-029099 and by the Centro 2020 Regional Operational Program - CENTRO-01-0145-FEDER-000012-HealthyAging2020)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC228

Cholinergic modulation of cell proliferation in the postnatal murine spinal cord.

N. Altuwaijri, S. Deuchars and J. Deuchars

University of Leeds, Leeds, UK

The proliferation and differentiation of endogenous neural stem cells and precursor cells in the postnatal central nervous system have been extensively reported. However, a full understanding on how to generate specific cell types to replace damaged or lost neurons and oligodendrocytes after spinal cord injury or other

neurodegenerative disorders such as multiple sclerosis is still unclear. In the spinal cord in particular, there is a lack of understanding of how the use of exogenous compounds such as those that enhance endogenous acetylcholine signalling can effectively drive the proliferation and differentiation of endogenous stem cells (the ependymal cells) and oligodendrocyte precursor cells. Using the thymidine analogue 5-ethynyl-2-deoxyuridine (EdU) as a marker of proliferating cells, we show that adult C57/Bl6 mice administered donepezil (i.p. 5 μ M) – an acetylcholinesterase inhibitor and the $\alpha 7$ nicotinic acetylcholine receptor (nAChR) positive allosteric modulator (PNU-120596, 1 μ M) had significantly ($P < 0.0001$) more EdU positive cells, compared to control, in the central canal, grey and white matter of uninjured spinal cord. Furthermore, in these animals, there was a significantly higher ($P < 0.0001$) number of EdU⁺ cells that also expressed the oligodendrocyte marker, PanQKI in both the grey and white matter compared to control. There was also a significantly ($P < 0.0001$) higher extent of colocalisation of EdU with the neuronal marker HUC/D in the spinal cord grey matter of animals treated with donepezil in combination with PNU-120596 compared to control animals. In a lysolecithin-induced focal demyelination mouse model, treatment with either PNU-120596 alone or in combination with donepezil significantly enhanced cell proliferation and oligodendroglial differentiation compared to lysolecithin alone treated animals. Thoraco-lumbar spinal cord slices cultured in the presence of donepezil and the muscarinic receptor inhibitor atropine revealed that most of the cholinergic proliferative response is mediated via nicotinic acetylcholine receptors in the central canal, grey and white matter; there was a significantly higher number of EdU positive cells in donepezil + atropine treated slices compared to donepezil alone. Slices cultured in the presence of donepezil, atropine and the non- $\alpha 7$ nAChR inhibitor DHbE had a significantly higher number of EdU positive cells compared to slices cultured with donepezil alone, donepezil + atropine or the nicotinic agonist cytisine + DHbE in the central canal of slices; suggesting that cell proliferation is mostly mediated through $\alpha 7$ nAChRs. Finally, the calcineurin-nuclear factor of activated T cells (NFAT) calcium signalling pathway downstream of $\alpha 7$ nAChRs plays a role in cells proliferation *in vitro*.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC229

Endogenous 5-HT promotes higher number of proliferating cell in hippocampus, brainstem and spinal cord but it is not mediated by 5-HTR4

N. Ghani, K. Greenin, G. Ford, I. Kearns, A. van der Schoot, J. Deuchars and S. Deuchars

University of Leeds, Leeds, UK

Neurogenesis is the differentiation of neural stem cells into neurones, astrocytes and oligodendrocytes. There is evidence for postnatal neurogenesis in the hippocampus, brainstem and central canal area of the spinal cord (Bauer et al., 2005,

Barnabé-Heider et al., 2010, Lois and Alvarez-Buylla, 1994). We therefore sought to determine the relationship between 5-HTergic fibres and neural stem cells using nestin-GFP mice. Then *in vivo* and *in vitro* experiments were conducted. Administration of 10mg/kg of 5-ethynyl-2'-deoxyuridine (EdU), a thymidine analogue, to allow detection of newly proliferated cells followed by immunofluorescent detection of differentiation markers for astrocytes ($\leq 100\beta$), oligodendrocytes (PanQKI) and neurones (HUC/D) were performed. In nestin-GFP mice, 5-HTergic fibres formed close appositions with nestin-positive neural stem cells in brain stem and spinal cord suggesting that 5-HT may modulate the stem cells. Intraperitoneal administration of fluoxetine hydrochloride (10mg/kg) for 10 days and EdU for 5 days in C57/Bl6 (n=8) mice promotes greater numbers of proliferating cells in dentate gyrus of hippocampus (50.0 ± 2.2 vs 30.7 ± 3.0 , $p \leq 0.05$), DVC (32.71 ± 3.45 vs 19.87 ± 1.64 , $p \leq 0.05$), raphe (3.8 ± 1.2 vs 0.63 ± 0.4 , $p \leq 0.05$), inferior olives of brainstem (17 ± 1.9 vs 9.5 ± 1.5 , $p \leq 0.05$) and dorsal horn of gray matter in thoracic spinal cord (15.3 ± 1.4 vs 10.0 ± 0.8 , $p < 0.05$). Furthermore, the proportion of new cells that became oligodendrocytes was significantly higher in both brainstem (26 ± 4.4 vs 15.4 ± 2.7 , $p \leq 0.05$) and thoracolumbar spinal cord (24.2 ± 2.2 vs 14.6 ± 1.7 , $p \leq 0.05$). To determine which 5-HTR is involved, *in vitro* experiments revealed that 4 hours incubation of $10\mu\text{M}$ of cisapride (5-HT₄ agonist) with $1\mu\text{M}$ EdU elicited higher numbers of proliferating cells in central canal of spinal cord (3.6 ± 0.3 vs 2.6 ± 0.2 , $p \leq 0.05$). *In vivo* experiments (n=8) by intraperitoneal administration of 1mg/kg of tegaserod maleate (5-HT₄ partial agonist) and EdU for 7 days elicited significantly fewer proliferating cells in dentate gyrus of hippocampus (32.6 ± 0.9 vs 40.3 ± 2.0 , $p \leq 0.05$) and selected areas in spinal cord, compared to control. None of the differentiation markers show any significant results in brainstem and spinal cord. However in hippocampus, activation of 5-HTR₄ significantly suppressed the differentiation of proliferating cells into neurones ($8.8\% \pm 0.8$ vs $13.7\% \pm 1.2$, $p < 0.05$). Further *in vitro* experiments using $1\mu\text{M}$ of tegaserod maleate revealed significantly lower numbers of proliferating cells in central canal of spinal cord (9.21 ± 0.8 vs 12 ± 1.2 , $p \leq 0.05$). In conclusion, this indicated the influence of endogenous 5-HT in promoting high levels of cell proliferation since fluoxetine blocks the 5-HT transporters, increasing 5-HT levels in the synaptic cleft. However, this positive effects were not mediated by 5-HTR₄.

BARNABÉ-HEIDER, F., GÖRITZ, C., SABELSTRÖM, H., TAKEBAYASHI, H., PFRIEGER, F. W., MELETIS, K. & FRISÉN, J. 2010. Origin of New Glial Cells in Intact and Injured Adult Spinal Cord. *Cell Stem Cell*, 7, 470-482.

BAUER, S., HAY, M., AMILHON, B., JEAN, A. & MOYSE, E. 2005. *In vivo* neurogenesis in the dorsal vagal complex of the adult rat brainstem. *Neuroscience*, 130, 75-90.

LOIS, C. & ALVAREZ-BUYLLA, A. 1994. Long-distance neuronal migration in the adult mammalian brain. *Science*, 264, 1145-1148.

University Sains Malaysia

Ministry of Education Malaysia

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Repeated hypoglycaemia attenuates cFos expression in corticotrophin-releasing hormone neurons of the paraventricular nucleus of hypothalamus

A. Sankar, T. Khodai, F. Mcewan, K. Tye and S. Luckman

Diabetes, Endocrinology and Gastroenterology, University of Manchester, Manchester, UK

Impaired awareness of hypoglycaemia (IAH) is characterised by reduced behavioural and endocrine counter-regulatory responses (CRR); deficits which develop following repeated hypoglycaemia (RH). These impairments lead patients to experience recurrent severe hypoglycaemic episodes, with associated morbidity and mortality. Treatments remain ineffective and our mechanistic understanding is incomplete. Corticotrophin-releasing hormone (CRH) neurons, in the paraventricular nucleus of the hypothalamus (PVH), modulate responses to physiological stressors, including a single hypoglycaemia (SH) episode. How CRH neurons respond to RH remains unclear.

Eight-week old male *Crh-cre::EYFP* mice were randomised to SH (n = 6), RH (n = 6) or saline (n = 5) groups. RH mice received repeated injections with insulin (1.75 U/kg, subcutaneous) three times a week for four weeks. In parallel, SH and saline groups received repeated injections of 0.9% NaCl. Blood glucose was recorded using tail-tip sampling. On the final injection day, SH and RH groups received insulin. 2 hours after injection, mice were anaesthetised with isoflurane (3-4 ml min⁻¹) and perfused transcardially with 4% paraformaldehyde. 30µm coronal brain sections were used to label EYFP and cFos immunoreactive neurons. Data (mean±SEM) were analysed using one-way ANOVA with Tukey's *post-hoc* comparisons, with significance set at *p* < 0.05. Insulin administration led to a consistent reduction in blood glucose, with a nadir of 3.8 ± 0.4 mmol/l on the final day of injection. In RH mice, the duration of recovery to baseline blood glucose, was observed to increase with successive hypoglycaemic episodes.

SH and RH significantly increased cFos in the PVH compared with saline (565 ± 37 vs 279 ± 31 cells, *p* < 0.005; 432 ± 53 vs 279 ± 31 cells, *p* < 0.05). Within the mid-PVH (bregma -0.82 to -0.94mm AP), which contained the greatest concentration of labelled cells, significantly higher cFos expression was noted with SH than RH (322 ± 15 vs 215 ± 32 cells/section, *p* < 0.05). The medial parvocellular dorsal division (mpd) of the PVH contained the greatest density of CRH neurons (150 ± 12 cells/section). CRH^{mpdPVH} neurons are highly responsive to SH, with 73 ± 5% of neurons expressing cFos. Co-localisation of cFos in CRH^{mpdPVH} neurons, was significantly higher for SH compared with RH (103 ± 15 vs 51 ± 10 cells/section, *p* < 0.05).

We conclude that CRH^{PVH} neurons, particularly in the mpdPVH, are activated by hypoglycaemia. However, with RH, these neurons become less responsive. These findings suggest that RH, akin to other forms of repeated stress, leads to adaptive

changes within stress-responsive neurons. Further understanding of the role of CRH^{PVH} neurons within the central glucose-regulatory network and their response to RH, may explain the CRR deficits which arise in patients with IAH.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC231

PrRP/GPR10 mutant mice are prone to obesity but have low blood pressure

C.H. Feetham and S. Luckman

University of Manchester, Manchester, UK

Prolactin-releasing peptide (PrRP) is expressed in three neuronal populations in the brain: the ventrolateral medulla (VLM), nucleus of the tractus solitarius (NTS) and dorsomedial hypothalamus (DMH) [1]. The receptor for PrRP, GPR10, is expressed at various sites involved in appetite and autonomic regulation. Mice with a conditional knock out for the PrRP peptide (loxSTOPlox [LSL]-*Prnp*) and those with a null mutation in the GPR10 receptor (*Gpr10*^{-/-}) have a late-stage obese phenotype and are hyperphagic [1,2]. In studies using rats, central injection of PrRP increased mean arterial blood pressure (MAP) [3], while hypotensive haemorrhage increased c-Fos expression in PrRP neurones of the VLM and NTS [4].

Baseline blood pressure (BP) recordings were made in conscious, pre-obese LSL-*Prnp* and *Gpr10*^{-/-} mice, using tail-cuff plethysmography, and hypotension was induced by the drug, clonidine (intraperitoneal (IP), 0.1mg/kg). Tracing studies were performed using *Prnp*-cre::EYFP mice injected with cre-dependent antero-grade tracer, AAV-h134r-mCherry, into the VLM. All surgery was performed under anaesthesia with isoflurane (induction 3%; maintenance 1-2%). Mice were later perfused with 4% paraformaldehyde under anaesthesia. Immunohistochemistry using DsRed and EYFP antibodies verified transduction of PrRP^{VLM} neurones. All results given are mean±SEM; unpaired *t*-test.

Gpr10^{-/-} (n=5) mice exhibit significantly lower baseline MAP than wild-type (WT) (n=8) counterparts (66±4mmHg vs 100±9mmHg; p<0.005). When injected with a low dose of clonidine, BP was too low to record by tail-cuff. LSL-*Prnp* (n=6) mice exhibit no difference in baseline MAP compared with WT (n=8) mice (99±9mmHg vs 101±7mmHg). However, when given IP clonidine, LSL-PrRP (n=6) mice were unable to defend their BP under hypotensive challenge, compared with WT (n=9) (relative change in MAP (-23±4mmHg vs -9±2mmHg; p<0.005). Anterograde tracing revealed projections to the bed nucleus of the stria terminalis (BNST), paraventricular hypothalamic nucleus (PVH), DMH, periaqueductal grey (PAG) and dorsal vagal complex. Projections to the BNST, PVH, and PAG were confirmed by retrograde tracing in *Prnp*-cre::EYFP mice.

These results demonstrate that PrRP and GPR10 are involved in cardiovascular regulation. The location of PrRP^{VLM} neurones means that they are likely to be

involved in mediating this role. In this study we have shown that PrRP^{VLM} neurones have strong projections to regions of the brain with known roles in cardiovascular regulation, providing further support to this assertion.

Dodd G.T. & Luckman S.M., 2013. Physiological roles of GPR10 and PrRP Signalling. *Frontiers in Endocrinology*, 4:20 doi: 10.3389/fendo.2013.00020

Dodd, G.T. et al., 2014. The thermogenic effect of leptin is dependent on a distinct population of prolactin-releasing peptide neurons in the dorsomedial hypothalamus. *Cell Metabolism*, 20, pp.1-11.

Samson, W. K., Resch, Z. T. & Murphy, T. C. (2000). 'A novel action of the newly described prolactin-releasing peptides: cardiovascular regulation', *Brain Research*, 858(1), pp. 19-25.

Uchida, K., Kobayashi, D., Das, G., Onaka, T., Inoue, K. & Itoi, K. (2010). 'Participation of the Prolactin-Releasing Peptide-Containing Neurones in Caudal Medulla in Conveying Haemorrhagic Stress-Induced Signals to the Paraventricular Nucleus of the Hypothalamus', *Journal of Neuroendocrinology*, 22(1), pp. 33-42.

This work was funded by the BBSRC.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC232

***Lagenaria bevilflora* fruit reduces free radicals in the carrageenan air-pouch model of inflammation in rats.**

O.T. Oyebanjo¹, A. Ajayi², G.S. Olayinka¹ and A.S. Onasanwo¹

¹Physiology, University of Ibadan, Ibadan, Nigeria and ²Pharmacology, University of Ibadan, Ibadan, Nigeria

Lagenaria bevilflora fruit is used in traditional medicine for the treatment of various ailments such as cold and schistosomiasis in man. Alternative therapies are needed in the treatment of various inflammatory conditions due to the side effects caused by most anti-inflammatory drugs (Onasanwo *et al.*, 2017). This study investigated the anti-inflammatory and anti-oxidant activities of the ethanol extract of *Lagenaria bevilflora* in rats. The ethanol extract of *Lagenaria bevilflora* fruit (EELB) was obtained by cold maceration. The anti-inflammatory activity of EELB was evaluated using the carrageenan induced air-pouch model of inflammation in rats. Briefly, 20ml sterile air was given subcutaneously for 6 days at the back of the rats. 2ml of 1% carrageenan solution was injected into the inflamed pouch on the 6th day (Sedgwick *et al*, 1985). EELB was administered orally for six days. Rats (120-150g; n=5) were anaesthetised with a mixture of ketamine (75mg/kg, i.p.) and xylazine (10mg/kg, i.p.) and sacrificed on the 7th day. The volume of the exudate obtained from the inflamed pouch was measured. Total and differential leukocyte counts were carried out using the exudates. Also, biochemical assays including protein concentration, malondialdehyde (MDA), catalase, nitrite and glutathione (GSH) were evaluated from the exudates of the inflamed pouch. Tumor necrosis factor-alpha (TNF-α)

and interleukin-6 (IL-6) cytokines were evaluated using enzyme-linked immunosorbent assay (ELISA). Values are expressed as Mean $\pm$ SEM. Data were analyzed by one-way analysis of variance followed by Newman-Keuls multiple comparison post-hoc tests using Graph-pad Prism software version 5.00 for Windows, Graph pad software, San Diego California USA. P values lower than 0.05 were considered statistically significant. EELB (50, 100 and 200 mg/kg) significantly reduced leukocyte count (33.02 $\pm$ 2.18, 30.62 $\pm$ 4.66, 30.38 $\pm$ 0.6 vs 42.41 $\pm$ 2.77; p<0.05), monocyte count (0.89 $\pm$ 0.07, 0.8 $\pm$ 0.07, 0.91 $\pm$ 0.02, vs 1.41 $\pm$ 0.12; p<0.05) neutrophils count (23.61 $\pm$ 2.29, 18.98 $\pm$ 2.56, 19.41 $\pm$ 0.50 vs 30.9 $\pm$ 2.77; p<0.05) and also exudate volume (2.48 $\pm$ 0.19, 2.6 $\pm$ 0.21, 2.44 $\pm$ 0.16 vs 3.2 $\pm$ 0.08; p<0.05). EELB (50, 100 and 200 mg/kg) significantly reduced protein concentration (215.7 $\pm$ 17.72, 233.7 $\pm$ 17.3, 185.5 $\pm$ 10.18 vs 308.0 $\pm$ 14.42; p<0.05) and nitrite levels (16.45 $\pm$ 2.83, 15.75 $\pm$ 1.56, 14.40 $\pm$ 1.49 vs 32.85 $\pm$ 1.31; p<0.05) in the exudates. Also, EELB (200 mg/kg) significantly reduced MDA (36.73 $\pm$ 3.09 vs 54.31 $\pm$ 4.08; p<0.05), TNF- α (174.4 $\pm$ 29.22 vs 521.8 $\pm$ 88.34; p<0.05) and IL-6 (272 $\pm$ 15.94 vs 392 $\pm$ 17.69; p<0.05) and elevated catalase (15.30 $\pm$ 0.56 vs 11.76 $\pm$ 0.65; p<0.05) significantly. EELB shows therapeutic potentials in reducing oxidative stress and inflammation as seen in various diseases such as rheumatism and arthritis.

Onasanwo *et al.*, (2017). *J Inter. Ethnopharmacology*. 2(6): 192-198.

Sedgwick *et al.*, (1985). *J Exp Pathol*. 66: 455-464

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC233

The effect of photoperiod and high fat diet on the cognitive response in photoperiod-sensitive F344 rats

G. Helfer¹ and S. Mclean²

¹*School of Chemistry and Biosciences, University of Bradford, Bradford, UK and* ²*School of Pharmacy and Medical Sciences, University of Bradford, Bradford, UK*

Seasonal animals have evolved diverse variations in physiology and behaviour to accommodate yearly changes in environmental and climatic changes. These changes are initiated by changes in photoperiod (daylength). Here we investigated the effect of photoperiod and high fat diets on the cognitive deficits, as measured by novel object recognition, in the photoperiod sensitive F344 rat, which exhibits marked natural changes in growth, body weight and food intake in response to photoperiod. 32 male juvenile F344 rats were housed on either long day (LD, 16 hrs light per day) or short day (SD, 8 hrs light per day) photoperiod conditions and fed either a high fat diet (HF, 45% fat by kcal) or a chow diet (nutrient matched). 8 rats/group were tested in the novel object recognition test before photoperiod and diet intervention and re-tested after 28 days of intervention. Briefly, rats were permitted to explore 2 identical objects for 3 minutes (acquisition trial); they were

then taken out of the arena and returned to their home cage for a 1 min inter-trial interval (ITI). This was followed by a 3 min retention trial where rats were exposed to a familiar object and a novel object. The exploration time spent at the objects was recorded by an experimenter blind to the intervention group. In both tests during the acquisition trials there was no significant difference in exploration levels of the left and right objects in any of the groups. Before the intervention, all 4 groups showed a significant increase in exploration of the novel object compared to the familiar object: SD-chow (11.6 ± 1.7 sec vs. 5.9 ± 1.5 , $P < 0.05$), SD-high fat (11.6 ± 2.4 sec vs. 7.6 ± 2.3 sec, $P < 0.05$), LD-chow (9.9 ± 2.3 sec vs. 3.6 ± 0.9 sec, $P < 0.05$), LD-high fat (8.1 ± 1.5 sec compared to 3.8 ± 0.6 , $P < 0.01$). However, following the photoperiod and diet interventions the retention trial revealed that only rats in the LD-chow group explored the novel object significantly more than the familiar object (8.6 ± 2.5 sec vs. 5.3 ± 1.6 sec, $P < 0.05$), whereas all the other 3 groups showed no significant preference. These results suggest that changing rats to SD impairs their ability to differentiate between the normal and familiar objects regardless of whether they eat chow or a high fat diet. The rats on LD-chow were still able to perform the task therefore their short-term recognition memory remained intact, whereas the high fat diet in the LD group induced an impairment in memory. These findings suggest that rats exposed to long photoperiod have different cognitive responses to rats exposed to short photoperiod and high fat diet.

We thank The Physiological Society for funding this research.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC234

Amygdalar output in Depression: The Stria Terminalis and HPA axis reactivity in Major Depressive Disorder.

L. Tobin-Schnittger^{1,5}, A. Nasa^{1,2}, A. Shah^{1,3}, C. Kennedy^{1,5}, E. Roman^{1,3}, D.W. Roddy^{1,5}, E. O'Hanlon¹, K. Levins⁴ and V. O'Keane¹

¹Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland, ²School of Medicine, Trinity College Dublin, Dublin, Ireland, ³School of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland, ⁴Department of Anaesthetics, Intensive Care and Pain Medicine, St Vincents University Hospital, Dublin, Ireland and ⁵Department of Physiology, University College Dublin, Dunlin, Ireland

Major Depressive Disorder (MDD) is one of the leading causes of global disability, yet the underlying biological mechanisms for this disorder are not well understood. The amygdala, as the key emotion maker in the brain, is known to have a role in MDD. Amygdalar dysfunction has been hypothesised to drive the aberrant hypothalamic-pituitary-adrenal (HPA) stress responses that are known in the disorder. The stria terminalis (ST) as the principal white matter tract connecting the amygdala to the hypothalamus may also have a role in MDD.

15 patients with MDD and 20 matched healthy controls underwent advanced diffusion-weighted magnetic resonance imaging at Trinity College Institute of Neuroscience. Using constrained spherical deconvolution tractography and a novel anatomically-driven protocol, the ST was isolated. Morning salivary cortisol samples (0, 30 and 60 minutes after waking) were obtained from all participants and cortisol concentrations determined using liquid chromatography-mass spectroscopy. The cortisol awakening response (CAR) was calculated using formulae for the area under the curve.

Analysis of covariance (ANCOVA) between depressed and controls revealed no difference for whole ST metrics. Multivariate ANCOVA along discrete sections of the tract showed increased mean and radial diffusivity (p-values of $0.019 + 0.036$ and $0.022 + 0.041$ respectively) in the portion of the ST close to the amygdala in MDD patients. Split-group partial correlations revealed an inverse relationship of the length (p-value of 0.003) and volume (p-value of 0.032) of the left ST with the CAR. Similarly, mean diffusivity, radial diffusivity and fractional anisotropy showed associations with the CAR along key sections of the tract.

These novel findings suggest that changes in the ST may account for neuroendocrine disturbances in MDD. Alterations in diffusion metrics may reflect diverse pathology including axonal degeneration and demyelination along sections of the tract. These findings suggest that the connection between the amygdala and hypothalamus may be functionally altered resulting in abnormal cortisol secretion and stress responses in MDD.

We would like to acknowledge all the patients and volunteers for their time and effort in taking part in the study. We'd also like to thank the staff at the Trinity College Institute of Neuroscience (TCIN) for their expertise and assistance.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC235

Role of glucocorticoids in the transmission of gestational stress signals from mother to foetus

S. Ying^{1,2}, J. Fernandes^{1,2} and P. Brunton^{2,1}

¹Roslin Institute, The University of Edinburgh, Edinburgh, UK and ²Centre for Discovery Brain Sciences, The University of Edinburgh, Edinburgh, UK

Chronic maternal social stress is known exert negative programming effects in the developing foetus (Brunton and Russell, 2010). The mechanisms by which this occurs remains unclear, although a long-held hypothesis contends that excess maternal glucocorticoid transfer to the foetus may play a potential role (Seckl and Meaney, 2004). However, a “protective barrier” in the placenta exists, due to the presence of the enzyme 11 β HSD2, which converts corticosterone (CORT) into the inactive metabolite 11-dehydrocorticosterone (11-DHC). 11 β HSD2, together

with 11 β HSD1 (which catalyses the reverse reaction of reactivating CORT), is also expressed in other tissues such as the foetal brain, and both enzymes act in tandem to control local glucocorticoid concentrations.

Here, we wanted to find out if maternal glucocorticoids are directly transferred from the maternal to foetal circulation following chronic maternal stress, and if the placental 11 β HSD2 “protective barrier” may be compromised. Pregnant rats (n=7) were exposed to 10 min social stress each day from gestational day (GD) 16 to 20 via the modified resident-intruder paradigm. Rats were killed on GD20 immediately after the final stressor. CORT and 11-DHC concentrations in the maternal and foetal plasma, placenta, foetal brain and liver were quantified using liquid chromatography-mass spectrometry. *In situ* hybridisation was also performed to quantify placental 11 β HSD2 as well as glucocorticoid receptor (GR) mRNA expression, which may indicate placental glucocorticoid sensitivity. In the foetal hippocampus, mRNA expression of 11 β HSD2 and 11 β HSD1 was also investigated.

Both plasma CORT and 11-DHC concentrations were dramatically increased in stressed pregnant dams compared to controls. Maternal stress significantly increased circulating CORT concentrations only in the female foetuses but not males, albeit to a smaller extent as compared to the maternal changes. CORT concentrations were significantly greater in the liver of both male and female foetuses following maternal stress; but no changes were detected in the foetal brain. 11-DHC concentrations were not different in foetal plasma nor brain/liver. In the placenta, stress resulted in elevated CORT concentrations and junctional zone 11 β HSD2 mRNA expression only in males, but not females. There were no changes in placental GR mRNA expression, nor foetal hippocampal 11 β HSD1 and 11 β HSD2 mRNA expression in either sex.

In conclusion, although repeated social stress increases the production of maternal CORT, the placental barrier appears intact, especially in the males, and the direct transfer of CORT from the maternal to foetal circulation is minimal. The data suggest maternal glucocorticoids are not directly involved in transmitting the programming effects of maternal stress to the foetuses, but may act in an indirect manner.

Brunton and Russell, 2010. Prenatal Social Stress in the Rat Programmes Neuroendocrine and Behavioural Responses to Stress in the Adult Offspring: Sex-Specific Effects. *J Neuroendocrinology*.

Seckl and Meaney, 2004. Glucocorticoid programming, *Ann N Y Acad Sci*.

We would like to thank Prof. Karen Chapman and Prof. Megan Holmes (Queen's Medical Research Institute, University of Edinburgh) for providing us with the plasmids containing rat 11 β HSD1 and 11 β HSD2 cDNA. Funding sources: University of Edinburgh Principal's Career Development Studentship (SY), British Society of Neuroendocrinology.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC236

High Frequency Spinal Cord Stimulation (HF-SCS) in a Sub-Acute Animal Model of Spinal Cord Injury (SCI)

A.F. DiMarco¹ and K.E. Kowalski²

¹Department of Physical Medicine and Rehabilitation, Case Western Reserve University / MetroHealth Medical Center, Cleveland, OH, USA and ²Medicine / Physical Medicine & Rehabilitation, Case Western Reserve University/MetroHealth Medical Center/Louis Stokes Cleveland VAMC, Cleveland, OH, USA

Objective: In previous studies, HF-SCS applied at the T2 spinal level was shown to result in physiologic activation of the both the diaphragm and inspiratory intercostal muscles in C2 spinal sectioned dogs. While the bulbo-spinal fibers were cut, they likely survived given the short duration of the day long acute experiments and that the mechanism of inspiratory muscle activation may have involved stimulation of these fibers. Since these fibers would not be viable in patients with chronic ventilator dependent SCI, it is important to determine if HF-SCS is effective following degeneration of these fibers.

Methods: In 2 anesthetized with pentobarbital sodium (initially 25mg/kg IV and supplemented with 1-2 mg/kg as required), C2 paralyzed, intubated and mechanically ventilated dogs, an electrode lead was positioned on the ventral epidural surface of the spinal cord at the T2 level to activate the inspiratory muscles. Animal temperature, end-tidal PCO₂ and oxygen saturation were continuously monitored. The effectiveness of HF-SCS in generating inspired volume (V) and negative airway pressures (P) was evaluated over a period of 5 days during which time the bulbo-spinal fibers would have degenerated. Since the effectiveness of HF-SCS may be adversely affected by deterioration in the condition of the animal, low frequency (50Hz) SCS (LF-SCS) was also performed and served as a control.

Results: All vital signs, oxygen saturation and end-tidal PCO₂ remained stable over the 5-day period. V and P also remained stable over the study period. For example, V and P were 771±25ml and 64±1cmH₂O with HF-SCS (3mA, 300Hz) during the initial and 674±59ml and 63±5cmH₂O and final 6 hours on day 5. Comparable values during LF-SCS (8mA, 50Hz) were 467±12ml and 48±1cmH₂O during the initial and 397±16ml and 40±1cmH₂O final 6 hours on day 5.

Conclusion: Since V and P in response to HF-SCS remained stable over a 5-day period following which the bulbo-spinal fibers would have degenerated, the mechanism of HF-SCS does not depend upon the viability of these tracts. HF-SCS therefore may be a useful method to restore ventilation in ventilator dependent tetraplegics.

DiMarco AF, Kowalski KE. High frequency spinal cord stimulation of inspiratory muscles in dogs: a new method of inspiratory muscle pacing. *J Appl Physiol* 107:662-669, 2009. PMID: PMC4073921.

DiMarco AF, Kowalski KE. Intercostal muscle pacing with high frequency spinal cord stimulation in dogs. *Respir Physiol Neurobiol* 171:218-224, 2010. PMID: PMC2874655.

DiMarco AF, Kowalski KE. Distribution of electrical activation to the external intercostal muscles during high frequency spinal cord stimulation in dogs. *J Physiol* 589:1383-1395, 2011. PMID: PMC3082098.

DiMarco AF, Kowalski KE. Spinal pathways mediating phrenic activation during high frequency spinal cord stimulation. *Respir Physiol Neurobiol* 186:1-6, 2013. PMID: PMC3602409.

DiMarco AF, Kowalski KE. Electrical Activation to the Parasternal Intercostal Muscles during High Frequency Spinal Cord Stimulation in Dogs. *J Appl Physiol* 118:148-155, 2015. PMID: PMC4297776.

Support: NIH-R01NS105785 and MetroHeath Foundation

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC237

Activation of the expiratory muscles via lower thoracic high frequency spinal cord stimulation (HF-SCS) in intact non-anesthetized animals.

A.F. DiMarco² and K.E. Kowalski¹

¹*Medicine / Physical Medicine & Rehabilitation, Case Western Reserve University/ MetroHealth Medical Center/Louis Stokes Cleveland VAMC, Cleveland, OH, USA and*

²*Department of Physical Medicine and Rehabilitation, Case Western Reserve University, MetroHealth Medical Center, Cleveland, OH, USA*

Objective: Lower thoracic SCS been shown to restore an effective cough mechanism in subjects with complete spinal cord injury. Unfortunately, the high stimulus amplitude requirements and potential activation of pain fibers significantly limits this application in subjects with intact sensation. Recent studies have shown that the expiratory muscles can also be effectively activated via low stimulus currents (1mA) but with high stimulus frequencies (500Hz) in anesthetized animals. The purpose of the present study was to assess the response to HF-SCS, in intact, non-anesthetized animals (pigs).

Methods: During an initial surgical procedure performed under sterile conditions (pigs were pre-medicated with telazol (1.5mg/kg IM), xylazine (1.5mg/kg IM), and ketamine (1.5mg/kg IM) and anesthetized with pentobarbital (1-2mg/kg/hr)) a stimulating disc electrode was placed on the dorsal surface of the spinal cord at the T9 level. 10-14 days post-operatively, electrical stimulation was applied 2-3 times a day to activate the expiratory muscles. While awake, expiratory muscle contraction was assessed during HF-SCS via abdominal muscle EMG and inductance plethysmography (IP) via bands positioned over the abdominal wall. Electrocardiogram and heart rate were also monitored. While anesthetized during a terminal procedure, airway pressure generation and abdominal movement were assessed. **Results:** During HF-SCS in the awake state, there was marked movement of the abdominal wall as assessed by IP and electrical activation of the external oblique muscles, which was positively correlated with stimulus amplitude (correlation

coefficients greater than 0.95 for both). HF-SCS was not associated with any changes in heart rate or vocalization. During a terminal procedure under general anesthesia, mean airway pressure generation during HF-SCS at 0.8, 1 and 1.5mA were 31 ± 6 , 46 ± 2 and 65 ± 2 cmH₂O respectively. The positive correlation between stimulus amplitude and excursion of the abdominal wall was not significantly different compared to the awake state.

Summary: HF-SCS applied to awake, non-anesthetized animals results in contraction of the abdominal muscles similar to that observed in anesthetized animals. The lack of changes in heart rate and vocalization suggests that HF-SCS was well tolerated and not painful.

Conclusion: Lower thoracic HF-SCS may be a useful method to restore an effective cough in patients with intact sensation.

DiMarco AF, Kowalski KE, Geertman RT, Hromyak DR. Lower thoracic spinal cord stimulation to restore cough in patients with spinal cord injury: results of a National Institutes of Health-sponsored clinical trial. Part I: methodology and effectiveness of expiratory muscle activation. Arch Phys Med Rehabil 90:717-725, 2009. PMCID: PMC2813808.

DiMarco AF, Kowalski KE, Geertman RT, Hromyak DR, Frost FS, Creasey GH, Nemunaitis GA. Lower thoracic spinal cord stimulation to restore cough in patients with spinal cord injury: results of a National Institutes of Health-sponsored clinical trial. Part II: clinical outcomes. Arch Phys Med Rehabil 90:726-732, 2009. PMCID: PMC2809374

Kowalski KE, Romaniuk JR, Brose S, Richmond MA, Kowalski T, DiMarco AF. High Frequency Spinal Cord Stimulation – New Method to Restore Cough. Respir Physiol Neurobiol 232:54-56, 2016. PMID: 27395446.

Kowalski KE, Romaniuk JR, Kowalski T and DiMarco AF. Effects of Expiratory Muscle Activation via High Frequency Spinal Cord Stimulation. J Appl Physiol 123:1525-1531, 2017. PMID: 28935824.

Kowalski KE, Romaniuk JR, Kirkwood PA, and DiMarco AF. Inspiratory Muscle Activation via Ventral Lower Thoracic High Frequency Spinal Cord Stimulation. J Appl Physiol. PMID: 30763163 DOI: 10.1152/jappphysiol.01054.2018. Feb 14, 2019.

Support: NIH-R01NS105785 and MetroHealth Foundation

Non-Financial Disclosure Statement: Dr. Anthony DiMarco owns patent rights for technology utilized in this research study.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC238

Effects of High Frequency Spinal Cord Stimulation (HF-SCS) Applied to the Ventral Surface of the Spinal Cord

K.E. Kowalski³, J.R. Romaniuk⁴, P.A. Kirkwood¹ and A.F. DiMarco²

¹Neurology, University College London, London, UK, ²Physical Medicine and Rehabilitation, Case Western Reserve University, MetroHealth Medical Center, Cleveland, OH, USA, ³Medicine/Physical Medicine and Rehabilitation, VA Medical Center/ Case Western Reserve University/ MetroHealth Medical Center, Cleveland, OH, USA and ⁴Research, VA Medical Center, Cleveland, OH, USA

Objective: HF-SCS applied to the ventral surface of the upper thoracic spinal cord (T2 level) results in physiologic activation of both the diaphragm and inspiratory intercostal muscles. The purpose of the present study was to evaluate the effects of HF-SCS (500Hz, 1mA) applied to the ventral surface of the entire thoracic spinal cord.

Methods: In 4 anesthetized with pentobarbital sodium (initially 25mg/kg IV and supplemented with 1-2 mg/kg as required) dogs, three eight plate stimulation leads, were inserted epidurally and positioned on the ventral surface of the spinal cord via laminectomies at the T6 and T10 levels. Changes in airway pressure (P) were assessed over different regions of the thoracic spinal cord following electrical stimulation (HF-SCS: 500 Hz, 1 mA, 0.2ms) at each electrode lead in separate trials, under conditions of airway occlusion and hyperventilation induced apnea.

Results: HF-SCS (500Hz, 1mA) resulted in negative P generation over the ventral surface of the entire thoracic spinal cord. Negative P generation was most pronounced at the T2 level (60 ± 2 cmH₂O) and T9 levels (46 ± 8 cmH₂O). Further examination of HF-SCS at the T9 level demonstrated that maximum negative P was dependent upon low stimulus currents (1-3 mA) and high stimulus frequencies (>300 Hz). Both the diaphragm and inspiratory intercostal muscles contributed to negative P generation.

Conclusion: The unique findings of the present investigation include the demonstration that spinal circuits which have connections to the inspiratory motoneuron pools exist over the ventral surface of the entire thoracic spinal cord. We postulate that responses are mediated, in part, by stimulation of the previously described intercostal to phrenic excitatory reflex. The large negative P generated at the T9 level suggests that HF-SCS in this region of the spinal cord may be a useful method to restore breathing in ventilator dependent tetraplegics.

DiMarco AF, Kowalski KE, Geertman RT, Hromyak DR. Lower thoracic spinal cord stimulation to restore cough in patients with spinal cord injury: results of a National Institutes of Health-sponsored clinical trial. Part I: methodology and effectiveness of expiratory muscle activation. Arch Phys Med Rehabil 90:717-725, 2009. PMID: PMC2813808.

DiMarco AF, Kowalski KE, Geertman RT, Hromyak DR, Frost FS, Creasey GH, Nemunaitis GA. Lower thoracic spinal cord stimulation to restore cough in patients with spinal cord injury: results of a National Institutes of Health-sponsored clinical trial. Part II: clinical outcomes. Arch Phys Med Rehabil 90:726-732, 2009. PMID: PMC2809374.

Kowalski KE, Romaniuk JR, Brose S, Richmond MA, Kowalski T, DiMarco AF. High Frequency Spinal Cord Stimulation – New Method to Restore Cough. *Respir Physiol Neurobiol* 232:54-56, 2016. PMID: 27395446.

Kowalski KE, Romaniuk JR, Kowalski T and DiMarco AF. Effects of Expiratory Muscle Activation via High Frequency Spinal Cord Stimulation. *J Appl Physiol* 123:1525-1531, 2017. PMID: 28935824.

Kowalski KE, Romaniuk JR, Kirkwood PA, and DiMarco AF. Inspiratory Muscle Activation via Ventral Lower Thoracic High Frequency Spinal Cord Stimulation. *J Appl Physiol*. PMID: 30763163 DOI: 10.1152/jappphysiol.01054.2018. Feb 14, 2019.

Support: Merit Review Award I01-RX-001488 from the U.S. Department of Veterans Affairs, Rehabilitation Research and Development Service.

Disclosure: Dr. DiMarco holds two U.S. patents for technology related to the content of this abstract: Method and Apparatus for Electrical Activation of the Expiratory Muscles to Restore Cough (5,999,855) and Bipolar Spinal Cord Stimulation to Activate the Expiratory Muscles to Restore Cough (8,751,004). Drs. DiMarco and Kowalski hold the U.S. patents for technology related to the content of this abstract: Respiratory Muscle Activation by Spinal Cord Stimulation (8,352,036).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC239

What if Parkinson's Disease Symptoms are cortical?

G.W. Arbuthnott

Brain Mechanisms for Behaviour, OIST Graduate University, Onna-son, Japan

No one doubts that the cause of Parkinson's Disease is the death of the dopamine neurons in the *substantia nigra-pars compacta*. Indeed, all the major treatments were designed to replace the missing neurotransmitter; until deep brain stimulation (DBS) came along. That breakthrough used the hypothesized basal ganglia circuit to interfere in a constructive way with the consequences of the dopamine loss.

While we explored the effects of stimulation in the subthalamic nucleus in animal models of the disease symptoms, it was obvious that as well as interfering in the basal ganglia, DBS also had dramatic actions in the cortex, (Li *et al.*, 2007). Each stimulus had a considerable chance of exciting cortical layer V cells antidromically along the axon that passed just under the electrode in isofluorine anaesthetised rats. Of course, this was in a small rodent – but the same phenomenon had already been described in patients. Single pulses at the subthalamic electrode induced a short latency (~3ms) positive going wave in the EEG: the signature of antidromic activation of deep cortical neurons.

In freely moving rats we first showed that recovery from akinesia depended on an antidromic response (Dejean *et al.*, 2009) in cortex, and then with other

collaborators we were able to show that the stochastic activation of these layer 5 cells was able to disrupt the correlation of cortical neuron firing with the beta wave in the EEG (Li *et al.*, 2012). Such disruption is a signature of curative actions on the disease symptoms.

Finally, the final output from the basal ganglia arrives back at the cerebral cortex (Arbuthnott *et al.*, 1990), is this where the symptoms originate? If it is, can we develop methods to fix the symptoms?

References

Arbuthnott, G., Macleod, N., Maxwell, D. & Wright, A. (1990) Distribution and synaptic contacts of the cortical terminals arising from neurons in the rat ventromedial thalamic nucleus. *Neuroscience*, 38, 47-60.

Dejean, C., Hyland, B. & Arbuthnott, G. (2009) Cortical Effects of Subthalamic Stimulation Correlate with Behavioral Recovery from Dopamine Antagonist Induced Akinesia. *Cereb. Cortex*, 19, 1055-1063.

Li, Q., Ke, Y., Chan, D.C., Qian, Z.M., Yung, K.K., Ko, H., Arbuthnott, G.W. & Yung, W.H. (2012) Therapeutic deep brain stimulation in Parkinsonian rats directly influences motor cortex. *Neuron*, 76, 1030-1041.

Li, S., Arbuthnott, G.W., Jutras, M.J., Goldberg, J.A. & Jaeger, D. (2007) Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation. *J Neurophysiol*, 98, 3525-3537.

Arbuthnott, G., Macleod, N., Maxwell, D. & Wright, A. (1990) Distribution and synaptic contacts of the cortical terminals arising from neurons in the rat ventromedial thalamic nucleus. *Neuroscience*, 38, 47-60.

Dejean, C., Hyland, B. & Arbuthnott, G. (2009) Cortical Effects of Subthalamic Stimulation Correlate with Behavioral Recovery from Dopamine Antagonist Induced Akinesia. *Cereb. Cortex*, 19, 1055-1063.

Li, Q., Ke, Y., Chan, D.C., Qian, Z.M., Yung, K.K., Ko, H., Arbuthnott, G.W. & Yung, W.H. (2012) Therapeutic deep brain stimulation in Parkinsonian rats directly influences motor cortex. *Neuron*, 76, 1030-1041.

Li, S., Arbuthnott, G.W., Jutras, M.J., Goldberg, J.A. & Jaeger, D. (2007) Resonant antidromic cortical circuit activation as a consequence of high-frequency subthalamic deep-brain stimulation. *J Neurophysiol*, 98, 3525-3537.

This work has developed over several years with support from FastTrack Award from the Michael J. Fox Foundation to D. Jaeger, a Grant from the New Zealand Neurological Foundation, and support from the Japanese government to OIST.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Lesions in the *Xenopus laevis* tadpole hindbrain reveal neural substrates for simple motor decision-making.

G. Messa and S. Koutsikou

Medway School of Pharmacy, University of Kent, Chatham Maritime, UK

Animal survival depends heavily on making the 'right' motor decisions. For the hatchling *Xenopus laevis* tadpole this is the choice between to swim or not swim (1). Tadpoles detect external stimuli through receptors in the skin. Sensory information is then transferred via sensory pathway neurons to the brain where it is processed until the motor decision is made. The tadpole's sensory pathway as well as the key Central Pattern Generator neurons, which drive swimming, are well-known (2). We have identified long and variable delays (~50-100ms) between firing in the sensory pathway neurons and the first spike in reticulospinal descending interneurons (dINs, 3). The latter signals the start of swimming. Such long and variable delays are key features of decision-making processes within the CNS (e.g. delays to eye movement; 4). However, the underlying neuronal circuitry is not well understood. In the tadpole, we proposed an additional type of neuron (hindbrain extension neurons; hexNs), presynaptic to dINs, which should spike at short latencies and show multiple firing (3,5), thus introducing variability and extending the delay to swim.

Here, we aim to determine the location(s) of hexNs and spatial distribution of their sensory signal that allows the tadpole to make motor decisions in a timely manner. In the behaving tadpole (stage 37/38), we monitored the latency to the start of swimming in response to trunk skin touch. Latency data were compared between control tadpoles and those with hindbrain lesions. Removing the rostral part of the brain and/or separating the left and right side of the hindbrain led to an overall significant increase in latency to swim in lesioned animals ($p=0.006$, Kruskal-Wallis test). Median latency to swim increased from 109 ms in control animals ($n=29$) to (i) 201 ms in tadpoles lesioned along the midbrain-hindbrain border ($n=32$), (ii) 163 ms in animals with transverse hindbrain lesion behind the otic capsule ($n=32$), (iii) 150 ms in animals with lesions along the hindbrain's midline ($n=30$).

In addition, the above hindbrain lesions led to a change in the tadpole's side preference for swim initiation. Control animals ($n=33$) showed 70% preference for the contralateral (contra) vs ipsilateral (ipsi) side (23 contra vs 10 ipsi responses). Tadpoles with rostral hindbrain transverse lesions ($n=30$) showed enhanced preference (87%) for the contralateral side (26 contra vs 4 ipsi responses). Similar increased preference for the contralateral side (85%) was observed in animals with midline hindbrain lesions ($n=30$; 24 contra vs 6 ipsi responses).

Our novel data indicate that the hexN population must be highly distributed across the hindbrain and essential to making motor decisions in a timely manner. Furthermore, hindbrain lesions tested 'forced' the animal to rely on a hard-wired mechanism, which favors the contralateral swim response.

Borisyuk R, Merrison-Hort R, Soffe SR, Koutsikou S, Li WC. To swim or not to swim: A population-level model of *Xenopus* tadpole decision making and locomotor behaviour. *Bio Systems*. 2017;161:3-14.

Roberts A, Li WC, Soffe SR. How neurons generate behavior in a hatchling amphibian tadpole: an outline. *Front Behav Neurosci*. 2010;4:16.

Koutsikou S, Merrison-Hort R, Buhl E, Ferrario A, Li WC, Borisyuk R, et al. A simple decision to move in response to touch reveals basic sensory memory and mechanisms for variable response times. *J Physiol*. 2018;596(24):6219-33.

Carpenter, R. H. S. A neural mechanism that randomises behaviour. *J Consciousness Studies* 1999; 6(1):13-22.

Roberts A, Borisyuk R, Buhl E, Ferrario A, Koutsikou S, Li WC, et al. The decision to move: response times, neuronal circuits and sensory memory in a simple vertebrate. *Proc Biol Sci*. 2019;286(1899):20190297.

This work was sponsored by a University of Kent Vice-Chancellor scholarship.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC241

Increased Expression of Transient Receptor Potential Vanilloid 1 Receptors in Zymosan-induced Inflammatory Pain Model in Absence Epileptic WAG/Rij Rats

A. Ayar, E. Saral, Z. Sahin, A. Kurt and A. Malkoc

Physiology, Karadeniz Technical University, Trabzon, Turkey

Considering the evident correlation between epileptogenesis and brain inflammation, and transient receptor potential vanilloid 1 (TRPV1) receptors involvement in inflammatory pain; we aimed at investigating possible involvement of the TRPV-1 receptors in acute pain in genetic model of absence epileptic WAG/Rij rats which has been shown to have increased nociceptive pain sensitivity (1). We also analyzed levels of IL-1 β which is a pro-inflammatory cytokine which has been implicated to be involved in inflammatory conditions including inflammatory pain. Adult male WAG/Rij rats were utilized for the study. Tissue samples were collected from peripheral and central pain pathways involving dorsal root ganglion (DRG), trigeminal ganglia (TG), reticular thalamic nucleus (RTN) and anterior cingulate cortex (ACC). Expression and levels TRPV1 and IL-1 beta were assessed by real-time polymerase chain reaction (RT-PCR) method. The PCR products are expressed in relative to respective control values.

Following stimulation with zymosan, expression of TRPV1 was determined to be significantly higher in TG (1.7 times, $P < 0.05$), RTN (1.6 times, $P < 0.05$) and ACC (3.4 times, $P < 0.05$) but the increase in DRG was not statistically significant ($n = 8$, for each).

In addition, the expression of IL-1 β was also significantly higher in TG, RTN, ACC and DRG ($n = 8$).

Data from this study provide evidence for a prominent acute inflammatory response and TRPV1 receptors plays a significant role in inflammatory hyperalgesia in absence epileptic rat model.

1) Velioglu SK, Gedikli O, Yildirim M, Ayar A. Epilepsy may cause increased pain sensitivity: Evidence from absence epileptic WAG/Rij rats. *Epilepsy Behav* 2017;75:146-150.

This study was supported by a scientific grant from TÜBİTAK (The Scientific and Technological Research Council of Turkey, Project No 214S206).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC242

Anti-depressant-like potentials of ethanol extract of the *Spondias mombin* in male mice

L.D. Adedayo^{1,2}

¹Physiology, Bowen Univeristy Iwo, Iwo, Nigeria and ²Physiology, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Depression is the most common psychiatric disorder and among the most common illness worldwide. Multiple neurotransmitters and components of the brain are involved with the disorder of depression. *Spondias mombin* (Linn) belongs to the family *Anacardiaceae* antiepileptic, antimicrobial, antipsychotic, antibacterial, antifungal, and antiviral properties. However, studies on antidepressant-like potential of *Spondias mombin* in mice This study investigated the antidepressant activity of the ethanol extract of *Spondias mombin* (ESM) and its mechanism(s) of action in mice.

Forty (40) male mice weighing between 18-24g were randomly divided into eight groups (n=5) of two phases. In the first phase, Group 1 received 10ml/kg of distilled water, Group 2 was given 5mg/kg of ESM, Group 3 received 10mg/kg of ESM and Group 4 was administered with Imipramine 10mg/kg. Group 1-3 received the administration orally and were pretreated one hour while Group 4 was administered intraperitoneally and pretreated 30 minutes before the experimentation, The second phase, Group 5-8 were selected and administered with Prazosin (1mg/kg), Haloperidol (1mg/kg), and Propanolol (10mg/kg) and 10mg/kg ESM respectively. The various blockers were given 15 minutes before the administration of the effective dose (10mg/kg ESM) in this phase. Anti-depressant activities were investigated using Tail Suspension Test (TST) and Force Swimming Test (FST) paradigm. The data were analyzed using GraphPad Prism.

The results showed that 10mg/kg possesses anti-depressant potentials with rapid reduction in immobility time (167.7±22.37 vs 88.5±10.52, and 183.7 ± 25.46 vs 80.5 ± 12.43 in TST and FST, p<0.05 respectively). The mechanisms of the antidepressants potentials of the ethanol extract of the *Spondias mombin* reverse the

antagonistic effects of prazosin (72 ± 1.21 vs 182.2 ± 4.89), propranolol (72 ± 1.21 vs 168 ± 1.705), haloperidol (72 ± 1.21 vs 206.4 ± 2.02) $p < 0.05$ respectively in the Tail Suspension Test paradigms.

The study revealed that ethanol extract of *Spondias mombin* possesses anti-depressant-like properties and that the mechanism of action may seem to act via α -1 adrenergic, beta-adrenergic and dopaminergic pathways.

Key words: Anti-depressants, *Spondias mombin*, Tail Suspension Test, Force Swimming Test

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC243

Monocytic-Wnt5a Dependent Impaired Revascularisation Is SRPK1 Dependent

S. Bhalla¹, K. Rajaji¹, C. Allen¹, Z. Blackley¹, J. Batson², J. Morris³ and D.O. Bates^{1,2}

¹Tumour and Vascular Biology Laboratories, The University of Nottingham, Nottingham, UK, ²Exonate Ltd, Cambridge, UK and ³School of Chemistry, University of New South Wales, Sydney, UK

Purpose: Vascular insufficiency in peripheral arterial disease (PAD) reduces limb blood flow and results in tissue ischemia. In response, circulating monocytes produce vascular endothelial growth factor (VEGF-A). Patients with PAD and mouse models of metabolic ischemic disease have increased Wntless-Type MMTV Integration Site Family 5a (Wnt5a) activity and decreased Wnt5a modulator SFRP5. This has been shown to increase the anti-angiogenic VEGF isoform in circulating monocytes from both humans and mice. VEGF splicing has previously been shown to be regulated by phosphorylation of splicing factor SRSF1 by the kinase, SRPK1. To determine whether this was the case in monocytes in mice with ischemia, we investigated the effect of SRPK1 inhibition with SPHINX31 on revascularisation after ischemia in mice with Wnt5A overexpression in monocytes.

Methods: All animal procedures met the requirements dictated by the Animals (Scientific Procedures) Act 1986/ASPA. 21 transgenic mice (C57/BLJ, 10-12 weeks, both genders) were used. Wildtype (WT) littermates and Wnt5a gain of function (LysM-Wnt5a^{GOF}) were subjected to left femoral artery ligation under anaesthesia with 2% isoflurane. Moor FLPI-2 laser perfusion speckle camera was performed on day 0 (pre- and post-surgery), 3, 7, 14 and 21 to measure the blood flow. LysM-Wnt5a^{GOF} received SPHINX31 (0.8mg/kg, i.p.) biweekly. After 21 days, mice were euthanised and the whole body was fixed perfused with 4% paraformaldehyde/phosphate buffered saline (PFA/PBS) under terminal anaesthesia (meditomidine 1mg/kg, ketamine 75mg/kg i.p.). The gastrocnemius muscle was collected, cryo-sectioned (5 μ M) and stained for blood vessels with Isolectin B₄ (IB₄) and arterioles with smooth muscle actin antibody (aSMA).

Results: The blood flow recovery in LysM-Wnt5a^{GOF} (day 3 $19 \pm 2.56\%$, day 7 $40 \pm 3\%$) was significantly slower than WT mice (day 3 $70 \pm 4\%$, day 7 $80 \pm 3\%$). This impaired revascularisation was rescued by SPHINX31 treatment on day 3 ($57 \pm 14\%$) and day 7 ($62 \pm 11\%$) post-surgery (N=7, $p < 0.0001$, two-way ANOVA). Staining of the muscles from these mice for arterioles indicated that impaired recovery of LysM-Wnt5a^{GOF} was due to reduced arteriogenesis.

Conclusion: Monocytes overexpressing Wnt5a in PAD models result in impaired collateralisation, insufficient angiogenesis and poor arteriogenesis. This was reversed, not enhanced, by SPHINX31 suggesting that the impaired revascularisation in PAD could be SRPK1 dependant and could be used as a potential therapeutic.

[1] Kikuchi R. et al. Nat Med. 2014; 20 (12): 1464-1471.

[2] Batson J. et al. ACS Chem. 2017; 12: 825-832.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC244

Cell signalling effects of Polyfect and Superfect dendrimer delivery systems on ErbB family of receptor tyrosine kinases

S. Akhtar¹, B. Bindu Chandrasekhar², I. Benter³ and A. El-Hashim⁴

¹College of Medicine, Qatar University, Doha, Qatar, ²Pharmacology and Toxicology, Kuwait University, Kuwait, ³Eastern Mediterranean University, Famagusta, Cyprus and ⁴Faculty of Pharmacy, Kuwait University, Kuwait, Kuwait

Commercially available polyamidoamine (PAMAM) dendrimer delivery systems, Superfect (SF) and Polyfect (PF) are routinely used as delivery vectors for plasmid DNA and siRNA nucleic acids but are known to have other biological effects. Aside from their drug delivery properties, these cationic PAMAMs, due to differences in their structural architecture, can differentially perturb basal signaling of the epidermal growth factor receptor (EGFR)- a member of the ErbB family of receptor tyrosine kinases that regulates multiple cellular functions. However, little is known of their effects on other members of ErbB family, namely ErbB2, ErbB3 and ErbB4 or on the signaling of EGFR upon stimulation with a ligand or following transactivation via ligand-independent mechanisms. In primary rat aortic vascular smooth muscle cells exposed to 0.4, 4 or 40 $\mu\text{g/ml}$ concentrations of dendrimers for 4h, SF dose-dependently stimulated (up to 2-fold) whereas PF inhibited basal phosphorylation (by approximately 50%) of all ErbB receptors as detected by Western blotting ($n=5$; $p < 0.05$). Mean values were compared using analysis of variance followed by post hoc test (Bonferroni). Similarly, SF stimulated whereas PF inhibited the activation/transactivation of EGFR by EGF-ligand- (10nM), high-glucose- (25mM) or Angiotensin II (1 μM). Similar differential effects of PF and SF on ErbB2, ErbB3 and ErbB4 were also observed in an immortalized human embryonic kidney cells line

(HEK-293) implying that this may be a general property related to their structural architecture. AG178, an inhibitor of EGFR phosphorylation, significantly enhanced the inhibitory effects of PF and attenuated SF-induced stimulation of EGFR ($p < 0.05$). Antioxidants (apocynin and tempol) significantly attenuated dendrimer-induced apoptosis and modulation of ErbBs ($p < 0.05$). These data highlight a new biological action of PF and SF PAMAMs in that they can differentially affect the activity of a whole major class of receptor tyrosine kinases (i.e. ErbB family of receptors) via oxidative stress-dependent pathways.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC245

Trimethylamine, a gut bacteria metabolite, increases in rat plasma with age and affects vascular smooth muscle cells viability.

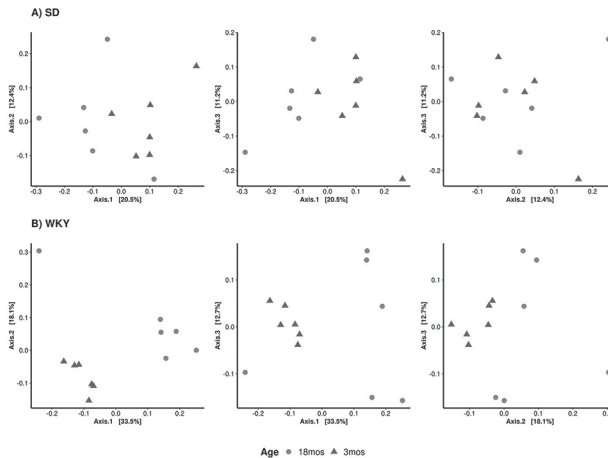
K. Jaworska¹, M. Konop¹, T. Hutch¹, K. Perlejewski³, M. Grochowska³, M. Radkowski³, A. Bielak-Zmijewska², G. Mosieniak², E. Sikora² and M. Ufnal¹

¹Department of Experimental Physiology and Pathophysiology, Medical University of Warsaw, Warsaw, Poland, ²Laboratory of Molecular Bases of Aging, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland, Warsaw, Poland and ³Department of Immunopathology of Infectious and Parasitic Diseases, Medical University of Warsaw, Warsaw, Poland

It has been suggested that trimethylamine oxide (TMAO), a liver oxygenation product of gut bacteria-produced trimethylamine (TMA), may be a marker and mediator of cardiovascular diseases. However, the mechanisms of plasma TMAO increase and its biological effects are obscure. We evaluated the effect of age, a cardiovascular risk factor, on rat plasma levels of TMA and TMAO, and cytotoxicity of the two molecules in human vascular smooth muscle cells (VSMCs).

The experiments were performed on (i) 3-month-old Sprague Dawley rats ($n=8$) and Wistar-Kyoto rats ($n=8$) and (ii) 18-month-old, male, Sprague Dawley rats ($n=8$) and Wistar-Kyoto rats ($n=8$), maintained on the same standard laboratory diet and in the same environmental conditions. The rats were anaesthetized i.p. with urethane 1.5 kg/bw (Sigma-Aldrich) for a direct arterial blood pressure and intestinal blood flow measurements. Next, blood samples were taken from the right ventricle of the heart and rats were killed by decapitation. A segment of the colon (7-8 cm, a middle part between the cecum and the rectum) was harvested for histological evaluation. The colon content (stool masses) was collected for biochemical and microbiome analysis. Gut bacteria were analyzed with a 16S ribosomal ribonucleic acid gene sequence analysis. TMA and TMAO were measured in the colon content, portal blood and systemic blood using chromatography coupled with mass spectrometry. Viability of VSMCs exposed to TMA and TMAO at concentrations found in rats, and higher, was evaluated with MTT test.

Older rats showed significantly different gut bacteria composition (Fig. 1) and higher, but not significantly, TMA stool level than younger rats. Systemic blood TMA level was significantly higher in older rats, which was associated with a significantly higher gut-to-blood TMA penetration (a significantly higher ratio of portal blood to stool concentration of TMA). There was no significant difference in arterial blood pressure between the groups. In contrast, older rats showed significantly lower intestinal blood flow and significantly reduced thickness of colonic mucosa which constitutes the physical barrier for gut-to-blood penetration of bacterial products. Older rats showed lower, but not significantly lower, TMA to TMAO liver oxidation and plasma TMAO level. In vitro, TMA at concentration of 500 $\mu\text{mol/L}$ (2-fold higher than in portal blood) significantly decreased VSMCs viability (Fig. 2). In contrast, TMAO at 1000-fold higher concentration than those observed under physiological conditions in rat plasma, had no effect on VSMCs viability. In conclusion, older rats show higher plasma level of TMA due to increased gut-to-blood penetration of the bacterial metabolite. TMA, but not TMAO, affects VSMCs viability at concentrations close to physiological ones. Therefore, we would hypothesize that it is TMA, not TMAO, that exerts detrimental effects on cardiovascular system and may contribute to increase in cardiovascular risk with aging.



Principal Coordinate Analysis (PCoA) clustering according to similarity of gut microbiota composition in stool samples. Percentage value (%) on each axis indicates % of the captured variation in the input data. PCoA analysis indicates that samples ordinated closer to one another are more similar than those ordinated further away. PCoA shows how examined samples differ between each other in tri-dimensional space, forming distinct clusters.

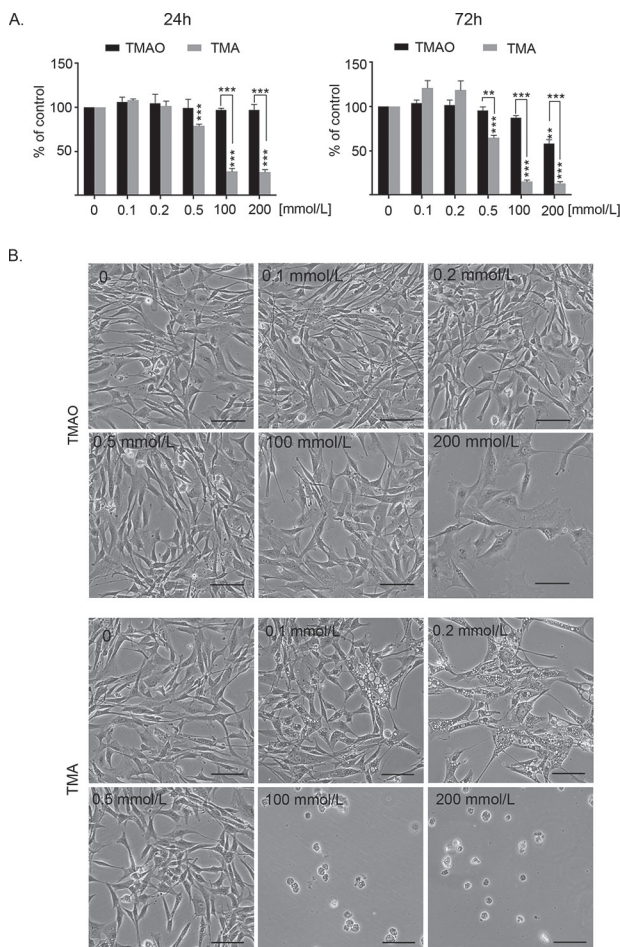


Fig. 2. A. Comparison of viability of human vascular smooth muscle cells (hVSMCs) treated with trimethylamine oxide (TMAO) or trimethylamine (TMA). MTT test was performed after 24 and 72h of treatment. Results were normalized to the control (untreated) cells; Graphs show mean $\pm$ SE, ** $P < 0.01$, *** $p < 0.001$; B. Representative pictures showing changes in the number and morphology of cells after 72 hours of treatment with of trimethylamine oxide (TMAO) or trimethylamine (TMA), bar 100 μ m.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Comparative study on the effects of Coir and *Cissus populnea* (Food gum) fibres as suture materials in ratsO.B. Igbokwe^{1,2}, A.P. Arikawe² and T.O. Azeez¹¹Biomedical Technology, Federal University of Technology, Lagos, Nigeria and
²Physiology, College of Medicine, University of Lagos, Lagos, Nigeria

Sutures provide mechanical strength to seal wounds and prevent pathogens invasion. These sutures are usually made from biological materials e.g. silk, catgut, and synthetic materials e.g. polytetrafluoroethane, nylon etc and production using these materials could be expensive, non-biocompatible and often times not available in some regions of developing nations. Thus physico-mechanical properties of Coir and *Cissus populnea* (food gum) fibres were assessed as substitutes to expensive biological and synthetic sutures. Coir fiber was processed from the hard internal shell and the outer coat of a coconut, while food gum was processed from the stem of the *Cissus populnea* plant. Both plant fibres in unmodified form have low tensile strength and limited pliability. The fibres were extracted using the water retting technique followed by a modification process using acetic anhydride and polyglycolic acid to improve tensile strength and their biocompatibility.

Mature male Sprague-Dawley rats weighing 300gm from the animal facilities of the College of Medicine, University of Lagos, Idi-Araba campus were housed in groups of six per cage and were divided into 3 groups; Control, Coir fibre, and Food gum fibre groups respectively. Rats were anaesthetized by a single intraperitoneal injection of chloral hydrate 400mg/Kg BW (Sigma-Aldrich, St. Louis, MO USA). After which, dorsum of each rat was shaved with electric clippers and skin sterilized with 70% alcohol. One 3 x 5cm region was marked by a sterile pen, a 3cm incision wound made and secured with full thickness suture method by monofilament nylon suture (Control group), modified Coir fibre (Coir fibre group), and modified food gum fibre (Food gum fibre group). The animals were allowed to fully recover from anaesthesia and surgery and 14 days later were humanely sacrificed using tribromoethanol (2, 2, 2-tribromoethanol 99%, Sigma Aldrich, 1 mL/100 g body weight of 2.5% solution, intraperitoneal route). Tensile strength measurements were carried out in vitro using universal testing machine instron 3369 and histopathological examination was also performed on tissue samples taken 14 days after incision.

Tensile strength was significantly different ($p < 0.05$) amongst the fibres, and was highest in the food gum fibre group. Histological evaluation revealed complete healing phase from both Coir and Food gum fibres but calcification was observed in the Coir fibre group while no calcification was observed in the Food gum fibre group and was absorbed. The study concludes that food gum fibre may be used as an absorbable suture.

Arun, H. S. Kumar and Anthony J. P. Clover. 2015. Intraperitoneal co-administration of low dose urethane with xylazine and ketamine for extended duration of surgical anesthesia in rats. Lab. Animal. Res., 31 (4): 174 – 179.

Cheng-San, Y., et al. 2010. The effect of suture size on skin wound healing strength in rats. *Jour., Med., and Biol. Eng.* 31 (5): 339 – 343.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC247

Dysmenorrhea relieved by the interactions of human body anatomical axes indicate an anti-inflammatory effect by normalization of tissue function

M. Ou¹, D. Ou² and C. Pang³

¹Obs & Gyn, Taipei City Hospital, Taipei City, Taiwan, ²Mechanical Engineering and Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA and ³Occupational therapy, National Taiwan University, Taipei City, Taiwan

Ou MC decrescendo phenomenon treatment (OuDPt) reduces pain or inflammation in an area by placing the contralateral hand along human body anatomical axis (HBAA) over the lesion (Proc Physiol Soc 2014-6).¹ More HBAA's interaction shows to be more competent to induce tumor regression, which evinces that the effect of OuDPt is associated with HBAA's interaction.² 59 patients with dysmenorrhea received OuDPt in menstrual period. Another 10 patients had recurrent dysmenorrhea about 6 months to 2 years after laparoscopic operation for chocolate cyst and endometriosis. Co-existing infection should be treated for that OuDPt may not be always effective against infection. These 10 patients received OuDPt in a 2 dimensional way twice daily and performed more in the menstrual period on the painful area.² All the dysmenorrhea of the 59 patients were alleviated by OuDPt. For the 10 patients after endometriosis operation, OuDPt also shows to alleviate the menstrual pain prominently to date that they do not need other analgesic treatment. The interaction of HBAA's in this study showed to alleviate dysmenorrhea which is mainly caused by inflammatory reaction. Alleviation of pain, cessation of uterine bleeding, improvement of organ dysfunction, and elimination of malignant changes by OuDPt suggests the restoration of normal tissue function.^{2,3,4} Normal cell polarity is essential to normal tissue function. Signaling system of embryonic axes has shown to impart polarization of individual cells and the tissue function normalization by HBAA's interaction in our studies may also be via the regulation of cell polarity. Recent studies show disruption of cell polarity increases inflammatory reaction.⁵

Ou MC, Ou D, Pang C. The anti-inflammatory effect by interactions of human body anatomical axes for dysmenorrhea caused by endometriosis. *J Assist Reprod Genet.* 2018; 35: 2097.

Ou MC, Ou D, Pang CC. Implication of tumor regression by a three-dimensional human body polarity system formed with human body anatomical axes. *Taiwan J Obstet Gynecol.* 2017; 56: 116-119.

Ou MC, Ou D, Pang C. The possible role of embryonic polarity axes for the normalization of tissue function induced by the interaction between human bilateral parts. *Integr Cancer Sci Therap.* 2015; 3: 329-335.

Ou MC, Ou D, Chu Pang C. The implication of tumor regression associated with tissue function normalization by human body anatomical axes interaction – A way for cancer prevention. *Int J Gynecol Cancer*. 2018; 28: 492.

Poernbacher I, Vincent JP. Epithelial cells release adenosine to promote local TNF production in response to polarity disruption. *Nat Commun*. 2018;9:4675.

The authors thank the helps from Ms. Ou, Yi Jen.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC248

Lower dietary antioxidant vitamins consumption and vascular endothelial dysfunction in older sedentary males; justification for dose adjustment?

M.T. Filippini¹, J. Brugniaux², C. Marley¹ and D. Bailey¹

¹Neurovascular Research Laboratory, University of South Wales, Pontypridd, UK and

²Université Grenoble Alpes, Grenoble, France

Background, aims:

Cardiovascular disease risk increases with advancing chronological age¹. Moderate aerobic exercise promotes a favourable cardiovascular state and helps in maintaining vascular health subsequent to an improvement in vascular endothelial function². However, diet plays an equally important role, if not oftentimes overlooked variable, in the prevention of cardiovascular diseases. In particular the consumption of primary water and fat-soluble chain-breaking antioxidants (vitamins C and E), given their collective capacity to constrain lipid peroxidation and attenuate oxidative stress³. The aim of this study was to determine if a link exists between inadequate dietary antioxidant intake in older, physically active and inactive, male adults and vascular endothelial health.

Methods:

Thirteen physically active older adult males (age 66±5 years), 12 sedentary age matched males (age 69±6 years) consented to the study. Participants were interviewed to collect a 24-hour structured dietary recall and data were analysed using NetWISP dietary analysis software (Version 4.0, Tinuviel Software; Anglesey, UK). Vascular function was assessed using flow-mediated dilation (FMD) according to international guidelines⁴. A high-resolution ultrasound machine (Acuson P50, Siemens) was used; FMD was expressed as a percentage change of the brachial artery from baseline (Studio; Computer Vision Group). Data were tested for normality using Shapiro-W-Wilks tests. T-test was performed to compare the groups. Significance level was established at $P<0.05$ and data are expressed as mean ± standard deviation (SD).

Results:

FMD was lower in sedentary older adults versus the matched active participants ($P<0.05$; table).

Dietary vitamin C and carotene consumption were also shown to be lower in the sedentary relative to the active group, yet within dietary guidelines (table). In contrast, dietary vitamin E intake was not different ($P>0.05$).

Conclusions:

The FMD response reported for the sedentary older adults was below the 7–10% identified as a normal healthy FMD response⁵. Furthermore, low intake of dietary antioxidants (vitamin C and carotene) may contribute, at least in part, to the observed reduction in vascular endothelial function. Despite dietary antioxidant consumption was not below the recommended guidelines, inadequate antioxidant vitamins intake may lead to excessive oxidative damage and has been associated with cardiovascular events. Is it time to review the guidelines?

Antioxidant intake and vascular function in active and sedentary older males

Group	Active (n = 13)	Sedentary (n = 12)	Significance (P Values)	Dietary Guidelines UK1
Vitamin C (mg)	117 ± 52	70 ± 29	0.010	40mg/day
Carotene (µg)	3929 ± 2121	2062 ± 1707	0.029	N/A
Vitamin E (mg)	8 ± 2	7 ± 3	0.186	3mg/day
FMD (%)	8.6 ± 4.5	5.1 ± 2.0	0.035	

Data are mean + SD; 1Department of Health (1991).

Landers-Ramos, *et al.* (2016) *Appl Physiol Nutr Metab.*, 41, 832–841.

Green, *et al.* (2018) *Cold Spring Harb Perspect Med.*, 8, a029819.

Varadharaj, *et al.* (2017) *Front Cardiovasc Med.*, 2017, 64, 1-11.

Black, *et al.* (2008) *Hypertension*, 51, 203-2.

Corretti, *et al.* (2002) *J Am Coll Cardiol.*, 39, 257–65.

DMB is supported by a Royal Society Wolfson Research Fellow (#WM170007).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC249

Maternal RBC alloantibodies in Pregnancy

R.J. Vatakencherry¹, S. L¹, V. S² and R. K³

¹Physiology, Amrita Institute of Medical Sciences; Kochi: Kerala; India, Kochi, India,

²Transfusion Medicine, Amrita Institute of Medical Sciences; Kochi: Kerala; India, Kochi, India and ³Obstetrics, Amrita Institute of Medical Sciences; Kochi: Kerala; India, Kochi, India

Background: At the majority of transfusion centres in India, antenatal antibody screening is done only in Rh (D)-ve mothers. Cases of hemolytic disease of new born by maternal alloimmunization to Rh antigens other than D, and to other blood group antigens were reported in Rh +ve women. This highlights the importance of routine antenatal antibody screening in Rh positive women in developing

countries like India. A close follow up throughout pregnancy is required if irregular antibodies are present, so that antigen negative, crossmatch compatible blood can be provided in a timely manner for intrauterine or exchange transfusions. Objective:1) To find the prevalence of irregular red cell antibodies(anti D and non anti D) in pregnant women 2) To analyse the significance of routine irregular red cell antibody screening in all antenatal women. Materials and methods:A Cross sectional study was carried out in 400 antenatal women attending the Obstetrics Department of Amrita Institute of Medical Sciences; Kochi, Kerala, India. Blood samples were screened for irregular antibodies. On those samples found to be positive, antibody identification was done. Results:Indirect Coombs test was positive in 3.5%(14) of the cases; among which, 3 cases were reported in Rh +ve women. After identification, these antibodies were anti D(28.6%), anti K(21.4%), anti c(14.3%), anti C+D(14.3%), anti Leb+Jk_a(7.1%), anti E(7.1%)and anti c+Jk_a(7.1%). Anti c+Jk_a, anti E and anti K(1 case) were reported in Rh +ve women, showing the importance of antibody screening and identification even in Rh+ve women. Conclusion:71.4% of the antibodies were non anti D. Eventhough the prevalence of irregular antibodies were lower in Rh+ve women, severity of the consequences justifies the need of routine antenatal antibody screening in Rh+ve women also.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC250

Global Reach 2018: High altitude acclimatisation improves neurovascular coupling in man

B.S. Stacey¹, R.L. Hoiland², H.G. Caldwell², C.A. Howe², T. Vermeulen², M.M. Tymko², G.A. Vizcardo-Galindo³, D. Bermudez³, F. Villafuerte³, P. Ainslie² and D. Bailey¹

¹Neurovascular Research Laboratory, Faculty of Life Science and Education, Univerisity of South Wales, Pontypridd, UK, ²Center for Heart, Lung and Vascular Health, University of British Columbia, Okanagan, Kelowna, BC, Canada and ³Laboratorio de Fisiología Comparada, Departamento de Ciencias Biológicas y Fisiológicas, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru

Neurovascular coupling (NVC) is responsible for the close temporal and regional linkage of cerebral blood supply to local cerebral metabolic requirements. The present study sought to examine the influence of acute simulated high altitude (SHA) and high altitude acclimatisation (HAA) on NVC in seven healthy male lowlanders (aged 28 ± 8 years). NVC was assessed at three time points: sea level (344m); after 30 minutes of dynamic end-tidal forcing to simulate an equivalent altitude of ~4,300m (SHA) and after two weeks acclimatisation to the same altitude (HAA, Cerro de Pasco, Peru). Posterior cerebral artery blood velocity (PCAv) was assessed using transcranial Doppler ultrasound during five consecutive trials of 30s eyes open with standardised visual stimulation (flashing checkerboard), followed

by 30s of eyes closed. The NVC response was characterised as the percent peak and average increase (relative to eyes closed) in PCAv during 25s of visual stimulation, averaged across the five trials. Distribution normality was confirmed by Shapiro Wilks W tests and data analysed using a repeated measures ANOVA. Significance was set at $P < 0.05$. SHA attenuated both peak ($10 \pm 2\%$ vs. $18 \pm 4\%$, $P = 0.021$) and average percent increases in PCAv ($3 \pm 2\%$ vs. $8 \pm 3\%$, $P = 0.015$) compared to sea level. Despite similar reductions in arterial oxygen saturation and partial pressures of oxygen and carbon dioxide, HAA increased both peak ($25 \pm 7\%$ vs. $18 \pm 4\%$, $P = 0.016$) and average ($13 \pm 5\%$ vs. $8 \pm 3\%$, $P = 0.045$) percent increases in PCAv during visual stimulation, when compared to sea level. The differential response of NVC to SHA and HAA (reduction and increase, respectively) may reflect influences of acid base status and nitric oxide availability considering their differences between conditions.

This work was supported by a Royal Society Wolfson Research Fellowship (#WM170007), grants from the Higher Education Funding Council for Wales (to D.M. Bailey) and a Canada Research Chair and NSERC discovery grant (to P.N. Ainslie)

Ethical Approval: The Clinical Research Ethics Board of the University of British Columbia (H17-02687 and H18-01404) and the Universidad Peruana Cayetano Heredia Comité de Ética (#101686) approved all experimental procedures and protocols in adherence with the principles of the Declaration of Helsinki (except registration in a database). All participants read an in-depth study information form and provided written informed consent.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC251

C₆₀ fullerenes as novel blockers of MaxiK channels in smooth muscle myocytes

M.I. Melnyk^{1,2}, I.V. Ivanova², D.O. Dryn^{1,2,3}, Y.I. Prylutsky³, V.V. Hurmach³, M. Platonov⁴, L. Al Kury⁵, U. Ritter⁶, A.I. Soloviev² and A.V. Zholos³

¹A.A. Bogomoletz Institute of Physiology, Kyiv, Ukraine, ²Institute of Pharmacology and Toxicology, Kyiv, Ukraine, ³ESC "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, Kyiv, Ukraine, ⁴Institute of Molecular Biology and Genetics, Kyiv, Ukraine, ⁵College of Natural and Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates and ⁶Technical University of Ilmenau, Institute of Chemistry and Biotechnology, Ilmenau, Germany

The large-conductance Ca²⁺- and voltage-activated K⁺ channels (BK_{Ca}, MaxiK) are widely expressed in different cell types, where they provide an important negative feedback control of membrane potential by generating outward hyperpolarizing current in response to increment in free intracellular Ca²⁺ concentration.

Correspondingly, maxi-K channels play many important physiological roles and they are considered to be important drug targets [1]. In particular, activation of these channels in vascular smooth muscles leads to membrane hyperpolarization and vasorelaxation, which makes them a key element of the regulation of vascular tone. On the other hand, carbon nanoparticles (CNPs), such as nanotubes and fullerenes, display multiple unique physical and chemical properties and hence there is a number of proposed diverse biomedical applications for CNPs, including modulation of the activity of various ion channels. We thus aimed to investigate the effects of water-soluble pristine C₆₀ fullerenes on MaxiK channels expressed in rat pulmonary artery and mouse ileal myocytes. The structure of C₆₀ fullerene particles in aqueous solution was studied using scanning tunnelling microscopy. Molecular modelling and docking was done based on 3NAF and 5TJ6 structures from the PDB database using SWISS MODEL, SDOCK+ embedded in the QXP package, GROMACS 4.6, and MembraneBuilder. Rat pulmonary artery rings were used in tensiometry studies, while single freshly dispersed rat pulmonary artery smooth muscle cells and mouse ileal myocytes were used for patch-clamp recordings in standard whole-cell and cell-attached configurations. Values are means±S.E.M., with differences evaluated using Student's *t*-test. C₆₀ fullerenes applied at 7.5 µg/ml inhibited the net outward potassium currents reducing current density from 132.4±9.6 to 75.0±9.0 pA/pF (n=9) at 120 mV (P=0.0005). The inhibitory effect was lost with high EGTA concentration (10 mM) in the pipette solution used to abolish MaxiK activity, indicating that C₆₀ fullerenes specifically inhibit MaxiK, but not voltage-dependent K⁺ (K_v) channels. In functional tests, C₆₀ fullerenes enhanced phenylephrine-induced contraction of pulmonary artery rings by about 25% and reduced endothelium-dependent acetylcholine-induced relaxation by about 40%. Both molecular docking simulations and analysis of single channel activity indicated that C₆₀ fullerenes blocked MaxiK channel pore in its open state. Notably, the inhibitory effect of C₆₀ fullerenes on the whole-cell current was due to significant reduction in channel open probability, as MaxiK single channel conductance remained unaltered: 125.2±5.5 pS in control and 125.7±9.6 pS with C₆₀ fullerenes (n=3). Thus, we characterised new pharmacological properties and novel molecular target for this class of bioaccessible and biocompatible nanostructured materials. Bentzen BH *et al.* (2014). *Front Physiol* 5: 389

Supported by the Ministry of Education and Science of Ukraine (#19BF036-01), Department of Targeted Training of Taras Shevchenko National University of Kyiv affiliated with the National Academy of Sciences of Ukraine (#7B) and National Academy of Medical Sciences of Ukraine (#DZ/28-2017)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC252

The Effect of Flow on Weibel-Palade Body Secretion and its Contribution to Atherosclerosis

A. Money, D. Beech, J. Schneider and L. McKeown

LICAMM, University of Leeds, Leeds, UK

Atherosclerosis is a chronic inflammatory disease characterised by the accumulation of fatty plaques at focal regions of the vasculature. This results in occlusion of the vessel and more severe cardiovascular diseases such as thrombosis. The focal nature of atherosclerosis is largely dictated by the haemodynamic forces exerted on the endothelial layer lining the vessels, known as shear stress. High shear stress, characterised by laminar flow, induces an atheroprotective endothelial phenotype. Low shear stress, characterised by disturbed flow often found at bifurcations and branching points, induces an atheroprone endothelial phenotype. The endothelial cells at regions exposed to low shear stress become activated and secrete pro-thrombotic and pro-inflammatory mediators that promote the progression of atherosclerosis. Many of these mediators are stored in endothelial-specific storage vesicles called Weibel-Palade bodies (WPBs).

The trafficking and secretion of WPBs is required in a normal response to vascular injury. WPBs therefore contain pro-inflammatory and pro-thrombotic cargo such as von Willebrand Factor (vWF), P-selectin and angiopoietin-2. Inappropriate and untimely secretion of WPBs however, leads to the unnecessary recruitment of platelets and immune cells resulting in an increased susceptibility of this region to cardiovascular disease. An *in vitro* Ibidi pump system was used to simulate shear stress at different regions of the vasculature by exposing cultured endothelial cells to different flow conditions. Endothelial cells exposed to high shear stress (12 dyn/cm^2) for 72 hours aligned with the direction of flow. The Golgi apparatus, responsible for the production of WPBs, increased in length by 23% ($n = 2$) compared to static conditions. The Golgi also became more fragmented as shown in figure 1, which has been shown to alter subsequent WPB morphology. This indicates that shear stress could be affecting the production of WPBs. The effect of varying shear stress on the subsequent WPB trafficking and secretion using the Ibidi pump system will also be measured using live-cell imaging, as well as the extent to which this contributes to lesion development in atherosclerosis using *in vivo* mouse models. Current research on the effect of flow on WPBs is limited. A better understanding of the molecular mechanisms which underlie the effect of flow on WPBs will allow us to exploit these mechanisms for potential therapeutic benefit in the treatment of atherosclerosis.

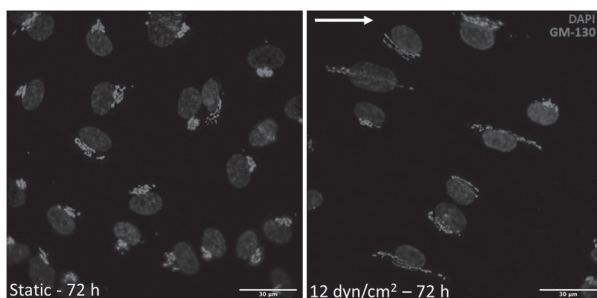


Figure 1 – Elongation and fragmentation of Golgi apparatus after exposure to high shear stress (right) for 72 hours (arrow indicates direction of flow), compared to static conditions (left).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC253

Sex Differences in Cerebral Perfusion during Insulin-Glucose Challenge

K.J. Carter¹, A. Ward¹, O. Wieben⁴, M.W. Eldridge², S.A. Hagen², B.J. Walker³, J.W. Lee³, A.M. Al-Subu² and W.G. Schrage¹

¹Kinesiology, University of Wisconsin-Madison, Madison, WI, USA, ²Pediatrics, University of Wisconsin-Madison, Madison, WI, USA, ³Anesthesiology, University of Wisconsin-Madison, Madison, WI, USA and ⁴Radiology, University of Wisconsin-Madison, Madison, WI, USA

Background: Insulin mediates endothelium-dependent vasodilation in peripheral tissues such as skeletal muscle and liver primarily via nitric oxide synthase (NOS), an effect reported to be greater in the skeletal muscle of women compared to men. Whether cerebral blood flow (CBF) increases in response to insulin or differs between the sexes remains unknown. We hypothesized that: a) insulin would increase CBF; b) CBF response to insulin would be NOS-mediated; and c) CBF response to insulin would be greater in women via greater NOS-mediated vasodilation.

Methods: Healthy normal weight young women (n=6; BMI 22±1 kg/m²; age 23±2 yrs) and men (n=6; BMI 22±2 kg/m²; age 22±2 yrs) underwent two magnetic resonance imaging (MRI) study visits with randomized intravenous infusion of saline or the NOS inhibitor L-NMMA (loading: 5 mg/kg/min; maintenance 1 mg/kg/min). Pseudo-continuous arterial spin labeling (ASL) quantified global and regional cerebral perfusion. Scans occurred at baseline and at 60 minutes during an oral glucose tolerance test (OGTT; insulin-glucose challenge). Significance was determined using a mixed three-way ANOVA (Time x Condition x Sex) and was set at p<0.05.

Results: Results are mean $\pm$ SD. OGTT increased glucose and insulin (both $p<0.05$; no interaction). Mean arterial pressure and end tidal CO₂ were similar at baseline and did not change during OGTT (both $p>0.05$; no interaction). Women tended to have higher heart rate (HR; 62 $\pm$ 6 vs. 58 $\pm$ 7 BPM; sex $p=0.05$), and HR increased with OGTT (both sexes $\Delta 5\pm 2$ BPM; $p<0.05$). Women displayed greater global perfusion compared to men (women 43 $\pm$ 5 men 35 $\pm$ 4 mL/100g/min; sex $p<0.05$) and a similar pattern was observed regionally in the frontal, temporal, parietal, and occipital lobes (sex $p<0.05$). L-NMMA decreased global perfusion (saline 40 $\pm$ 7 vs. L-NMMA 38 $\pm$ 7 mL/100g/min; condition $p<0.05$) and perfusion to the frontal and parietal lobes (condition $p<0.05$). Despite the main effect of L-NMMA, during OGTT, global and temporal lobe perfusion decreased with saline but increased with L-NMMA (interaction; both $p<0.05$).

Conclusions: Women exhibited greater global and regional perfusion and NOS inhibition reduced global perfusion similarly between the sexes. Surprisingly, some regions increased perfusion with NOS inhibition during OGTT. Therefore, insulin-glucose surge interacts with NOS in a regionally-specific manner to alter cerebral circulation, but this effect appears similar between sexes.

Support provided by the American Diabetes Association grant ADA 1-16-ICTS-099

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC254

Perinatal exposure of dams to a high salt diet impaired vascular function and elevated biomarkers of inflammation in the offspring

A.K. Oloyo, S. Olley, E. Ohihoin, A. Adejare, K. Ismail-Badmus, E. Asamudo and O. Sofola

Physiology, University of Lagos, Idi-Araba, Surulere, Nigeria

Heritability of salt-sensitive hypertension and high susceptibility of offspring's blood pressure (BP) to maternal perinatal high salt diet (HSD) [Gray *et al.*, 2015; Maruyama *et al.*, 2015] suggests that hypertension has its origin early in life. However, the initiating factor for early origin of salt-sensitive hypertension and the mechanism(s) that underlie it is not clear. To investigate the mechanism(s) underlying the foetal programming of salt-sensitive hypertension, pregnant Sprague-Dawley rats were exposed to perinatal normal (0.3%) or high (8%) salt diet from day 1 of pregnancy till term. The following experiments were conducted in the offspring at 12 weeks of age: Blood pressure (BP) was measured via arterial cannulation under urethane and α -chloralose anesthesia (5ml/kg body weight i.p) using ADInstrument powerlab. Isolated abdominal aorta reactivity to noradrenaline (NA) and acetylcholine (ACh) in the presence or absence of endothelial nitric oxide synthase (eNOS) inhibition by L- nitro arginine (L-NA) was determined and serum concentrations of C-reactive proteins (CRP), TNF- α and IL-6 was measured

using commercially available kits. Mean arterial BP was elevated ($p < 0.05$) in the offspring of dams exposed to perinatal HSD (116 ± 3 vs. 103 ± 2 mmHg). Maximum contractile response to noradrenaline was similar ($72.22 \pm 5.57\%$ vs. $79.45 \pm 5.76\%$) in both groups of offspring of dams fed a normal and high salt diet respectively. However, maximum relaxation response to acetylcholine with (54.23 ± 3.92 vs. $39.45 \pm 3.98\%$) or without L-NA (84.23 ± 4.92 vs. $61.32 \pm 4.76\%$) was significantly reduced in the offspring of dams that were exposed to HSD. Perinatal HSD elevated CRP (511.7 ± 13.52 vs. 771.7 ± 25.74 ng/ml), IL-6 (112.5 ± 2.7 vs. 120.8 ± 2.43 pg/ml) and TNF- α (2200 ± 123.8 vs. 3900 ± 198.3 pg/ml) in the offspring. Findings from this study suggest that perinatal feeding of HSD to the dams elevated BP in the offspring by increasing vascular tone as well as systemic and vascular inflammation. Gray, C. et al (2015). Scientific Reports | 5:09753 | DOI: 10.1038/srep09753.

Maruyama, K. et al., (2015). Life Sciences 136. 42–51.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC255

In situ dynamic structure of Rab46 – a novel endothelial protein.

S.D. Wiktor, D. Beech, L. McKeown and A. Breeze

University of Leeds, Leeds, UK

Weibel-Palade bodies (WPBs) are endothelial-specific secretory vesicles that serve a role of vascular ‘emergency kit’.¹ WPBs store haemostatic and pro-inflammatory mediators that can be released on demand, enabling rapid response to vascular injury. However, untimely and inappropriate exocytosis of WPBs can contribute to the development and progression of atherosclerosis, and thus secretion from endothelial cells has to be a tightly coordinated process. Rab GTPases are important regulators of all aspects of intracellular membrane trafficking. Rab46 is a novel multi-domain Rab GTPase that localizes to WPBs.² In addition to the conserved Rab domain, Rab46 contains a coiled-coil domain responsible for interactions with other proteins and EF-hand domain that acts as a built in Ca^{2+} sensor. Recently Rab46 has been shown to act directly as dynein adaptor in T cells.³ Moreover, findings from our lab demonstrated that Rab46 is a key regulator of context-dependent differential WPB cargo secretion. As the conformational changes observed during activation and inactivation of Rab proteins might provide binding pockets that could be modulated by small molecules, an understanding of the 3D atomic structure of Rab46 could provide novel therapeutic targets for cardiovascular disease. Since no structural studies on Rab46 have been performed to date, this project aims to investigate the structure, folding and dynamic properties of Rab46.

3D structures for the EF-hand, coiled-coil and Rab domains of Rab46 were predicted and analysed using protein structure prediction servers (I-TASSER and Phyre2) and Maestro software. Full-length protein and its individual functional domains were

cloned into pET32a-LIC expression vector using In-Fusion® Cloning system. The histidine-tagged recombinant proteins were expressed in BL21 and Rosetta 2 *E. coli* strains and purified by immobilized metal ion affinity chromatography, followed by size exclusion chromatography allowing to obtain high purity protein at a final concentration of 0.3 mM, suitable for natural abundance ¹⁵N HSQC NMR experiments.

Future work will focus on Rab46 structure elucidation using low resolution structural and biophysical techniques. Isotopically-labelled recombinant proteins will be produced for investigation of the dynamics and inter-domain interactions of Rab46 using high-resolution solution NMR. Structures of the domains obtained from homology modelling will be investigated to identify functionally important residues and guide new experiments such as site-directed mutagenesis. Moreover, coiled-coil domains of other dynein adaptors will be analysed using *in silico* approaches to explore the nature of the interaction between Rab46 and dynein. Ultimately, in-cell NMR will be used to observe conformational changes in response to agonists in the native environment.

Schillemans, M., Karampini, E., Kat, M. and Bierings, R. (2018). Exocytosis of Weibel–Palade bodies: how to unpack a vascular emergency kit. *Journal of Thrombosis and Haemostasis*, 17, pp. 6-18.

Wilson, L., McKeown, L., Tumova, S., Li, J. and Beech, D. (2015). Expression of a long variant of CRACR2A that belongs to the Rab GTPase protein family in endothelial cells. *Biochemical and Biophysical Research Communications*, 456(1), pp.398-402.

Wang, Y., Huynh, W., Skokan, T., Lu, W., Weiss, A. and Vale, R. (2019). CRACR2a is a calcium-activated dynein adaptor protein that regulates endocytic traffic. *The Journal of Cell Biology*, doi.org/10.1083/jcb.201806097.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC256

Angiogenesis following different physiological stimuli elicits distinct structural and functional outcomes in rat skeletal muscle.

R. Kissane^{1,2}, P. Tickle² and S. Egginton²

¹University of Liverpool, Liverpool, UK and ²University of Leeds, Leeds, UK

Microvascular distribution is a key in determining efficacy of peripheral O₂ transport. Angiogenesis, growth of capillaries from a pre-existing network, is driven by a range of chemical and mechanical factors. Although often described in global terms, we have demonstrated that neovascularisation in skeletal muscle is effected on a lengthscale approximating the diameter of individual fibres. To characterise the complex local feedback required for such tight regulation of the growth process, it is necessary to quantify both structural and functional responses following stimulation of angiogenesis in a physiological context.

Sustained skeletal muscle hyperaemia (predominantly mechanical stimulus) was induced by prazosin (PRAZ: 50 mg l⁻¹) given *ad libitum* in drinking water, while overload (OV: mechanical stimulus that also invokes an anabolic, chemical stimulus) induced by surgical extirpation of the tibialis anterior, results in extensor digitorum longus (EDL) hypertrophy. Animals were sampled after 7 or 14 days. Simultaneous recordings were made of hindlimb (femoral artery) perfusion with a Transonic® flow probe and EDL twitch force by an Aurora® force transducer system. Fatigue resistance was quantified with 10Hz twitches (0.3ms pulse width, supramaximal voltage) for 180s, and fatigue index (FI) calculated as end-stimulation tension/peak tension at the start of the test. Muscles were frozen in liquid N₂ cooled isopentane, and 10µm cryosections were stained with *G. simplicifolia* lectin-1 (Vector).

FI was improved in 7d and 14d OV EDL compared to control ($P<0.05$) and PRAZ ($P<0.01$) animals (all $n=5$), but there was no effect of PRAZ on FI compared to control ($P=0.94$), and no difference in FI between 7d and 14d treatments. Resting femoral flow was enhanced with PRAZ relative to control ($P\leq 0.05$) and OV animals ($P<0.05$), with no difference between end-stimulation blood flow among treatments ($P>0.05$). When considering global microvascular supply, capillary to fibre ratio (C:F) at 7d (1.90 ± 0.08 , $n=5$) and 14d OV (1.85 ± 0.11 , $n=3$), as well as 14d PRAZ (1.72 ± 0.07 , $n=4$), were all significantly ($P<0.05$) higher than control tissue (1.43 ± 0.02 , $n=6$). Fibre cross-sectional area (FCSA) after 14d OV (2660 ± 350 µm²) was significantly higher than control (1629 ± 86 µm²). Examining local capillary distribution, by the capillary domains technique (Al-Shammari et al. 2019. *J Appl Physiol* 126:544-557), allowed capillary-spacing heterogeneity to be quantified as LogSD of supply area to account for the log-normal distribution. LogSD after 7d OV (0.16 ± 0.01) was significantly ($P<0.01$) reduced compared to control (0.19 ± 0.01) and 7d PRAZ (0.18 ± 0.01).

These data show that a haemodynamic signal alone can elicit an angiogenic response, with little effect on muscle fatigability and a stochastic location of new vessels, while a combination of mechanical and chemical stimulation improves muscle function and is associated with directed location of capillaries to optimise O₂ delivery.

Al-Shammari, A. A., Kissane, R. W. P., Holbek, S. Mackey, A. L., Anderson, T. R., Gaffney, E. A., Kjaer, M. and Egginton, S. (2019) Integrated method for quantitative morphometry and oxygen transport modeling in striated muscle. *Journal of Applied Physiology*, 126, 544-577

Supported by the BHF.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

A novel GTPase protein (Rab46) regulates differential trafficking of Weibel-Palade bodies

K. Miteva, L. Pedicini, D. Beech and L. McKeown

Medicine & Health, University of Leeds, Leeds, UK

Weibel-Palade bodies (WPBs) are rod-shaped organelles, stored as heterogeneous populations in the endothelium, containing cargo that is rapidly released upon demand ¹. WPBs have numerous constituents that play roles in primary haemostasis, inflammation and angiogenesis such as von Willebrand Factor (vWF), P-selectin and angiopoietin-2, respectively ^{2,3}. Several functionally distinct endothelial agonists induce WPB trafficking and exocytosis by raising intracellular Ca^{2+} . It has been previously speculated that endothelial cells can selectively secrete WPB cargo in an agonist-appropriate manner, however, the underlining mechanisms are poorly understood ⁴.

The aim of the project was to examine differential WPB trafficking using high-resolution cellular imaging alongside intracellular Ca^{2+} measurements and biochemical techniques.

In order to investigate differential WPB trafficking, we treated HUVECs with histamine and thrombin (both potent WPB secretagogues). After 5 mins we observed that histamine-treated cells (immunostained with vWF as a marker for WPBs), but not thrombin, evoked clustering of a subpopulation of WPBs that were positive for Rab46 (CRACR2A-a: a newly identified Ca^{2+} -sensing GTPase ⁵) to the microtubule organising centre (MTOC). Therefore, we reasoned that histamine may evoke differential WPB trafficking, the release of P-selectin is necessary for a histamine response and P-selectin resides in WPBs that do not contain the pro-angiogenic tie-2 ligand, angiopoietin-2 ³. Image analysis revealed that in basal and stimulated conditions, Rab46 localised to WPBs that are devoid of P-selectin (control $8.32\% \pm 0.38$, histamine $7.38\% \pm 1.87$) but contain angiopoietin-2 (control $27.58\% \pm 1.48$, histamine $32.27\% \pm 1.52$). Stimulation of HUVECs with histamine, promoted significant MTOC localisation of WPBs carrying angiopoietin-2 (control 0.53 ± 0.02 , histamine 0.73 ± 0.03 ; 2-way ANOVA p value < 0.001) but not P-selectin (control 0.48 ± 0.03 , histamine 0.59 ± 0.03 ; 2-way ANOVA p value = 0.23). In addition, in the absence of stimulation, only angiopoietin-2, but not P-selectin, is confined to the MTOC when co-localised with constitutively active Rab46 (Q604L). Quantification of P-selectin at the cell surface in HUVECs, transfected with control or Rab46 siRNA, demonstrated that histamine-evoked surface release of P-selectin was Rab46-independent. However, Angiopoietin-2 was decreased in supernatants from histamine-stimulated cells where Rab46 was depleted. Further investigation revealed an inhibitory effect on angiopoietin-2 protein levels as well as high-resolution imaging showed that in cells where Rab46 was depleted, angiopoietin-2 was no longer localised to WPBs.

Taken together these findings support that Rab46 is a novel regulator that couples inflammatory stimuli to differential trafficking of WPBs and that it may be necessary for angiopoietin-2 recruitment.

Rondaij, MG *et al.* (2006). *Arteriosclerosis, Thrombosis, and Vascular Biology* 26, 1002-1007.

McEver RP *et al.* (1989). *J Clin Invest* 84, 92-99.

Fiedler U *et al.* (2004). *Blood* 103, 4150-4156.

Lowenstein CJ *et al.* (2005). *Trends Cardiovasc Med* 15, 302-308.

Wilson LA *et al.* (2015). *Biochem Biophys Res Commun* 456, 398-402.

Authors thank Bioimaging Facility staff (Leeds) for their support and Dr Izzy Jayasinghe for advice on advanced image analysis.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC258

Interaction between a new Rab GTPase (Rab46) and dynein complex regulates Weibel-Palade body trafficking in response to inflammatory stimuli

L. Pedicini, K. Miteva, D. Beech and L. McKeown

Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK

Endothelial cells selectively release pro-coagulant and pro-inflammatory mediators stored in Weibel-Palade bodies (WPBs) in order to regulate vascular function¹. WPBs provide an intracellular storage pool for the pro-thrombotic protein von Willebrand factor (vWF) and the pro-immunogenic protein P-selectin. Exposure to thrombotic or inflammatory stimuli induce WPBs exocytosis. However, inappropriate exocytosis of WPBs can promote the pro-thrombotic environment evident in cardiovascular disease. The mechanisms underlying differential cargo release in order to produce physiologically distinct responses are poorly understood. Recent studies have unravelled the role of some Rab GTPase family members in regulating the exocytosis of WPBs².

Here, we describe a novel Rab GTPase (Rab46:CRACR2A-a) in endothelial cells³ that has GTPase and Ca²⁺ binding activities and is located on WPBs. Super-resolution microscopy confirmed that individual WPBs contain vWF that is juxtaposed to Rab46 whilst quantitative imaging analysis suggested that Rab46 may regulate a subpopulation of WPBs because only 49% of WPBs were positive for both vWF and Rab46 (n/N=3/8).

To explore the role of Rab46 in endothelial cells we used imaged-based analysis of differentially stimulated cells in the presence and absence of Rab46 expression. Our data indicated that WPBs are able to distinguish between histamine and thrombin signals. Interestingly, histamine (30µM) but not thrombin (2U/ml) stimulation evoked a movement of WPBs towards the MTOC. This trafficking was dependent of Rab46 as depletion of Rab46 with specifically targeted

siRNAs reduced histamine-evoked WPB clustering (0.35 ± 0.05 siRNA treated vs 0.64 ± 0.04 control siRNA, p -value < 0.05).

Furthermore, we demonstrated that retrograde WPB trafficking to the MTOC is dependent on the integrity of microtubules and dynein activity. Experiments performed with nocodazole, a microtubule disrupting agent and ciliobrevin, a dynein inhibitor, abolished histamine-dependent Rab46 clustering (0.9 ± 0.06 histamine vs 0.6 ± 0.06 nocodazole, vs 0.6 ± 0.01 ciliobrevin, p -value < 0.01). Values are means $\pm$ SEM, compared by ANOVA.

To investigate the molecular mechanisms underlying Rab46-dependent retrograde trafficking we performed pull-down experiments of heterologously expressed Rab46 nucleotide-binding mutants and mass spectrometry analysis. Dynein heavy chain was identified as a candidate effector protein and further biochemical experiments confirmed the interaction between endogenous Rab46 and dynein both in presence or absence of histamine stimulation ($n=3$). Taken together, these results suggest that after acute histamine stimulation, dynein-bound Rab46 mediates retrograde transport of a subset of WPBs along microtubules to the MTOC.

These observations indicate Rab46 as a key regulator of differential WPB cargo secretion and understanding the Rab46/WPB signalling axis could provide novel therapeutic targets for cardiovascular disease.

Wagner DD. Cell biology of von Willebrand factor. *Annu Rev Cell Biol.* 1990; 6:217-46.

Nightingale T. & Cutler D. The secretion of von Willebrand factor from endothelial cells; an increasingly complicated story. *J. Thromb. Haemost. JTH* 11 Suppl 1, 192–201 (2013).

Wilson L. A., McKeown L., *et al.* Expression of a long variant of CRACR2A that belongs to the Rab GTPase protein family in endothelial cells. *Biochem. Biophys. Res. Commun.* 456, 398–402 (2015)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC259

Heterogeneity in the endothelium enables parallel processing of multiple stimuli

M.D. Lee, C. Wilson and J.G. McCarron

University of Strathclyde, Glasgow, UK

The endothelium is the innermost layer of all blood vessels and changes in the behaviour of this single layer of cells underlie almost all cardiovascular disease. To regulate cardiovascular function, the endothelium must integrate hundreds of signals. These signals can arrive from substances circulating as well as neighbouring cells. However, how the endothelium detects and relays the information stored in each of these signals to initiate a physiological output is not fully understood. The problem in integrating signals is made even more complex as the central relay system within an endothelial cell is a single ion, Ca^{2+} . Here, we show the

endothelium manages a multitude of extracellular signals by utilising spatially-distinct endothelial cells. Furthermore, the Ca^{2+} signal generated by each activator has unique distinguishing characteristics.

To determine how multiple extracellular signals are detected in the endothelium we analysed the Ca^{2+} activity from ~1000 endothelial cells from intact arteries. Male-Sprague-Dawley (150-250g) rats were euthanized by CO_2 overdose (Schedule 1; Animals (Scientific Procedures) Act 1986) and second order mesenteric arteries were extracted, cut open and pinned flat. The endothelium was loaded with a Ca^{2+} indicator Cal-520/AM and imaged using fluorescence microscopy. Ca^{2+} activity was separately analysed in each of the 1000 individual endothelial cells using a custom built software package written in Python.

For each activator (ACh, ADP, ATP & Histamine) full concentration response curves were carried out to determine the EC_{25} concentration (the concentration at which 25% of cells responded). There was variation in the EC_{25} for the four agonists (ACh = 15.6 nM; ADP = 119.8 nM; ATP = 1.2 mM; Histamine = 6.3 mM). The addition of the four activators (applied separately) at their respective EC_{25} concentration, to the same field of endothelium revealed the endothelium utilises sub-populations of endothelial cells to detect each activator. Endothelial cells were organised in clusters and Ca^{2+} signals propagated between cells as Ca^{2+} waves. The cells responsible for the detection of a specific activator were spatially unique and there was no significant overlap with cells that were primed to detect another agonist. For example, of the 25% of cells that responded to ACh only 21.5% responded to ADP, 19.6% responded to ATP and 18.5% responded to Histamine ($p < 0.05$, $n = 5$). There was no significant overlap in the sensitivity of the endothelium to the different agonists for all the various permutations (ADP vs ACh, ATP & Histamine; ATP vs ACh, ADP & Histamine; Histamine vs ACh, ADP, ATP; $p < 0.05$, $n = 5$). Each activator generated a unique Ca^{2+} signal that was specific to the agonist. By using spatially-distinct cells, unique signal characteristics and communication, the endothelium is able to detect and relay the information stored in the multitude of extracellular activators to initiate physiological outputs.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC260

Vasotracker: flexible, open source pressure myography

C. Wilson¹, M.D. Lee¹, P. Lawton², C.D. Saunter², J.M. Girkin² and J.G. McCarron¹

¹SIPBS, University of Strathclyde, Glasgow, UK and ²Durham University, Durham, UK

Pressure myography uses diameter measurements of isolated, pressurized arteries to assess smooth muscle and endothelial cell function. Here, we introduce the open-source pressure myograph system and analysis software, VasoTracker, which can be built in house for ~10% of the cost of commercial systems. We report on the

development of the VasoTracker platform and demonstrate its use by assessing endothelium-dependent vasodilation.

The complete VasoTracker system includes everything expected of a commercial pressure system (heated vessel chamber, temperature controller, pressure monitor, computer, camera, microscope, and acquisition/analysis software). The freely available VasoTracker software is a stand-alone interface for image acquisition and blood vessel diameter tracking that, although designed to work with VasoTracker hardware, can be used with any microscope/camera system supported by μ Manager. The software acquires, displays and records video images, and automatically tracks the diameter of blood vessels – all in real time. Temperature and pressure data, and user interventions (such as drug additions) can also be logged. VasoTracker enables the investigation of artery function using a wide array of protocols, such as those investigating vascular contraction, dilation, or the myogenic response. We used VasoTracker to investigate whether TRPV4 channels contribute to vascular relaxation induced by the muscarinic receptor agonist, acetylcholine (ACh). Second-order mesenteric arteries were isolated from male Sprague Dawley rats (10-12 week old; 250 - 300 g) euthanized by intraperitoneal injection with pentobarbital sodium (200 mg kg⁻¹). Arteries were mounted on a VasoTracker pressure myograph system, pressurized to 70 mmHg, and pre-constricted using phenylephrine. The selective TRPV4 agonist, GSK1016790A (GSK; 20 nM; applied intraluminally, ~ 100 μ l min⁻¹) relaxed PE-constricted vessels (n = 5). These GSK-evoked relaxations were reversed by the selective TRPV4 channels antagonist, HC067047 (20 μ M; 70.5 $\pm$ 6.1 % relaxation for control; 8.8 $\pm$ 2.1 % for HC067047; n = 5; p < 0.05; paired t-test). ACh also relaxed pre-constricted arteries (n = 5). However, ACh-evoked relaxations were unaffected by HC067047 (90.0 $\pm$ 5.5 % relaxation for control; 82.6 $\pm$ 8.1 % for HC067047; n = 5; p < 0.05; paired t-test). These data suggest that TRPV4 channels, whilst present in rat mesenteric arteries, do not contribute to ACh-evoked vasodilation.

In releasing VasoTracker, we provide the vascular community with an inexpensive, transparent and fully customizable tool for studying blood vessel function. Details on how to build and use the system, and the VasoTracker software are freely available from the publicly accessible website, www.vasotracker.com. We hope that the open documentation will enable researchers to expand and enhance the platform for tailored vascular reactivity experiments.

We thank the Wellcome Trust (204682/Z/16/Z; 202924/Z/16/Z) and the British Heart Foundation (PG/16/54/32230; PG16/82/32439) for their support.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

Transcutaneous oxygen pressure relation to skin blood flow and oxygen consumption in recovery after submaximal exercise

N. potocnik¹ and P. potocnik²

¹*Institute of Physiology, University of Ljubljana, Medical faculty, Ljubljana, Slovenia and*

²*University of Ljubljana, Medical faculty, Ljubljana, Slovenia*

Transcutaneous oxygen pressure (tcpO₂) measurements allow quantitative estimation of cutaneous oxygen delivery. At skin temperatures higher than 43°C, tcpO₂ is believed to equal the arterial oxygen partial pressure (pO_{2a}). However, the relationship is complex and affected by several factors as sympathetic tone, age, edema, oxygen consumption (VO₂), although the tcpO₂ is directly related to skin blood flow (1). In our previous study (2) it was shown that different skin sites (glabrous (G) and nonglabrous (NG) skin) with their characteristic microvessel anatomy and innervation (3) determine skin blood perfusion measured by laser Doppler flowmetry (LDF). The aim of this research was to find the differences in the relationship between both local indicators of skin blood perfusion (LDF and tcpO₂) with respect to skin site and the relationship between systemic (VO₂) and local (tcpO₂) oxygen kinetics.

9 physically active healthy subjects (age 25.9±3.3), non-smokers, with comparable maximal oxygen uptake (VO_{2max}) were recruited to participate in the study. Recovery after submaximal cycling was applied as a provocation to change sympathetic tone and oxygen consumption. After exercise (10 minutes of submaximal cycling) the subjects were asked to continue sitting at rest for additional 25 minutes to recover. Simultaneous changes in tcpO₂ and laser Doppler skin blood flow in finger pulp (G) and dorsal forearm (NG) together with heart rate, arterial blood pressure and oxygen consumption (VO₂) were assessed. Linear regression was applied to find the correlations between measured parameters. tcpO₂ correlated differently with LDF with respect to skin site: positive linear correlation was found in NG skin (R=0.309, p=0.013) and negative in G skin (R= -0.268, p=0.001) while VO₂ correlated positively with tcpO₂ in both skin types (R=0.388, p=0.035 in NG and R= 0.331, p=0.008 in G). Our study revealed that the cutaneous oxygen delivery was not uniquely dependant on LDF. In NG skin it changes proportionally to LDF as expected. The relationship in G skin could be explained by the increased blood flow through arterio venous anastomoses, the main target for conductional heat elimination, which contributes to LDF but not to oxygen delivery to the skin.

Ballard J.L. (2007) Transcutaneous Oxygen Tension: Principles and Applications. In: AbuRahma A.F., Bergan J.J. (eds) Noninvasive Vascular Diagnosis. Springer, London

Potočnik N, Lenasi H. The responses of glabrous and nonglabrous skin microcirculation to graded dynamic exercise and its recovery. Clin Hemorheol Microcirc. 2016 Nov 4;64(1):65-75.

Krogstad AL, Elam M, Karlsson T, Wallin BG. Arteriovenous anastomoses and the thermoregulatory shift between cutaneous vasoconstrictor and vasodilator reflexes. J Auton Nerv Syst. 1995 Jun 25;53(2-3):215-22.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC262

Ultrasound and Microbubble Gene Delivery for Targeting Altered Placental MicroRNAs in Preeclampsia

S. Frazier¹, H. Morgan², M. McBride¹, A. Bradshaw¹, H. Mulvana³ and D. Graham¹

¹*Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK,* ²*University of Nottingham, Nottingham, UK,* ³*Nottingham, UK and* ³*School of Engineering, Glasgow, UK*

Targeted gene delivery using ultrasound (US) and microbubbles (MB) is in development as a clinically applicable gene therapy (GT) technique. There is limited research targeting the placenta, which is a potential therapeutic strategy for preeclampsia (PE) since PE is a complex genetic disorder with ineffective management strategies. Differentially regulated placental microRNAs (miRNAs) in PE may represent suitable targets for GT. The aims of the study were 1) to develop an US and MB gene delivery protocol, with applicability to the placenta, by conducting a proof-of-concept study to demonstrate site-specific gene transfection and 2) to identify differentially expressed placental miRNAs in 3rd trimester PE patients, through a systematic literature review, and conduct a comparison of expression in a PE rodent model recently published by our group (1). Female CD1 mice were anaesthetised with 5% isoflurane and maintained on 1.5% isoflurane during procedures. MB (SonoVue) and plasmid (PGL3) were administered systemically to treatment mice (n=3), followed by exposure of the heart to US (B-mode, H14, 1.8 M.I., 1cm focal depth, 2 minutes), using Siemens Acuson Sequoia-512 system and 15L8 probe. Plasmid was injected intramuscularly to mouse gracilis muscle as a positive control (n=1). Luciferase assay was performed and normalised to protein concentration measured by Pierce BCA protein assay to evaluate gene transfection; values are mean CPS/ μ g of protein $\pm$ SD (n=4 technical replicates per tissue). Significantly differentially expressed placental miRNAs in PE patients were identified as candidate miRNAs based on detection by ≥ 3 screening studies. Expression of candidate miRNAs was measured by qRT-PCR in PE rat model placentas; values are mean RQ (RQ min-max) vs. non-PE controls (n=4 biological replicates per group). Luciferase activity was detected in the gracilis muscle (6.17 $\pm$ 4.51 CPS/ μ g of protein) of the positive control mouse. Luciferase activity was detected in the atria of treatment mouse 2 (14.6 $\pm$ 19.5 CPS/ μ g of protein) and ventricles of treatment mouse 3 (6.43 $\pm$ 5.84 CPS/ μ g of protein), providing evidence of site-specific gene transfection. Less luciferase activity was detected in the atria and ventricles of treatment mouse 1 (3.52 $\pm$ 1.95 and 1.04 $\pm$ 0.20 CPS/ μ g of protein, respectively). The systematic literature review identified nine candidate miRNAs. MiR-223 and miR-181a were significantly differentially expressed in PE rat model placentas (RQ: 1.46(0.12-0.13)-fold upregulation and 0.81(0.05-0.05)-fold downregulation, respectively, $p < 0.05$,

unpaired t-test). MiR-223 and miR-181a, which have been shown to play roles in trophoblast migration and invasion (2) (3), represent targets for US and MB gene delivery. Future studies will optimise the US and MB gene delivery protocol for translation to targeting the placenta in our PE rodent model, a novel application of the GT technique.

1. Morgan, H. L., Butler, E., Ritchie, S., Herse, F., Dechend, R., Beattie, E., McBride, M., & Graham, D. (2018). Modeling Superimposed Preeclampsia Using Ang II (Angiotensin II) Infusion in Pregnant Stroke-Prone Spontaneously Hypertensive Rats. *Hypertension*, 72(1), 208-218.

2. Meng, H., Xu, L., Jing, G., Qian, L., & Qi, M. (2017). MiR-223 promotes trophoblast cell survival and invasion by targeting STAT3 in preeclampsia. *Int J Clin Exp Med*, 10(4), 6577-6585.

3. Wu, L., Song, W., Xie, Y., Hu, L., Hou, X., Wang, R., Gao, W., Zhang, J., Zhang, L. Li, W., Zhu, C., Gao, Z., & Sun, Y. (2018). MiR-181a-5p suppresses invasion and migration of HTR-8/SVneo cells by directly targeting IGF2BP2. *Cell Death Dis*, 9, 16.

I would like to thank the Medical, Veterinary and Life Sciences Doctoral Training Programme funded by the College of Medical, Veterinary and Lifes Sciences, University of Glasgow and the British Heart Foundation.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC263

Purinergic involvement in the regulation of placental vasculature

P. Fodor, B. White and R. Khan

Division of Medical Sciences and Graduate Entry Medicine, The University of Nottingham, Derby, UK

Extracellular ATP (eATP) has been shown to trigger an inflammatory cascade, resulting in the production of cytokines including interleukin (IL)-1 β through the activation of the leukocyte purinergic receptor P2X₇^[1, 2]. However, less is known about the molecular mechanisms linking the role of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome which mediates IL-1 β processing, with eATP-P2X₇ in the regulation of the feto-maternal vasculature^[3]. The aim of this study was to compare IL-1 β production with that of the proinflammatory cytokines IL-18 (also produced via processing of its proform) and IL-6 by chorionic plate arteries (CPAs), stem villous arteries (SVAs) and trophoblast on exposure to pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). Ethical approval for the study was obtained from the Derbyshire Research Ethics Committee (Ref: 09/H0401/90). All placental samples were obtained from patients attending the Royal Derby Hospital, who provided informed, written consent prior to undergoing either a normal vaginal delivery or elective caesarean section at term gestation (>37 weeks). To characterise the inflammatory response of isolated CPAs, SVAs and trophoblast to the exposure of ATP (10mM), Lipopolysaccharide (LPS) (1mg/mL)+ATP(10mM)

and LPS(1mg/mL)+ 2'(3')-O-(4-Benzoylbenzoyl) adenosine 5'-triphosphate triethylammonium salt (BzATP) (300µM), enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of IL-1β, IL-18 and IL-6 in the media (N=9). Isolated trophoblast tissue (100mg, N=3) was incubated in the presence of various concentrations of glucose (5.5, 10, 20, 30mM) for 4 and 48 hours. Western blotting was used to investigate the modulation of P2X₇ receptor expression in these tissues. The ELISA experiments show that from all isolated tissues from term placenta, the trophoblast contributes the most towards IL-1β production. In terms of IL-6 levels, there is a statistically significant increase of the cytokine in LPS+ATP (p=0.0009) and LPS+BzATP (p=0.0039) relative to control in SVAs using a one way ANOVA with Tukey's multiple comparisons test. P2X₇ receptor expression increases between control (0mM glucose) and 5.5mM glucose in trophoblast at both 4 hours and 48 hours incubation. However, at high glucose levels (10-30mM), the expression of P2X₇ decreases. This study provides evidence that ATP+LPS stimulation of CPAs, SVAs and trophoblast can elicit more IL-1β production when compared to LPS+BzATP. Future work needs to be done to assess the functional effects of the P2X₇ receptor, as well as characterising the inflammatory response in the placenta vasculature.

[1] Chiao C.W., Tostes R.C., Webb R.C., 2008, P2X₇ receptor activation amplifies lipopolysaccharide-induced vascular hyporeactivity via interleukin-1β release, *Journal of Pharmacology and Experimental Therapeutics [e-journal]*, 326(3), pp. 864-870

[2] Khan R.N., Hay D.P., 2014, A clear and present danger: inflammasomes DAMPING down disorders of pregnancy, *Human Reproduction Update [e-journal]*, 21(3) pp. 388-405

[3] Sathanoori R., Swärd K., Olde B., Erlinge D., (2015), The ATP Receptors P2X₇ and P2X₄ Modulate High Glucose and Palmitate-Induced Inflammatory Responses in Endothelial Cells, *PLOS One [e-journal]*, 10(7)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC264

Maternal and offspring outcomes associated with duration of second stage of labour: A systematic review.

A. Ingram and N. Smith

School of medical science, University of Aberdeen, Aberdeen, UK

Objective:

To review systematically the evidence on prolonged second stage of labour and the maternal outcomes and neonatal outcomes.

Data sources:

Medline, Cochrane, Embase and CINAHL found 3580 papers by two-person review using an inclusion and exclusion criteria for both maternal and neonatal outcomes. 1 additional paper was identified by supervisor.

Methods:

An initial search was conducted on the online databases. Patients who had singleton vaginal births were included, along with studies of women who underwent emergency caesarean sections as complications of labour for differing reasons. A meta-analysis was performed to combine results on two outcomes, namely neonatal intensive care unit (NICU) admission and birth asphyxia. All other outcome results were presented using narrative synthesis.

Quality:

Quality was assessed using inclusion and exclusion criteria alongside CASP cohort study checklists.

Results:

This systematic review identified 16 papers to be eligible for inclusion. 13 of the 16 studies reported maternal outcomes. Post-partum haemorrhage, caesarean hysterectomy, chorioamnionitis, 3rd and 4th degree perineal tears, caesarean delivery and episiotomy were associated with prolonged second stage of labour. Post-partum haemorrhage showed between a 2 to 5-fold increase across the studies. Caesarean hysterectomy was linked with between a 6- and 7-fold increase and chorioamnionitis showed a 4 to 5-fold increase when labour was prolonged in the second stage in more than one study. 3rd and 4th degree perineal tears and episiotomy both were associated with an approx. 2-fold increase when second stage labour duration was prolonged. Caesarean delivery showed a 34-fold increase in incidence was second stage of labour became prolonged in one study. Ten studies papers were identified which reported neonatal outcomes. Prolonged second stage of labour was associated with a 1.35-1.85 -fold increase in neonatal intensive care unit admission. Duration greater than 4 hours was associated with a 2.5-fold increase in asphyxia.

Conclusion:

Prolonged labour second stage of labour is associated with some adverse maternal and neonatal outcomes, specifically: post-partum haemorrhage, caesarean hysterectomy, chorioamnionitis, 3rd and 4th degree perineal tears, caesarean delivery, episiotomy, neonatal intensive care unit admission and birth asphyxia. Reduction and better understanding of these outcomes involves future global standardization of the definition of prolonged second stage. This systematic review provides evidence to inform health professionals' and pregnant women's decision-making regarding management of labour when second stage is prolonged.

Laughon, S., Berghella, V., Reddy, U., Sundaram, R., Lu, Z. and Hoffman, M. (2014). Neonatal and Maternal Outcomes With Prolonged Second Stage of Labor. *Obstetrics & Gynecology*, 124(1), pp.57-67.

Rouse, D., Weiner, S., Bloom, S., Varner, M., Spong, C., Ramin, S., Caritis, S., Peaceman, A., Sorokin, Y., Sciscione, A., Carpenter, M., Mercer, B., Thorp, J., Malone, F., Harper, M., Iams, J. and Anderson, G. (2009). Second-stage labor duration in nulliparous women: relationship to maternal and perinatal outcomes. *American Journal of Obstetrics and Gynecology*, 201(4), pp.357.e1-357.e7.

Matta, P., Turner, J., Flatley, C. and Kumar, S. (2018). Prolonged second stage of labour increases maternal morbidity but not neonatal morbidity. *Australian and New Zealand Journal of Obstetrics and Gynaecology*.

Quiñones, J., Gómez, D., Hoffman, M., Ananth, C., Smulian, J., Skupski, D., Fuchs, K. and Scorza, W. (2018). Length of the second stage of labor and preterm delivery risk in the subsequent pregnancy. *American Journal of Obstetrics and Gynecology*, 219(5), pp.467.e1467.e8.

Grantz, K., Sundaram, R., Ma, L., Hinkle, S., Berghella, V., Hoffman, M. and Reddy, U. (2018). Reassessing the Duration of the Second Stage of Labor in Relation to Maternal and Neonatal Morbidity. *Obstetrics & Gynecology*, 131(2), pp.345-353.

I would like to express my appreciation to Dr Mairead Black for her guidance during this systematic review. I would also like to thank Nicole Smith for her work ethic and knowledge throughout our joint systematic review. Without Dr Black and Nicole this overall review would not have been possible.

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC265

Proteomic signatures of human placental vascular maturation

P. Palmowski¹, A. Treumann¹, R. Watson¹, J. Taggart¹, S. Robson², N. Europe-Finner¹ and M. Taggart¹

¹*Institute of genetic Medicine, Newcastle University, Newcastle upon Tyne, UK and*

²*Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK*

Vasculogenesis is the mechanisms by which new blood vessels are formed. Yet, the molecular changes underlying human blood vessel formation and maturation remain unresolved. The placenta is an organ forming de novo during pregnancy to support fetal growth. Extensive vascular formation and maturation is therefore crucial to this organogenesis and a successful pregnancy outcome and aberrant placental vascular form and/or function may contribute to common obstetric complications that impair fetal well-being and lifelong cardiovascular health so, possibly compromise cardiovascular health [Leijnse et al., 2018; Burton et al., 2016]. In this study, we have performed proteomic analysis of human placental arteries at different stages of pregnancy to investigate the nature of protein changes underlying human blood vessel maturation.

Following written informed consent (LREC 10/H0906/71), human placenta biopsies were obtained from 1st (7-12 weeks gestation, n=9) and 3rd (39-40 weeks gestation, n=7) trimesters of normal pregnancy. Chorionic plate arteries were isolated, homogenized, trypsin digested and analysed in triplicate with liquid chromatography mass spectrometry (LC-MS) using label-free SWATH (Serial Window Acquisition of All Theoretical spectra) acquisition [Ludwig et al., 2018; Rosenberger et al., 2014]. Proteins were quantified (≥ 5 fragment ions per peptide ion, ≥ 2 unique peptide ions per protein) in PeakView and differential protein abundance determined by unpaired t-test with multiple corrections FDR, followed by pathway analysis in STRING.

3586 proteins were quantified, with 1073 proteins differing between the 1st and the 3rd trimester (FDR 0.01 and >1.5-fold change). 482 proteins were up- and 591 down-regulated at term. Notable changes involved: (i) down-regulation of proteins regulating post-transcriptional processing (e.g. ribosomal and spliceosome-related proteins); (ii) up-regulation of those involved in ECM/myofilament/cytoskeletal integrity; (iii) up-regulation of Serpin superfamily proteins. Of these proteins, many were found to display upward or downward trajectories of expression across the early pregnancy timeline examined of 7 to 12 weeks gestation indicative of processes required for a commitment to a mature vascular cell phenotype.

Our study reveals an enormously dynamic proteomic milieu associated with human placental blood vessel maturation and provides a comprehensive characterization of the major underlying protein abundance changes. We identify key molecules (and processes) involved from early in pregnancy. We speculate that these molecules and pathways are also attractive candidates as markers of aberrant human vascular modelling whether that be in situations of (i) pregnancy complications accompanied by placental vasculopathy or (ii) systemic vascular diseases/injury resulting in smooth muscle de-differentiation (e.g. atherosclerosis).

Leijnse *et al.*, (2018) *Preg Hypertension* 13, 87-94.

Burton *et al.*, (2016) *Physiol Rev* 96, 1509-1565.

Ludwig *et al.*, (2018) *Mol Syst Biol* 14: e8126

Rosenberger *et al.*, (2014) *Sci Data* 1: 140031

Supported by MRC (MR/L0009560/1)

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PC266

Oleic acid inhibits oxytocin stimulated myometrial contractions in strips from pregnant rat myometrium.

N.M. Hag¹, M.J. Elmes² and A. Shmygol¹

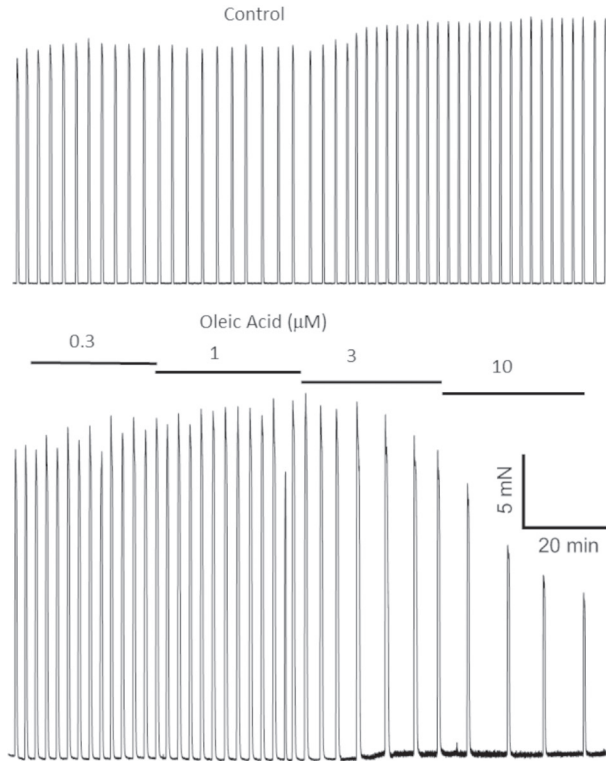
¹*Department of Physiology, United Arab Emirates University, Al Ain, United Arab Emirates* and ²*Division of Food, Nutrition and Dietetics, University of Nottingham, Loughborough, UK*

Obesity is a major health problem throughout the developed world, especially among women of childbearing age. In pregnancy, obesity is associated with an increased risk of prolonged and dysfunctional labour and increased risk of caesarean section, post-date pregnancy and post-partum haemorrhage. Our recent work using a rat model of maternal obesity has revealed decreased expression of Cx43, a major gap junction protein responsible for the synchronisation of uterine contractions, that was accompanied by desynchronised and weak contractions.

of the myometrial strips *ex vivo* [1]. Feeding the rats a high fat high cholesterol diet also leads to a significant rise in plasma concentrations of oleic acid, a mono-unsaturated fatty acid implicated in modulation of many regulatory processes in excitable cells [2]. The aim of the present study was to investigate the *in-vitro* effects of oleic acid on myometrial contractility in lean pregnant rats. The experiments were approved by the UAEU Animal Ethics Committee (ERA_2016_4261). The rats were humanly killed by cervical dislocation after CO₂ anaesthesia. Strips of longitudinal myometrium were dissected under the dissection microscope and mounted in an automated organ bath (TOBS 750, DMT, Denmark). After one hour equilibration, 0.25 nM oxytocin was applied and 40 min later water soluble oleic acid (Sigma O1257) was added accumulatively at concentrations ranging from 0.1 to 100 mM every 20 minutes. Time-matched control strips were exposed to vehicle only (Methyl- β -cyclodextrin (MCD), Aldrich 332615). The contractility of the myometrium was characterised by activity integral measured as area under the curve during the last 10 min at each concentration of oleic acid or MCD that was normalised to the activity integral of each strip before the additions of oleic acid or vehicle were commenced. The normalised activity integral values were compared between different concentrations using One-way ANOVA followed by Kruskal-Wallis test.

The results of our experiments show that oleic acid caused a dose-dependent inhibition of myometrial contractile activity at concentrations above 3 mM (Fig. 1, bottom trace) while the addition of MCD at concentration equivalent to that in 100 mM oleic acid caused small increase in activity integral (Fig. 1 top trace). At lower concentrations, there were no statistically significant changes in contractile activity. As illustrated in Fig. 1, the decrease in activity integral in response to oleic acid was due to decrease in both, the amplitude and the frequency of myometrial contractions.

In conclusion, our data show a direct inhibitory effect of oleic acid on myometrial contractility, which at least in part, may explain poor myometrial contractility in obese rats observed in our previous studies.



Representative tracings of myometrial contractions recorded from longitudinal strips exposed to vehicle (upper trace) of progressively increasing concentrations of oleic acid (marked by solid bars with concentrations above them).

Muir, R., Ballan, Clifford, J., McMullen, S., Khan, R., Shmygol, A., Quenby, S. and Elmes, M. (2016) Modelling maternal obesity: the effects of a chronic high-fat, high-cholesterol diet on uterine expression of contractile-associated proteins and ex vivo contractile activity during labour in the rat. *Clinical Science* 130, 183–192.

Muir R, Liu G, Khan R, Shmygol A, Quenby S, Gibson RA, et al. (2018) Maternal obesity-induced decreases in plasma, hepatic and uterine polyunsaturated fatty acids during labour is reversed through improved nutrition at conception. *Scientific Reports* 8(1):1–11.

Supported by the UAEU New Investigator Award and by the College of Medicine and Health Sciences

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

A

Abboussi, O. C108
 Abdo, M. PC204
 Abdul Kadir, L. PC046*
 Abdulkadir, Z.S. PC142
 Abdulla, M.H. C029
 Abdussalam, T.A. PC179*
 Aberdein, N. C086*
 Aboajah, N.A. PC170
 AbouDaya, H. C011*
 Abubakar, A. PC189
 Adagbada, E. PC019, PC137
 Adams, D.J. PC207
 Adams, Z. C055, PC020
 Adamson, A. PC029
 Adaniwimowan, E. PC040
 Adedapo, A.A. PC006, PC089*
 Adedayo, L.D. PC242*
 Adeghate, E. PC172
 Adejare, A. PC254
 Adejumobi, O.A. PC006*
 Adele, B.O. PC156
 Adeleye, A.I. PC170
 Adeleye, O.E. PC170*
 Adeoye, B. PC089
 Adetayo S., A. PC142
 Adetomiwa, A.S. PC170
 Adewoye, E.O. PC156
 Adeyanju, O.A. PC134*
 Adeyemi, A. PC155
 ADEYEMI, T.E. PC181*
 Adeyileka, B. PC057
 Agace, W. SA059
 Agouni, A. PC205*
 Ahmad, S. C007
 Ahmed, K.M. PC190*
 Aiku, A.O. PC019*, PC137*
 Ainerua, M.O. C001*
 Ainslie, P. PC099, PC100, PC250
 Aird, T.P. C071
 Aiyedun, J.O. PC090
 Ajayi, A. PC219, PC232
 Ajime, T. PC187*
 Ajonijebu, C. C098
 Ajonijebu, D.C. C108*
 Akande, O.O. PC215
 Akangbou, P. PC038
 Akaniro-Ejim, N. PC087, PC186
 Akhtar, S. PC244*
 Akiyama, K. PC160
 Akter-Miah, T. PC117
 Al Bakour, Z. C118*

Al Kury, L. PC021, PC251
 Al-Moubarak, E. PC016*
 Al-Owais, M. PC042*, PC043*
 Al-Salam, S. PC011
 Al-Shanti, N. PC093
 Al-Subu, A.M. C120, PC253
 Alaagib, N.A. C019*
 Alada, A. PC155, PC173*
 Alamdari, N. C069, C070
 Albloshi, A.M. PC128
 albuhtori, M. PC059*
 Alcaide-Corral, C.J. PC031
 Aldhous, M. PC201
 Alexeenko, V. C006*
 Alghamdi, A.M. PC014*
 Alghamdi, F. PC141*
 Alhassan, A. PC220
 Ali, I.I. PC011
 Alkawadri, T. C113*
 Alkhalil, M. PC106
 Allaway, D. PC158
 Allen, C. PC243
 Allison, B.J. C077
 Alm, P.S. C082
 Alnoor, T.A. PC177*
 Alshehri, Z. PC009*
 Alshwaira, N.R. PC044*
 Altaf, Q. PC009
 Altekena, F. PC177
 Althaus, M. C046, PC082
 Altintas, A. C079
 Altuwaijri, N. PC228*
 Alvarez, D. PC183
 Alves, F. PC026
 Alzahrani, A. PC008*, PC017
 AMAO, O.S. PC195*
 Ambali, H.M. PC090*
 Ambrosio, L. PC125, PC126
 Ameen, S.A. PC090
 Amici-Dargan, S. C033
 Amosah, J. C042*
 Anderson, G.C. C017*
 Anderson, M. C097
 Ando, S. PC184
 Ang, R. C018
 Anyiyeloye, M. PC038, PC154, PC168
 Arber, S. PL04*
 Arbuthnott, G.W. PC239*
 Arc-Chagnaud, C. SA016
 Archer, S. C042
 Areta, J.L. PC097*
 Arikawe, A.P. PC246

Arnott, A. PC073
 Arrowsmith, S. C115*
 Arvaniti, A. C088
 Asamudo, E. PC254
 Ashford, M. C117
 Ashley, B. PC202
 Ashley, J. PC103, PC126
 Ashley, J.D. PC125*
 Aspden, J. PC209
 Atherton, P. C061, PC095, PC101,
 PC146, PC174, PC180
 Atherton, P.J. C059, C067, PC113,
 PC119, PC130
 Atkinson, L. C100
 Attwell, D. C114
 Ayagama, T. PC030*
 Ayar, A. C126, PC241*
 AYAR, A. PC133
 Aye, C. PC151
 Ayo, J.O. PC189
 Azeez, O.M. PC090, PC142*
 Azeez, T.O. PC246
 Aziz, M. C059, PC119, PC180

B

Babiker, R. PC169*
 Bach, K. SA009
 Bach, V. PC161
 Backx, K. PC107
 Baczko, I. PC022
 Badaam, K. PC076*
 Badejo, J.A. C003*
 Badhwar, S. C109*
 Bagley, L. PC060
 Bailey, D. PC102, PC105, PC123,
 PC184, PC248, PC250
 Bailey, D.M. C072*
 Bailey, M.A. C043, C110
 Bailey, S. C009
 Baines, D. C037, C050, PC050,
 PC051, PC081
 Baker, D. PC178
 Baker, R. C055
 Balchin, C. PC112*
 Baldwin, M.M. PC118*
 Bamford, J.C. PC069
 Banaszkiwicz, A. PC083
 Banks, G. PC193
 Banks, R.W. C103, PC210, PC214*
 Barak, O.F. PC099*
 Baranidharan, G. C107
 Baranova, T. PC039
 Barbieri, R. SA053
 Barclay, N. PC115
 Bari, M. PC131
 Barlow, J. C068*
 Barres, R. C079
 Barrett-Jolley, R. C104, PC046,
 PC222, PC226
 Barrett, D. C083
 Barrett, P. PC193
 Barrow, J. PC064*, PC065*,
 PC066*, PC067*
 Basic, M. C048
 Bass, J.J. C067, PC146*
 Bast-Habersbrunner, A. SA032
 Bates, D.O. PC243
 Batis, N. PC017
 Batista, C.C. C089*, PC196
 Batson, J. PC243
 Bay, S. PC218
 Beard, N.A. C007
 Beattie, J. C125
 Becker, N. C095
 Beckett-Brown, N. PC110*, PC120
 Beckler, M. PC026
 Bednall, R. PC158
 Bednarek, M. PC138
 Beech, D. PC252, PC255, PC257, PC258
 behl, S. PC163*, PC164*
 Bello, U. PC189
 Beltrame, T. PC092*
 Benedetto, R. SA052*
 Benson, A. SA012*, PC042
 Benter, I. PC244
 Bentley, L. PC157, PC193
 Bermudez, D. PC100, PC250
 Bertelli, S. SA053*
 Bescos, R. C065*
 Bewick, G.S. C103*, PC210, PC214, PC216
 Bhagat, O. PC013*
 Bhalla, S. PC243*
 Bidasee, K. PC172
 Bielak-Zmijewska, A. PC245
 Bielinska, K. PC005, PC083
 Biggart, M.G. PC050*
 Billert, M. PC198*, PC199, PC200
 Bindu Chandrasekhar, B. PC244
 Biobaku, K.T. PC090, PC142
 Birch, K.M. PC118, PC132
 Bittner, R. SA056
 Black, A.D. C060*
 Black, S. C107*

Blackley, Z. PC243
 Blasco-Lafarga, C. PC096*, PC124
 Blasco-Lafarga, N. PC096, PC124
 Bleich, A. C048
 Boller, A. SA015
 Bolze, F. SA032
 Bond, H.L. PC117
 Booth, D. PC070
 Borlaug, B.A. SA044
 Borovik, A. C066*, PC108
 Bosson, J. SA003*
 Botting, K.J. C077
 Bourbia, N. PC193
 Bouzerara, A. PC161
 Bovell, D. C023*
 Bowen, M. C006
 Boyett, M.R. SA033*, C005, PC036
 Brackenbury, W.J. C044
 Bradshaw, A. PC262
 Bradshaw, K. PC077*
 Brain, K. PC008
 Brain, K.L. C077
 Brand, K. C009
 Breeze, A. PC255
 Breitenbucher, J. C118
 Bretherton, B. C100*, C107
 Brinkmeier, H. SA056
 Brinkwirth, N. C095
 Broadbent, S.D. PC217*
 Brook, M.S. C059, C067*, PC113, PC119
 Brooks, P.M. PC128
 Brown, A.M. PC212, PC221*
 Brown, C. PC088
 Brown, M. SA011*
 Brownbill, P. C075
 Brüggemann, A. C095
 Brugniaux, J. PC102, PC248
 Brunton, P. PC235
 Bryson, K. PC069
 Budge, H. PC182
 Buendia, I. SA028
 Bulmer, D.C. SA009
 Burchell, A. C010
 Burggren, W.W. PC206
 Burrows, P. PC063
 Burton, F.L. C016
 Burton, G.J. C077
 Burton, R. PC030
 Bussayajirapong, A. C040
 Bussey, C.T. SA034*
 Butler, R. PC045
 Byrne, T. PC053

C

Cabrita, I. SA052
 Cai, Z. SA027
 Calaghan, S. PC034*
 Caldwell, H.G. PC250
 Caldwell, J. SA048*, PC018
 Callejo, G. SA009
 Calverley, T.A. PC105, PC123*
 Camm, E.J. C084, C106
 Campbell, A. C111
 Campbell, T. C102*, PC053
 Cannon, D.T. PC131*
 Cano-Abad, M. SA028
 Cao, L. PC008
 Capel, R. PC030
 Carrasco, D.I. SA055
 Carretero, A. SA016
 Carrie, A.A. PC063
 Carrillo-Franco, C. PC208
 Carrillo-Franco, L. PC208
 Carson, B.P. C071*
 Carter, K.J. C120, PC253*
 Carter, N. C094
 Carvalho, J. PC096
 Castrucci, A.D. PC185
 Catai, A. PC092
 Cataliotti, A. C013
 Catherine, B. PC113
 Causer, A.J. C009*
 Caves, R.E. C004
 Cegielski, J. C067, PC113*
 Celik, N. PC226*
 Chadwick, M.R. PC118
 Chan, N. PC063
 Chandler, C. C022
 Chandran, D.S. C109
 Chandran, S. PC216
 Chang, Y. PC049*
 CHANNA, M. PC181
 Chant, B. C010, PC020
 Chaturvedi, N. PC122
 Chavda, J. PC052
 Chen, C. C103
 Chen, R. SA029
 Chen, X. PC050
 Chen, Y. SA039*
 Cheng, H. C004
 chernyavsky, I. C075
 Chiang, M. PC049
 Chichger, H. C054
 Chilian, W.M. SA026
 Chipperfield, A.J. SA061*

CHO, Y. PC024
 Choate, J. PL01*
 Chodorge, M. PC178
 Choisy, S.C. C004
 Choudhury, N. C097*
 Christian, M. SA031*
 CHUEMERE, A.N. PC038*, PC104,
 PC114, PC121, PC154*, PC168*
 Chung, G. PC088
 Church, C. PC178
 Church, H.L. C056*
 Cid, L. C041
 Cindrova-Davies, T. C077
 Ciroma, F.L. PC175*
 Clark, J.E. C036*
 Clarke, J. SA048, PC035
 Clarke, L.L. SA054*
 Cleal, J. PC202*
 Cleasby, M.E. PC146
 Clennell, B. C004
 Clough, G.F. SA061
 Coate, T.M. PC128
 Cockcroft, J.R. SA021, SA022, SA023,
 SA024, PC107
 Cocks, M. C056
 Coenen, F. PC226
 Coffman, K. SA013*
 Colquhoun, D. C031*
 Conde, S. C012*, C089, PC054,
 PC159, PC196
 Coney, A. PC008, PC017
 Connolly, S. C022
 Coombey, F. PC078*
 Coombs, G.B. PC100
 Cooper, C. PC071
 Cooper, R. PC117
 Cope, T.C. SA055*
 Cordellat, A. PC124
 Cormier, S. C002
 Cornejo, I. C041
 Cornwell, W.K. SA021, SA022, SA023,
 SA024*
 Correias, A. SA016
 Correia, C. SA041
 Coull, B. C117
 Cowpland, L. PC073
 Cox, J.J. SA008
 Cox, R. PC157
 Crane, I.J. PC059
 Crombie, E. PC095
 Cross, L. PC061
 Crossland, H. PC101*

Crotty Alexander, L.E. SA018*
 Cruickshank, S. SA059, PC057
 Cuhna, M. SA028
 Cui, Z. C052*
 Cummings, M. C009
 Cunningham, K. SA039
 Cutler, C. C065
 Czopek, A. PC047

D

Dalibalta, S. PC109*
 Danboyi, T. PC220*
 Daniels, W. C108
 Dapper, D.V. PC037*, PC038, PC104*,
 PC114*, PC121*, PC154, PC168
 Dare, A. PC140*
 Dass, M. SA038
 Davidson, A. PC065
 Davies, B. PC105
 Davies, D. PC061
 Davies, G.R. PC178*
 Davies, K. C084*
 Davies, K.I. PC104
 Davis, C. C022
 Davis, D. PC102
 Davison, G. PC109
 Dawid-Milner, M. PC208
 de Assis, L.V. PC185
 de Castro Barbosa, T. C082
 de la Rosa, A. SA016
 Deane, R. PC079
 Deepak, K.K. C109, PC013
 Degens, H. C064, C116, PC187
 Dekeryte, R. C073
 Delibegovic, M. C085, PC150
 Delpiano, L. C045
 Demolder, M.J. SA006*
 Dempsey, C.E. PC010, PC016
 Demydenko, K. PC025*
 Denholm, B. PC012*
 Denison, F. PC201
 Deuchars, J. C100, C107, PC209*,
 PC228, PC229
 Deuchars, S. C100, C107, PC209,
 PC228, PC229
 Dhaun, N. PC047
 DHENNIN-DUTHILLE, I. C038
 Diaz-Ramos, A. PC151
 Diaz, M. PC012
 Dibb, K. SA048, C014, PC018,
 PC033, PC035

DiMarco, A.F. PC236*, PC237*, PC238
 Dirks, M.L. C069, C070
 Djajadikarta, Z.J. C063*
 Djekkoun, N. PC161
 Dobi, S. C111*
 Dockerill, C. PC106*
 Dodgson, J. PC138, PC178
 Doherty, M.K. PC151
 Donaldson, J. PC167*
 Donaldson, L.F. SA010*
 Dong, B. C002
 Donowitz, M. C040
 Dorrello, N. SA039
 Dove, R. PC052
 Dovey, J.L. C090*
 Doyle, E. C047
 Dragicevic, E. PC026
 Drake, A. PC201
 Drapala, A. PC005
 Dries, E. SA049*
 Dryn, D.O. C121, PC251
 Duarte, J.R. PC096
 Duchen, M. SA028
 Duffy, E. PC061
 Dujic, Z. PC099
 Dulajova, S. C051
 Dulhunty, A. C007
 Dumbell, R. PC157*, PC193*
 Duncker, D. SA027
 Dunn, W.B. C061
 DUTTA CHOWDHURY, S. PC136*
 Dweck, M.R. PC031
 Dyson, J. C118
 Dziekiewicz, M. PC083

E

E Hall, J. C086
 Earley, S. C123
 Eddie-Anunobi, O.F. PC156
 Edupuganti, O. C049
 Egana, M. C062
 Egginton, S. C116, PC256
 Eid, A. PC205
 Eisner, D. SA048, C014,
 PC033, PC035
 El Khayat El Sabbouri, H. PC161
 El-Hashim, A. PC244
 Elder, H.Y. PC023
 Eldridge, M.W. C120, PC253
 Ellis, J. C081
 Elmes, M.J. PC266

Elneel, A. PC001
 Else, K. SA059
 ELWAZIR, Y.M. PC204*
 Emediong, I.E. PC156
 Emem-Chioma, P. PC104
 Endo, M. PC139
 Enrick, M. SA026
 Epplin, M.P. PL03
 Erlich, A. C075
 Erlwanger, K.H. PC167
 Erram, J. PC131
 Espinosa, A. PC183
 Etim, U. PC089
 Europe-Finner, N. PC265
 Evans, B. C054
 Evans, H. C086
 Eziuzo, C. PC037

F

Facchi, C. PC029
 Falayi, O. PC089
 Fall, L. PC105, PC123
 Fallon, C.M. C049*
 Falodun, T. PC134
 Fares, Y. PC079
 Farouk, M. PC204
 Farquharson, C. PC153
 Farrash, W.F. PC095*, PC119
 Farrell, A. PC080
 Fasanmade, A. PC019, PC137
 Fazeali, M. PC135
 Fedorenko, O.A. PC186
 Feetham, C.H. C104, PC231*
 Felder, E. PC055
 Felder, T. PC055
 Felton, M. PC002*
 Ferguson, C. PC118, PC132
 Fernandes, J. PC235
 Fernandez-Cuervo, G. PL03
 Fernández-Puente, E. PC148
 Fertig, N. C095, PC026
 Figueroa-Mujica, R.J. PC100
 Filimonova, N. PC224
 Filipovic, M. PC176
 Filipponi, M.T. PC248*
 Finn, G. C022
 Fischer, R. PC030
 Fitzpatrick, E. C002
 Flatman, P.W. C043
 Fodor, P. PC263*
 Forbes, C. PC106

Ford, G. PC229
 Ford, S.G. C077
 Forhead, A.J. C084
 Forker, C. PC064
 Forman, R. SA059
 Formenti, F. PC106
 Forsythe, I.D. C097
 Foulke-Abel, J.D. C040
 Fowden, A.L. C084, C106
 Frade, M. PC092
 Fraser, J.A. C006, C007
 Frazier, S. PC262*
 Freestone, N.S. C021*, PC058*
 Freitas, F. C114*
 Frick, M. SA038
 Frisbee, J. SA058*
 Fromme, T. SA032
 Fuentes, G. C039
 Furuse, M. PC084

G

Gabler, S. SA032
 Gabriel, B. C079*, C082
 Gadegaard, N. C016
 Gagnon, D. PC098*, PC110, PC115*,
 PC120, PC176*
 Gallagher, I.J. C061, PC146
 Gallant, E.M. C007
 Galli, G.L. C015
 Gandevia, S.C. C063
 Gardiner, M. PC065
 Garnett, J.P. SA038
 Garnier-Amblard, E. PL03
 Garrud, T.A. C077
 Garthe, I. PC097
 Gathercole, L. C088
 GAUTIER, M. C038
 Gavazzo, P. SA053
 Gawrys-Kopczynska, M. PC005
 Gay-Quéheillard, J. PC161
 Gayan-Ramirez, G. PC187
 Geneci, G. PC138
 George, M. C095
 Georgiadou, P. PC017
 Gerwin, L. SA056
 Gettings, S.M. C046*, PC082
 Ghadhanfar, E. PC003*, PC004
 Ghani, N. PC229*
 Gharahdaghi, N. C059*, PC095,
 PC119*, PC174
 GHOSH, R. PC136

Gildea, N. C062
 Gillespie, A.M. PC069
 Gillotin, S. PC217
 Ginesi, L. PC074*
 Giommi, A. PC041*
 Girkin, J.M. PC260
 Giuraniuc, C. PC210*, PC216
 Giussani, D.A. C077
 Gladman, J. PC113, PC130
 Gleghorn, L. C045
 Goacher, P. PC074
 Goetze, T.A. C095
 Gohl, A. C047
 Gois, M. PC092
 Gómez-Cabrera, M. PC096
 Gomez-Cabrera, M. SA016*, PC124
 Gomez-Niño, A. PC054
 Gonçalves, J. PC227
 Gonzalez-Garcia, M. PC208*
 Gonzalez-Obeso, E. PC159
 Gonzalez-Rodriguez, L.G. C087
 Gordillo, A. PC054
 Gordon, E. C002
 Gorin, Y. PC205
 Gorissen, S. C067
 Gorman, L. PC053*
 Gourine, A.V. C018
 Graham, D. PC262
 Grainne, C. PC052
 Grant, A. C022
 Gray, G. PC031
 Gray, J. C023
 Gray, M. C008, C045
 green, S. C062
 Greenhaff, P.L. C061, PC130
 Greenin, K. PC229
 Greensmith, D.J. PC028*, PC060
 Greenstein, A. C123
 Gregory, J.S. C026*, PC069*
 Griffin, C. C123
 Griffin, J. PC178
 Griffiths, C. PC052
 Grigg, J. SA019
 Grochowska, M. PC245
 Grøntved, L. SA041*
 Guanzon, D. C076
 Guibourdenche, J. PC161
 Guibourdenche, M. PC161*
 Gurgel, A.R. PC041
 Gutierrez, L. PC144
 Gutteridge, A. SA009
 Gyulkhasyan, T. SA026

H

Haarmann, C.	C095	Hettiarachchi, N.	PC042
Hadgraft, N.E.	PC028	Heussaff, O.	C047
Hag, N.M.	PC266	Hey, F.	PC202
Hagen, S.A.	C120, PC253	Hezelgrave, N.	C076
Hague, F.	C011	Hickson, M.	C065
Hakamata, A.	PC192	Hills, C.E.	C078
Hakobyan, T.	SA026	Hinton, T.	C010, C055, PC020*
Hall, S.	C033*	Hirsch, M.	PC045
Hamann, M.	C099	Hislop, J.	C035, C118
Hammond, D.E.	PC151	Hockley, J.	SA009*
Hancox, J.C.	SA001, C004, PC010, PC016	Hoffman, P.	C073
Handyside, J.	PC071	Hoffmann, U.	PC092
Hansen, R.	C058	Hoiland, R.L.	PC099, PC250
Hanson, S.	PC074	Holden, A.	PC042
Hardy, E.	PC174	Hollywood, M.	C113
HARDY, M.E.	C034*	Holmes, A.P.	PC008, PC017*
Hardy, T.A.	PC132*	Holroyd, S.	C023
Harkes, T.	C081	Hong, T.	SA047*
Harper, A.A.	PC207*	Hope, K.	C055
Harris, S.	C088*	Hope, K.A.	C010*, PC020
Harrison, M.	PC158	Horbunov, O.	PC224
Hart, E.C.	C010, C055, PC020	Hornigold, D.C.	PC138
Hartell, N.	C099	Houghton, M.	PC166
Hartley, P.	PC012	Housley, S.N.	SA055
Harvey, N.	PC202	Houweling, P.	PC129
Hashimoto, T.	PC184	Howard, L.	SA014*
Hassan-Danboyi, E.	PC220	Howarth, F.C.	PC011, PC021*, PC172
Haughney, D.	PC069	Howe, C.A.	PC100, PC250
Haupt, C.	SA056	Howells, S.	C034
Hayashi, H.	PC084, PC085*, PC086	Huang, C.L.	C006, C007, PC045
Haydock, R.	PC158	Huethorst, E.	C016*
Head, S.I.	PC129*	Hughes, A.D.	PC122
Heagerty, A.	SA059	Hughson, R.	PC092
Healy, M.	C023	humeda, H.	PC001
Heilker, R.	SA038	Humeda, H.	PC177
Heisler, L.	PL02*	Hundley, V.	PC002
Helen, W.	PC052	Hunter, A.	PC071
Helfer, G.	PC197*, PC233*	Hurley, M.E.	PC027*
Hellgren, K.T.	C015*	Hurmach, V.V.	PC251
Helyer, R.	SA004*	Hurst, J.	C064
Hempstock, W.	PC084*	Hurst, W.	PC064
Henderson, V.	PC071	Hussell, T.	PC048
Hendrickse, P.	C116	Hutch, T.	PC245
Heritage, S.R.	C106	Hutchings, D.C.	C014*
Hernansanz-Agustin, P.	SA028	Hutsch, T.	PC005, PC083
Herrick, S.	PC048		
Herrod, P.J.	PC095, PC119, PC174*		
Hesketh, K.	C056		
Hess, R.M.	C084		
Hession, K.	PC180		
Hetherington, C.D.	PC216*		

I

Ian, M.	PC052
Ibironke, G.F.	PC215
Idris, I.	C059, PC119, PC180
Igbokwe, O.B.	PC246*

Ige, A.O. PC156
 Ihalainen, S. C051
 Ilesanmi, L.K. PC140
 Ilochi, O. PC038, PC154, PC168
 Immanuel, C. C002
 Ingram, A. PC264*
 Inneh, C. PC040*
 Inns, T.B. PC127*
 Iraki, J. PC097
 Irons, J.R. PC045
 Ishibashi, A. PC184
 Ishida, K. PC106
 Ishii, T. PC160, PC192*
 Ishizuka, N. PC084, PC085, PC086*
 Ismail-Badmus, K. PC254
 Itani, N. C087*
 Ivanova, I.V. PC251
 Ivy, J.R. C043*
 IWHIWHU, F.O. C098*
 Iyama-Boma, C.O. PC114, PC121

J

Jackson, A.P. PC045
 Jackson, S. C050
 Jacob, J.G. PC081*
 James, A.F. C004*
 JAMES, S. PC052
 Jameson, T.S. C069, C070*
 Janjic, N. PC099
 Jaryal, A.K. C109
 Jaszczwili, M. PC198, PC199, PC200*
 Jaworska, K. PC005, PC083*, PC245
 Jayabalan, M. PC032
 Jayasinghe, I. PC027, PC034
 Jeevaratnam, K. C006
 Jenkins, H.J. C076*
 Jenkins, S. SA029
 Jenkinson, A. C035
 Jensen, R. PC188
 Jimoh, A. PC189, PC220
 Johnson, B. SA013, SA044
 Johnson, D.L. PC112
 Johnson, W. PC179
 Johnstone, D. C093*
 Johnstone, E. C075
 Jolly, J. C022
 Jones, G. PC105
 Jones, J.F. C102, PC053
 Jones, K. PC202
 Jones, N.K. C110*
 Jones, R. C080*

Jones, S. PC122*
 JOST, N. PC022*
 Juhasz, K. PC026
 Juhasz, V. PC022
 Jury, J. PC073

K

K, R. PC249
 Kablan, S. PC218
 Kaddam, L. PC001, PC177
 Kalakoutis, M. SA015
 Kalnysh, V. PC224
 KALSI, H.S. PC052*
 Kalsi, K.K. C050*
 Kalvisa, A. SA041
 Kamli-Salino, S. C073, PC150*
 Kamran, M. PC135
 Kantamneni, S. PC197
 Kanwar, M. SA021, SA022,
 SA023*, SA024
 Karamat, M. PC009
 Karavadra, M. C045
 Kassem, N.A. PC079*
 Katwan, O. C074
 Kay, I. PC060
 Kay, J.W. PC023
 Kazmierczak, S. PC199
 Kearns, I. PC229
 Keely, S. C040, C049
 Kellawan, J. PC103*, PC125, PC126
 Kennedy, C. PC234
 Kennedy, C.B. C105
 Kennedy, S. C074
 Keown, M. C027, C028*
 Kerimi, A. PC166
 Kerr, K.M. C119
 Kersten, S. SA042*
 Khalaf, M. C009
 Khan, F. C117
 Khan, H.A. C034
 Khan, I. PC081
 Khan, Q.U. PC191*
 Khan, R. PC263
 Kharazmi, F. PC135
 Khatri, A. PL03
 Khodai, T. PC230
 Khorsi-Cauet, H. PC161
 Kim, D. C083
 King, L. C054
 King, R. C060
 Kini, A. C048*

Kiriaev, L.	PC129	Latifi, S.	C023
Kirkman, J.	C035	Law, J.	PC182
Kirkwood, P.A.	PC238	Lawrence, C.	SA059
Kirton, H.M.	PC027	Lawrence, S.M.	C044
Kissane, R.	PC256*	Lawson, S.D.	PC114, PC121
Kitmitto, A.	C005	Lawton, P.	PC260
Klingenspor, M.	SA032*	Lazzari, C.	PC211*
KO, W.	C053*	Le Maitre, C.	C086
Koido, S.	PC086	Le, H.Q.	SA038
Kolawole, T.	PC154	LeBlanc, A.J.	SA025*
Kolstad, T.R.	C013*	Lechner, A.	SA040
Kolz, C.L.	SA026	Lee, C.	C103
Kompella, S.N.	SA001, C001	Lee, D.Y.	PC205
Konop, M.	PC005, PC083, PC245	LEE, J.	PC024
Kopach, O.	PC213*	Lee, J.W.	C120, PC253
Kopp-Scheinpflug, C.	C097	LEE, M.	SA051*
Koprowski, P.	C101	Lee, M.D.	PC259*, PC260
Koschate, J.	PC092	Lees, M.	C060
Kotler, I.	C057	Leeson-Payne, A.T.	C103
Koutsikou, S.	PC240	LEFEBVRE, T.	C038
Kowalski, K.E.	PC236, PC237, PC238*	Leon, R.	SA028
Kramer, H.	PC030	Lettieri, M.	PC029*
Krause, F.	PC178	Levins, K.	C105, PC234
Kravenska, Y.	C101*	Lewallen, M.	PC206*
Kristian, P.	PC052	Lewis, C.T.	C119*
Kristiansen, M.R.	PC188	Lewis, J.	PC158*
Kröger, S.	SA056	Lewis, R.	C075, PC202
Krook, A.	C079, C082	Lewisohn, M.	PC061
Krueger, T.	PC047	Li, J.	PC091*
Kruth, M.	PC216	Li, X.	PC151
Kueh, S.	PC129	Li, Y.	SA032
Kumar, P.	PC008, PC009, PC017	Liebeskind, L.S.	PL03
Kunzelmann, K.	SA052	Lightfoot, A.	PC117
Kurt, A.	C126*, PC241	Lillo, P.M.	PC147*
Kwakye, A.	C057	Lin, J.	C008*
Kyza, I.	C081	Linares, S.	PC092

L

L, S.	PC249	Lionikas, A.	PC128*
Laber, S.	PC157	Liotta, D.C.	PL03
Labram, B.	PC048	Lippiat, J.	PC043
Lagos, D.	C044	Litherland, G.	C113
Lai, A.	C076	Little, R.	PC166*
Lajczak-McGinley, N.K.	C040, C049	Liu, H.	SA039, PC049
Land, S.C.	PC070*	Liu, J.	SA054, C052
Lane, C.	C123	Llanos, P.	PC147
Laneelle, D.	C072	Lofthouse, E.	PC202
Lang, R.	C094	Lopez-Gonzalez, M.	PC208
Lanner, J.	SA015	Lopez, M.	SA028
Laranjinha, J.	PC227	Louch, W.E.	SA050*, C013
Lasisi, T.J.	PC155	Louise, C.	PC052

Lourenço Marques, C.F. PC227*
 Lucatelli, C. PC031
 Luckman, S. PC230, PC231
 Lugo Leija, A. SA030
 Lund, J. C057
 Lundy, F. C113
 Lusiantari, R. PC162
 Lutz, A. PC055
 Lyall, G.K. PC132
 Lyons, T. C047
 Lyu, Q. C077

M

M Anton, P. PC161
 Mabandla, M. C108
 MacAskill, M.G. PC031*
 MacLean, D. PC110, PC120
 MacMillan, F. PC061*, PC063*
 Macrae, V.E. PC153
 Madders, G.W. C014
 Mader, T. SA015
 Maes, K. PC187
 Mahmood, S. C073, PC145*
 Makarchuk, M. PC224
 Malcolm, C. C035*
 Maliqueo, M. PC183
 Malkoc, A. PC241
 Mallinson, J. C080
 Mann, B. C004
 Manning, J. PC201
 Mansley, M.K. C043
 Marczylo, T. SA017*
 Marini, P. PC065
 Marley, C. PC102*, PC105, PC123,
 PC184, PC248
 Marr, C. C006
 Marra, V. C097
 Marris, C. SA001*
 Marsh, D. PC110, PC120
 Martin-de-Saavedra, D. SA028*
 Martin-Fernandez, M. PC045
 Martin, K. C093
 Martinez-Murillo, R. SA028
 Martinez-Ruiz, A. SA028
 Mastitskaya, S. C018*
 Matsakas, A. C068, PC144, PC152
 McBride, M. PC262
 McCaffery, C. C117
 Mccaig, C. PC094
 McCarron, J.G. PC259, PC260
 McCaul, C. PC053

McConnell, A. PC002
 McCormick, D. PC101, PC127, PC130*
 McCue, A. PC110, PC120*
 McDermott, A.D. C062*
 McDonnell, B.J. SA021, SA022,
 SA023, SA024
 Mcewan, F. PC230
 McGahon, M. C025
 McGarvey, L. C113
 McGonnell, L. PC222
 McGuigan, J.A. PC023*
 McKeown, L. PC252, PC255,
 PC257, PC258
 McKinley, A. PC073
 Mclean, S. PC233
 McMurray, G. SA009
 McNiff, C. C004
 McPhee, J. C064, PC093, PC101
 McStea, A. PC045
 Meah, V.L. PC107*
 Meakin, P.J. C117*
 Melnyk, M.I. C121, PC251
 Melo, B.F. C012, C089, PC196
 Menaldino, D.S. PL03
 Méndez, L. PC148
 Menolascina, F. PC047
 Merkus, D. SA027*
 Messa, G. PC187, PC240*
 Messa, G.M. C064*
 Metcalfe, J. PC138
 Mijacika, T. PC099
 Mijuskovic, A. C115
 Miklavc, P. PC055*
 Mikus, C. C069, C070
 Miles, G. PC216
 Millan, F. SA016
 Miller, M.R. SA002*
 Milne, E. PC153
 Milnes, J.T. C004
 Mitchell, R. PC144
 Miteva, K. PC257*, PC258
 Miyashita, L. SA019*
 Mody, N. C073, PC145, PC150
 Mohamed, O. PC021
 Mohammed, A. PC189*
 Mohammed, M. PC177
 Molopo, M.C. PC167
 Molsberger, A. PC094
 Momodu, O. PC040
 Money, A. PC252*
 Monteagudo, P. PC124
 Monteiro, E.C. C012

Mora, S. PC149, PC151*
 Moraes, M.N. PC185*
 Moreno-Ortega, A. SA028
 Morgan, H. PC262
 Morgan, R. PC222*
 Morgan, T. PC031
 Morley, J. PC129
 Morrice, J. PC069
 Morrice, N. PC145
 Morris, J. PC243
 Morrison, D. C060
 Morsy, S. PC204
 Mort, E.J. C106*
 Morton, N. PC153
 Mosieniak, G. PC245
 Mulle, C. PC214
 Müller, T. PC055
 Mullins, J.J. C110
 Mulvana, H. PC262
 Mungall, W. PC031
 Muniz, A. PC149*
 Munten, S. PC098, PC115, PC176
 Murphy, P. C025
 Murray, A.J. C077, C084
 Murray, F. C035, C118
 Musa, O.A. C019
 Musa, R.M. PC001*
 Muso, M. PC157
 Mutch, N. C125
 Muttagina, A.F. PC015

N

Nabofa, W. PC173
 NADAR, A. PC181
 Nagy, Z. PC022
 Najem, H. PC033
 Nakayama, M. PC085
 Namvar, S. PC048*
 Nandi, M. SA060*
 Narang, R. C109
 Narayanasamy, K.K. PC027
 Nardelli, P. SA055
 Nasa, A. C105, PC234
 Nathan, C. PC209
 Nathanielsz, P.W. C087
 Navarro, E. SA028
 Naylor, J. PC138*
 Negulyaev, V. C066
 Nelson, M. C123
 Nevin, A. C062
 Newby, C. PC052

Newby, D. PC031
 Nichols, B. PC034
 nielsen, J. PC188
 Nielsen, P. C058
 Niemeyer, M. C041
 Nieznanska, H. C101
 Nieznanski, K. C101
 Nightingale, A. C010, C055, PC020
 Nikolaou, N. C088
 Niu, Y. C077
 Nixon, G. C085, C119, PC056
 Nolan, P.M. PC193
 Nordén, E. C013
 Norman, J. PC201
 Norman, R. PC027, PC034
 Normand, H. C072
 Norstedt, G. PC021
 North, K. PC129
 Nowak, K. PC200
 Nowak, K.W. PC198, PC199
 Nuñez, O. PC147
 Nurmasitoh, T. PC162*
 Nusair, A. PC211
 Nye, G.A. C075*, PC117

O

O'Brien, F. C104*, PC046, PC226
 O'Brien, K. C077
 O'Donovan, D.J. PC203
 O'Hanlon, E. C105, PC234
 O'Hara, J. C060
 O'Hare, D. PC064
 O'Keane, V. C105, PC234
 O'Malley, D. C092
 O'Shea, D. C062
 Obaid, A. PC088*
 Obergrussberger, A.R. C095*, PC026*
 Obeso, A. PC054, PC159*
 Odetola, A.O. PC156*
 Ogbona, S. PC019, PC137
 Ogoh, S. C072, PC184
 Ogunpolu, B. PC089
 OH, I. PC024*
 Ohanyan, V. SA026*
 Ohba, K. PC139
 Ohihoin, E. PC254
 Oigbochie, V. PC040
 Ojeka, S.O. PC114
 Okada, K. PC160
 Oladejo, R.O. PC225*
 Oladipo, S. PC006

Olaniyan, O.T. PC140
 Olaso-Gonzalez, G. SA016
 Olatunji-Bello, I.I. PC171
 Olayaki, L.A. PC179, PC194*
 Olayinka, G.S. PC215*, PC232
 Oldham, S. PC138
 Olea, E. PC054, PC159
 Olley, S. PC254
 Olorunfemi, O. PC168
 Oloyo, A.K. PC254*
 Olson, T.P. SA044
 Olukunle, J.O. PC170
 Olumide, O.M. PC171*
 Olusanya, A.W. PC195
 Oluwanisola, A. PC194
 Omobowale, T. PC006, PC089
 Onasanwo, A. PC225
 Onasanwo, A.S. PC215, PC219,
 PC223*, PC232
 Orlova, E. C066, PC108*
 Orriss, I. PC153
 Ørtenblad, N. PC188*
 Ortiz, M. PC183
 Orton, L.D. C096
 Ou, D. PC247
 Ou, M. PC247*
 OUADID-AHIDOUCH, H. C011, C038
 Ousingsawat, J. SA052
 OWEMIDU, I.O. PC219*
 Owens, T.S. PC105*, PC123
 Owoeye, O.B. PC111
 Oyagbemi, A. PC006, PC089
 Oyebanjo, O.T. PC232*
 Oyeleke, M. C098
 Ozturk, G. PC218*
 Ozturk, R.I. PC218

P

Pabla, P. C080, C083*
 Palma, C. C076
 Palmowski, P. PC265
 Palomero, J. PC148*
 Pan, K. C053
 Pang, C. PC247
 Parkinson, S. C025
 Parsons, M. PC193
 Parthasarathi, K. C002
 Patel, C. C109
 Patel, K. PC144
 Paton, J.F. C010, PC020, PC210
 Paulsen, G. PC097

Pavis, G.F. C069*, C070
 Peart, D. C081
 Pedicini, L. PC257, PC258*
 Peers, C. PC042, PC043
 Peffers, M. PC222
 Peltonen, J. PC110, PC120
 Peltz, T. C043
 Perez-Perez, R. PC151
 Perez, T. C087
 Perkins, J. PC065
 Perlejewski, K. PC245
 Perszyk, R. PL03
 Pervolaraki, E. SA012, PC027
 Peters, R. PC112
 Phelan, M. PC151
 Phelan, O. C047
 Phillips, B. C057, C059, C061, C067,
 PC095, PC113, PC119, PC127, PC158,
 PC174, PC180
 Phillips, H. PC174
 Phillips, S. C067
 Philp, A. PC146
 Piasecki, J. PC093*, PC101
 Piasecki, M. C064, PC101, PC127, PC130
 Piercy, R. PC222
 Pillon, N. C079
 Pimlott, S.L. PC031
 Pinali, C. SA048, PC033
 Plakane, L. PC116
 Platonov, M. PC251
 Platt, S. C082
 Pocock, T. C027, PC060*
 Podyacheva, E. PC039*
 Polanco, J. PC103
 Pollock, M. PC075
 Pors, K. PC197
 potocnik, N. PC261*
 potocnik, P. PC261
 Potter, J.A. C078
 Powell, A. C022
 Powers, R.K. SA055
 Poyner, E. PC069
 Præstholt, S. SA041
 PRAMANIK, S. PC136
 Pramaningtyas, M.D. PC015*, PC162
 Prego, C.S. PC196
 Preston, J. PC079
 Preston, T. C060
 PREVARSKAYA, N. C038
 Price, G.W. C078
 Prieto-Lloret, J. C012, PC054, PC159
 Prime, S. PC217

Pritchard, H.A. C123*
 Prylutsky, Y.I. PC251
 Pusch, M. SA053
 Pyke, K.E. PC100
 Pyner, S. C102

Q

Quast, K. SA038
 Quinn, C. PC033*
 Qureshi, A. PC021

R

Radkowski, M. PC245
 RADOSLAVOVA, S. C038*
 Rae, M.G. C029*, C092
 Raedel, D. SA038
 Rafferty, G. PC106
 Rajaji, K. PC243
 Rajanna, T. PC211
 Raji, Y. PC111
 Rajnicek, A.M. C051*, PC094
 Ramasawmy, P.E. C055
 Ramírez, M.A. C039
 Ramzan, I. PC180
 Ranat, R. PC174
 Rance, T. PC211
 Randhawa, G. PC052
 Rapedius, M. C095
 Rashdan, N. PC153
 Ratcliffe, L. C010
 Rathmacher, J. PC174
 Ravi, R. C094*
 Rawlins, E. SA037*
 Ray, C.J. PC008, PC009, PC017
 Redmond, C. C022
 Reed, M.W. PC045
 Rees, J. PC045
 Reinhardt, O. PC026
 Reynolds, R. PC201
 Ribeiro, M.J. C012
 Ribeiro, M.P. PC132
 Rich, L.R. PC212*, PC221
 Richardson, A. C097
 Rickard, A. SA005*
 Rigual, R. PC054
 Rinke-Weiß, I. C095
 Ritter, U. PC251
 Roberts, C. PC069
 Roberts, F.L. PC153*
 Roberts, M. C042

Robinson, S. C097
 Robson, L. C020*
 Robson, S. PC265
 Rocha, J. C062
 Rocher, A. PC054*, PC159
 Rochford, J. C085, C103
 Rock, D. PC217
 Rock, J. SA040*
 Roddy, B. PC075
 Roddy, D.W. C105, PC234
 Roderick, H. PC025
 Rodriguez-Cuenca, S. PC151
 Roe, S.M. C025*
 Roermann, D. C048
 Roldán, A. PC096, PC124*
 Roman, E. C105, PC234
 Romaniuk, J.R. PC238
 Romero, M. PC149
 Ronan, D. C047
 Rosenblum, H. SA021, SA023
 Rosenblum, H.R. SA022*, SA024
 Rossmanith, S. SA056
 Rote, E. C009
 Rotordam, M.G. C095
 Rowe, I. C051, PC057
 Rowe, J. C060
 Rudrappa, S. C059, PC119
 Ruggieri, M. C042
 Rusakov, D.A. PC213
 Rutherford, S. C033

S

S, V. PC249
 Sabra, M. PC079
 Sacks, H. SA030, PC182
 Sacramento, J.F. C012, C089, PC196*
 Saeed, A.M. C019, PC169
 Saghy, L. PC022
 Sahin, E. PC133*
 Sahin, Z. PC241
 Saint-Criq, V. C045*
 Salihoglu, A.K. C126
 Salihu, M.A. PC140
 Salomon, C. C076
 Salt, I. C074*, PC141
 Salvador-Pascual, A. SA016
 Salvage, S.C. C007*, PC045*
 Sampson, A.P. C024
 San Miguel, M. C093
 Sánchez-Martín, M. PC148
 Sands, A. PC064

Sani, I.	PC007	Shen, X.	C013
Sankar, A.	PC230*	Shennan, A.	C076
sankar, S.	PC032	Shenton, F.	C102
Saral, E.	PC241	Shepherd, A.	C009
Sardon-Puig, L.	C079	Shepherd, S.	C056
Saronee, F.	PC121	Sheridan, H.	C049
Sato, S.	PC139	Shiels, H.	SA001, C001, PC016
Saunter, C.D.	PC260	Shil, A.	C054*
Saxena, P.	PC041	Shinohara, Y.	PC184
Saxton, S.	SA059	Shittu, S.A.	PC111*, PC155
Sayda, M.H.	PC180*	Shittu, S.T.	PC155*
Saynor, Z.L.	SA045*, C009	Shmygol, A.	PC011*, PC021, PC266*
Schelske, M.	C058	Shocking, L.	SA026
Schempp, R.	PC055	Shoda, J.	PC160
Schnabl, K.	SA032	Shute, J.	C009
Schneider, J.	PC252	Shute, L.	C094
Schoenberger, M.	C046	Siamantouras, E.	C078
Scholz, M.	PC062*	Sian, T.	C057*, PC119
Schrage, W.G.	C120, PC253	Siersbaek, M.	SA041
Schreiber, R.	SA052	Sikora, E.	PC245
Schroeder, V.	SA038	Silverthorn, D.U.	SA007*
Schruf, E.	SA038*	Sindeeva, O.A.	PC213
Schultz, M.G.	PC122	Singh, A.	SA029*
Schwingshackl, A.	C002	Singh, J.	PC165*, PC172*
Scott, D.	C026, C035, PC066, PC067, PC071*, PC073*, PC075*, PC077, PC078	Sipido, K.	PC025
Scragg, J.	PC043	Sjaastad, I.	C013
Scully, D.	C068, PC144*, PC152	Skinner, R.	PC103
Secher, N.	C058	Skrzypski, M.	PC198, PC199, PC200
Seidler, U.	C048	Slater, G.	PC097
Sekar, M.	PC032*	Sloan, N.	PC031
Sepulveda, F.V.	C041*	Smets, A.	C055*
Seresse, O.	PC110, PC120	Smirl, J.	PC102
Sergeant, G.	C113	Smite, Z.	PC116*
Serre, J.	PC187	Smith, C.	PC033, PC035*
Sethupathi, P.	C087	Smith, E.	SA009
Sfyri, P.	C068, PC144, PC152	Smith, G.L.	C016, C111, PC041
Shafiq, S.	C086	Smith, J.A.	C079, C082*
Shah, A.	C105, PC234	Smith, K.	C059, C061, C067, PC095, PC101, PC113, PC119, PC146, PC158, PC180
Shaikh, S.	PC076	Smith, M.	PC036
Shandell, M.A.	C044*	Smith, N.	PC264
Shang, L.	PC143*	Smith, P.A.	PC087*, PC186*
Sharma, K.	PC205	Smith, R.	PL05*
Shastri, L.	PC106	Smyth, J.S.	C040*
Shattock, M.J.	C017	Snoeck, H.	SA039
Shave, R.E.	PC107	Sobrevia, L.	C039*
Sheader, E.	C027*, C028, PC060	Soeller, C.	C013
Sheard, T.M.	PC027, PC034	Sofidiya, O.M.	PC195
Sheel, W.	SA043*	Sofola, O.	PC254
Shelley, C.	PL03	Sogebi, E.A.	PC170
Shelley, J.H.	PC125, PC126*	Soloviev, A.I.	PC251

Soltani, N. PC135*
 Sørrhus, E. C001
 Sorop, O. SA027
 Sottile, V. SA030*
 Sowton, A. C077
 Spath, N. PC031
 Speake, T. C027
 Squires, P.E. C078*
 sritharan, T. C005*
 Stacey, B.S. C072, PC105,
 PC123, PC250*
 Staunton, C. C104
 Stavropoulos-Kalinoglou, A. PC112
 Steele, D. PC027, PC042,
 PC043
 Steenhorst, J.J. SA027
 Steggall, M. PC105, PC123
 Steinert, J.R. C097
 Steinz, M. SA015*
 Stephens, F. C069, C070, C083
 Stephenson, K.A. C092*
 Stewart, E.L. SA038
 Stewart, G. C047, PC080*
 Stewart, I. PC073
 Stewart, K. C110, PC069
 Stierstorfer, B. SA038
 Stöhr, E.J. SA021*, SA022, SA023,
 SA024, PC107
 Stokes, T. C067
 Stölzle-Feix, S. C095, PC026
 Stone, B. PC103, PC125, PC126
 Strath, A. PC057
 Strauss, J. C056
 Strowig, T. C048
 Strubberg, A. SA054
 Subhan, M. C094
 Sudol, A. PC010
 Sugioika, S. PC084
 Sukhorukov, G.B. PC213
 Sultan, A. C023
 Sultan, Z. PC033
 Sumya, U. C125*
 Sun, J. PC125, PC126
 Sutherland, A. PC031
 Sutton, G. PC047*
 Svensson-Frej, M. SA059
 Svent, M. C099*
 Swaminathan, A. PC187
 Swanger, S.A. PL03
 Sweeney, E. C081*
 Swiderska, A. PC017
 Symonds, M. SA030, PC182

Szewczyk, A. C101
 Szewczyk, N.J. C059, PC119, PC146

T

Taggart, J. PC265
 Taggart, M. C008, PC265*
 Tahrani, A.A. PC009
 Takahashi, K. PC139*
 Takahashi, S. PC160
 Takaishi, T. PC106
 Tallis, J. C064
 Tamiya, K. C072
 Tamuno, I. PC114
 Tanko, Y. PC189
 Tansey, E. C025
 Tarasova, O. C066, PC108
 Tarran, R. SA020*, C008, C037,
 PC050, PC081
 Tavares, A.A. PC031
 Taylor, B.J. SA044*, PC118, PC132
 Taylor, J. PC036*
 Taylor, J.L. C063
 Taylor, P.D. C087
 Taylor, T. SA009, C080
 Telliez, M. C038
 Teng, B. C002
 Thakore, P. C123
 Thanaj, M. SA061
 Thoma, A. PC117*
 Thomas, U. PC026
 Thompson, A.J. PC045
 Thompson, D. C073*, PC145, PC150
 Thornbury, K. C113
 Tickle, P. C116*, PC256
 Tillin, T. PC122
 Tinker, A. C018
 Tinwell, J. C001
 Tjörnhammer, R. C079
 Tobin-Schnittger, L. C105*, PC234*
 Toledo-Rodriguez, M. PC186
 Tomlinson, J. C088
 Toms, L. PC018*
 Tong, W. C077*
 Torrens, C. C024*
 Torres, R. PC092
 Trafford, A. SA048, C014,
 C015, PC018, PC029, PC033, PC035
 Traynelis, S.F. PL03*
 Tremblay, J.C. PC100*
 Treumann, A. PC265
 Tribe, R.M. C076

Trihan, J. C072
Troosters, T. PC187
Tseng, Y. PC178
Tsintzas, K. C080, C083
Tsocheva, I. PC052
Tsukamoto, H. PC102, PC105, PC184*
Tsukamoto, H.J. PC123
Tucker, S.J. C032*, PC068*, PC072*
Tuharska, Z. C117
Turcani, M. PC003, PC004*
Tye, K. PC230
Tymko, M.M. PC100, PC250
Tynan, C. PC045

U

Ufnal, M. PC005*, PC083, PC245*
Ugalde, C. PC151
Uwayama, J. PC192

V

Valdebenito, G.E. PC183*
Valero, P. C039
Valli, H. C007
van de Wouw, J. SA027
van den Brink, J. C013
van der Schoot, A. PC229
Vande Velde, G. C046
vanDongen, B. C001
VANLAEYS, A. C038
Varro, A. PC022
Vasiljevs, S. PC051*
Vatakencherry, R.J. PC249*
Velickovic, K. SA030
Venables, L. PC105, PC123
Venetucci, L. PC029
Vera, E. C041
Vermeulen, T. PC250
Verpoorten, S. C068, PC144, PC152*
Verschoor, P.J. C085*
Vidal-Puig, A. PC151
Vilén, L. PC110
Villafuerte, F. PC100, PC250
Vina, J. SA016
Vinogradova, O. C066, PC108
Virag, L. PC022
Vizcardo-Galindo, G.A. PC100, PC250
Vlahovic, D. PC099
Vogiatzis, I. SA046*
Volianitis, S. C058*

Vozniuk, V. PC224*
Vunjak-Novakovic, G. SA039

W

Wabitsch, M. PC166
Waddell, C. C014
Wagenmakers, A. C056
Walker, B.J. C120, PC253
Walker, N. SA054
Wall, B.T. C069, C070
Walmsley, G. PC222
Walsh, P.A. PC203*
Walshe, I. C081
Walters, M. PC103
Walton, E. PC061
Walton, T. PC031
Wang, L. PC045
Wang, Y. SA035*, PC036
Waqar, T. PC172
Warabi, E. PC160*, PC192
Ward, A. C120*, PC253
Washio, T. C072
Waters, C. C002*
Watkins, B. SA056*
Watson, K. PC069
Watson, R. PC265
Wayne, D. PC182*
Waziri, B.I. PC007*
Webb, S.D. C096*
Webster, M.J. SA038
Wehinger, S. PC147
Wells, T. C090
White, B. PC263
White, E. SA012, PC027
white, K. C001
Whitfield, P.D. PC151
Whysall, K. PC046
Widmer, H. PC057*
Widyaningrum, A. PC162
Wieben, O. C120, PC253
Wiktor, S.D. PC255*
Wilbrey, A.L. SA009
Wilcox, A.G. PC193
Wilkinson, D. PC095, PC101,
PC113, PC146
Wilkinson, D.J. C059, C061*,
C067, PC119
Wilkinson, K.A. SA057*
Will, S. PC138
Willershäuser, M. SA032

Williams, J.P. C061
 Williams, L. PC031
 Williams, P. PC105
 Williams, S. PC122
 Williamson, G. PC166
 Wilson, C. PC259, PC260*
 Wilson, H.M. C119
 Wilson, O. C060, PC112
 Winchester, W.J. SA009
 Winter, J. C017
 Wiseman, J.W. C110
 Witchel, H.J. C030*
 Withers, S. SA059*
 Wojciechowicz, T. PC198, PC199*,
 PC200
 Wolugbom, J. C098
 Wood, A.F. C022*
 Wood, J.N. SA008*
 Wood, N. PC112
 Woodall, M. C037*, PC081
 Woode, R.A. SA054
 Wooding, P.B. C077
 Woods, L. C014
 Workman, A.J. C111, PC041
 Wray, S. C115
 Wrobel, J. PC050
 Wu, C. C042
 Wu, Q. PC197
 Wüst, R. PC187

Y

Yahaya, M.A. PC007
 Yakubu, M. PC006, PC089
 Yamasaki, E. C123

Yanagawa, T. PC160, PC192
 Yang, C. PC091, PC151
 Yang, L. PC201*
 Yang, Z. PC027
 Yayman, S.G. PC094*
 Ying, S. PC235*
 Yip, C. C053
 Yokota, Y. PC139
 Young, M. SA036*
 Yuan, H. PL03
 Yung, B. C077

Z

Zajko, V. PC061
 Zambrano, E. C087
 Zechini, L. PC012
 Zhang, H. PC044
 Zhang, J. PC056*
 Zhang, L. C077, PC106
 Zhang, R. C053
 Zhang, X. C091*
 ZHANG, Y. PC010*, PC016
 Zhang, Z. PC143
 Zhao, B. C048
 Zhao, J. SA008
 Zheng, K. PC213
 Zheng, Y. PC226
 Zholos, A.V. C121*, PC251*
 Zhong, C. C047*
 Zibara, K. PC079
 Zierath, J. C079, C082
 Zolkiewski, L. PC157
 Zorzano, A. C079, PC149, PC151
 Zyma, I. PC224