C01

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

Sol Olivera[1], Delyth Graham[1], Dilys Freeman[1]

[1]School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, United Kingdom

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

Pregnant stroke-prone spontaneously hypertensive rats (SHRSP) were implanted with an osmotic minipump for the continuous delivery of 0.9% saline (control) or 660-700 ng/kg/min angiotensin II (ANGII) at gestational day 10.5 (n=6/group). Pregnancies progressed until birth and offspring were weighed between 1 and 7 days of age. Echocardiography and systolic blood pressure (SBP) measurements were carried out regularly on the offspring (n=16-19/group) between 5 and 17 weeks of age (W5-17), and tissues were harvested for wire myography at sacrifice (W18-19). Animal procedures were performed according to regulations established in the Animals (Scientific Procedures) Act 1986, under the Home Office approval of Project Licence PP0895181.

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

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

C02

Ventricular repolarization abnormalities and arrhythmogenesis in catecholaminergic polymorphic ventricular tachycardia

Spyros Zissimopoulos[1], Ana María Gómez[2], Ewan Douglas Fowler[3]

[1]Swansea University, Swansea, United Kingdom, [2]INSERM U769, Université Paris-Sud 11, Paris, France, [3]Cardiff University, Cardiff, United Kingdom

Introduction

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

Methods

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

Results

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

Conclusion

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

C03

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

Rhea Takhar[1], Kun Liu[2], Ni Li[1], Sang Soo Seo[3], Thamali Ayagama[1], David Paterson[1], Neil Herring[1], Dan Li[1]

[1]1. Burdon Sanderson Cardiac Science Centre, Department of Physiology, Anatomy and Genetics, University of Oxford. United Kingdom, Oxford, United Kingdom, [2]1. Burdon Sanderson Cardiac Science Centre, Department of Physiology, Anatomy and Genetics, University of Oxford. United Kingdom, Oxford, United Kingdom, [3]2. Immunocore Limited, Milton Park, Abingdon, Oxon. United Kingdom, Oxford, United Kingdom

Diabetic Cardiomyopathy (DCM) is a subset of heart failure that is a consequence of diabetes mellitus[1]. The contractile dysfunction of heart failure is characterised by blunted beta-adrenergic-cAMP signalling[2]. Insulin is known to reduce cAMP via phosphodiesterase (PDE)[3], which experiences dysfunction in the diabetic state, owing to insulin resistance. Recently insulin and Fibroblast Growth Factor 1 (FGF1) signalling has been shown to be convergent[4] and appears to be protective against DCM[5]. We tested whether cAMP signalling is impaired in a model of DCM using human induced pluripotent stem cell (hiPSC) derived cardiomyocytes, and investigated whether cAMP kinetics is altered by FGF1.

Healthy hiPSC (OX1-19, obtained from The James Martin Stem Cell Facility, Oxford) were differentiated into cardiomyocytes and cultured in either normal or high-glucose, high-fatty acid media. Cytoskeletal and nuclear staining were used to assess cell and nuclear area. ATP based cell viability and glucose reuptake were assessed using a luminescence-based assay. BNP release was measured by a NT-proBNP ELISA kit and gene expression levels were detected by bulk RNA-sequencing. Real time cAMP kinetics were investigated with a fluorescence resonance energy transfer (FRET) based sensor Epac-S^{H187}. DCM model cells expressed phenotypic changes consistent with the disease. Cells cultured in either 11 mM (n=122 cells, 2 wells) or 25 mM glucose (n=102 cells, 2 wells) had a significant increase in cell area (μm^2) compared to control (n=236 cells, 2 wells), ($p<0.0001$, Kruskal-Wallis). DCM cells (n=7 separate wells) had a significant increase in the concentration of NT-proBNP, a key hypertrophic marker, in the media assay compared to control cells (n=7 separate wells, $p<0.0001$, one-way ANOVA). DCM cells (n=7 separate wells) had a significantly lower cell viability compared to control (n=7 separate wells, one-way ANOVA, $p<0.001$). RNA-seq informed the KEGG pathway analysis were enriched in PI3-Akt, cAMP signalling, Ras-Raf (ERK-MAPK), and hypertrophic cardiomyopathy pathways. Hypertrophic genes, collagens, and ANP and BNP genes, both of which are released in failing heart, were upregulated, as was PDE4DIP. cAMP signalling in response to forskolin was lowered in the DCM model cells (n=21, 7.21±0.05%) compared to control (n=20, 9.53±0.12%, $p<0.05$). This was reversed when DCM cells were treated with FGF1 (1 ng/ml, n=19, 13.65±0.09%, $p<0.0001$).

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

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

References 1. Rubler, S. et al. (1972). Am J Cardiol 30, 595–602. 2. Bastug-Özel, Z. et al. (2019). Cardiovasc Res 115, 546–555. 3. Marchmont, R. J. & Houslay, M. D. (1980). Nature 286, 904–906. 4. Sancar, G. et al. (2022). Cell Metab 34, 171-183.e6. 5. Wang, D. et al. (2021). Signal Transduct Target Ther 6, 133.

C04

Neuropeptide Y signalling in human induced pluripotent stem cell derived cardiomyocytes

Carla Handford[1,2], Kun Liu[1], Ni Li[1], Thamali Ayagama[1], David Paterson[1], Dan Li[1], Neil Herring[1]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, [2]University of Oxford (undergraduate student), Oxford, United Kingdom

Background: Acute myocardial infarction and chronic systolic heart failure result in heightened cardiac sympathetic activation[1], driving release of the co-transmitter neuropeptide-Y (NPY)[2], with circulating levels correlating with morbidity and mortality in both conditions[3]. The acute physiological effects of NPY on cardiac excitability are well studied[4], but the effects of chronic NPY exposure, which may be important for subsequent ventricular remodelling, are poorly characterised. We therefore investigated this using human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs).

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

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

1. Borovac, J. A., D'Amario, D., Bozic, J. & Glavas, D. Sympathetic nervous system activation and heart failure: Current state of evidence and the pathophysiology in the light of novel biomarkers. *World journal of cardiology* **12**, 373-408 (2020).
2. Tan, C. M. J. *et al*. The Role of Neuropeptide Y in Cardiovascular Health and Disease. *Frontiers in physiology* **9**, 1281 (2018).

3. Gibbs, T. *et al*. Neuropeptide-Y Levels in ST-Segment–Elevation Myocardial Infarction: Relationship With Coronary Microvascular Function, Heart Failure, and Mortality. *Journal of the American Heart Association* **11**, e024850 (2022).
4. Kalla, M. *et al*. The cardiac sympathetic co-transmitter neuropeptide Y is pro-arrhythmic following ST-elevation myocardial infarction despite beta-blockade. *Eur. Heart J.* **41**, 2168-2179 (2020).

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

C05

ST-elevation myocardial infarction (STEMI) alters iron status

Samira Lakhal-Littleton[1], Mayra Vera-Aviles[2], Goran Mohammad[3], Kaspar Broch[4], Thor Ueland[5], Pal Aukrust[6], Ola Kleveland[7], Geir Andersen[8], Lars Gullestad[9]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, [2]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, [3]Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, [4]Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway, [5]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway, [6]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway, [7]Clinic of Cardiology, St. Olav's Hospital, Trondheim University Hospital, Trondheim, Norway, [8]Department of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway, [9]Department of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway

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

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

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

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

C06

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

Svetlana Mastitskaya[1], Felipe Freitas[1], David Attwell[1]

[1]UCL, London, United Kingdom

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

Methods: Pentobarbital anaesthetized Sprague-Dawley rats were subjected to 45 min LAD occlusion followed by 15 min reperfusion. RPc was induced by 15 min occlusion of both femoral arteries. Systemic GLP-1R blockade was achieved by i.v. injection of the specific antagonist Exendin(9-39) (50μg/kg, Tocris) prior to RPc. At the end of the experiment, rats were perfused with FITC-albumin, and cardiac capillary perfusion was analysed using FIJI software (ImageJ 1.53c, NIH) following immunostaining. For dissection of molecular mechanisms, live cardiac tissue from NG2-DsRed mice (right free ventricular wall) was perfused intralumenally with modified Tyrode's solution via a glass cannula introduced into the right coronary artery, and imaged using a Zeiss confocal LSM780 microscope. The GLP-1R agonist Exendin-4 (Ex4, 100nM, Tocris), and/or the ATP-sensitive K^+ (K_{ATP}) channel inhibitor glibenclamide (20μM, Insight Biotechnology) were applied in oxygenated Tyrode's solution. Oxygen/glucose deprivation was achieved by replacing glucose with sucrose in the Tyrode's solution and bubbling with 95% N_2/5% CO_2. Capillary diameters were measured using FIJI software.

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

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

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

C07

Ultrastructural dynamics of contracting cardiomyocytes

Joachim Greiner[2,3], David Kaltenbacher[2,3], Thomas Kok[2,3], Peter Kohl[2,3], Eva Rog-Zielinska[2,3]

[1]Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg-Bad Krozingen, and Faculty of Medicine, University of Freiburg, Germany, Freiburg, Germany, [2]Institute for Experimental Cardiovascular Medicine, University Heart Center Freiburg-Bad Krozingen, Freiburg im Breisgau, Germany, [3]Faculty of Medicine, University of Freiburg, Freiburg im Breisgau, Germany

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

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

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

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

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

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

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

C08

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

Agathe Lermant[1], Gwen Rabussier[2], Henriëtte Lanz[2], Lindsey Davidson[1], Yashika Relan[1], Iain Porter[1], Colin Murdoch[1]

[1]University of Dundee, Dundee, United Kingdom, [2]Mimetas, Oegstgeest, Netherlands

Motivation

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

Methods

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

Results

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

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

Conclusion

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

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

C09

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

David Brennan[1], John Babraj[2], Sarah Cottin[1]

[1]Abertay Univeristy, Dundee, United Kingdom, [2]Abertay University, Dundee, United Kingdom

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

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

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

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

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

MAP= (SBP+DBP*2)/3

PP= SBP-DBP

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

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

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

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

C10

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

Andrew S. Butler[1], Stephen C. Harmer[1], Jules C. Hancox[1]

[1]School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom

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

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

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

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

A method for simultaneous electrical stimulation of iPSC-MLs and whole-cell recording has not previously been published to the best of our knowledge. Accordingly, we have developed a novel method for localized stimulation of iPSC-MLs which allows concurrent whole-cell patch clamp recordings to be made at a user-defined stimulation rate. Using this method >95% of RA-iPSC-MLs and DMSO-iPSC-MLs could be paced at 1 Hz. RA-iPSC-MLs (n = 53) paced at 1 Hz exhibited a more atrial-like phenotype than DMSO-iPSC-MLs (n = 45) as characterised by abbreviated repolarisation at APD_{30} (40.5 ± 4.0 [RA] vs. 128.8 ± 4.6 ms [DMSO]; $p < 0.0001$) and APD_{90} (220.8 ± 13.3 [RA] vs. 283.4 ± 10.2 ms [DMSO]; $p < 0.001$); and a lower AP and plateau amplitude (101.6 ± 1.6 mV and 82.2 ± 2.8 mV respectively) than DMSO-iPSC-MLs (113.0 ± 2.1 mV and 110.0 ± 2.2 mV; $p < 0.0001$ for both). Prolongation of APD_{50} by application of UCL1684 was significantly larger in RA-iPSC-MLs (18.7 ± 3.0%; n = 12) than in DMSO-iPSC-

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

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

1. Ismaili, D., et al. (2023). Front Physiol 14, 1132165 2. Zhang, Q., et al. (2011). Cell research 21(4), 579-587 3. Devalla, H.D., et al. (2015). EMBO Mol Med 7(4), 394-410 4. Skibsbye, L., et al. (2014) Cardiovascular Research 103(1), 156-167 5. Hancox, J.C., et al. (2016) Expert Opin Ther Targets 20(8), 947-58

C11

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

Lara Scofano[1], Rumaitha Al-hosni[1], Catherine S. Choi[1], Zuzanna Borawska[1], Claire Smith[1], Dawn Shepherd[1], Zeki Ilkan[1], Frances Platt[1], Paolo Tammaro[1], Kathryn E. Acheson[1]

[1]*University of Oxford, Oxford, United Kingdom*

Background The TMEM16A Ca^{2+}-activated Cl^- channel plays a key role in the control of vascular tone and blood flow. TMEM16A has a pore with sections exposed to plasmalemmal lipids (1-3). This structural arrangement may confer the channel sensitivity to plasmalemmal lipids, including phosphatidylinositol 4,5-bisphospate (PIP_2) (4). The lysosomal NPC1 protein regulates cellular distribution of lipids (5). Loss-of-function mutations in *NPC1* lead to Niemann-Pick disease Type C (NPC) a prematurely fatal neurodegenerative disorder with a range of systemic alterations including vascular (5). Here, we ask whether TMEM16A is modulated by NPC1 and examine the impact of this modulation on the tone of isolated systemic arteries, where TMEM16A is highly expressed.

Methods Whole-cell patch-clamp recordings of native and cloned TMEM16A currents, isometric tension recordings, confocal imaging and Förster Resonance Energy Transfer (FRET) were used in this study. For patch-clamp recordings, the external solution contained (mM): 150 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 D-mannitol, and 10 HEPES (pH 7.4); the pipette solution contained (mM): 130 CsCl, 10 EGTA, 1 $MgCl_2$, 10 HEPES and 8 $CaCl_2$ (pH 7.3). The tone of isolated artery rings obtained from mice carrying *Npc1* deletion (*Npc1*$^{-/-}$) before and after 5-week treatment (4g/kg/week) with 2-hydroxypropryl-β-cyclodextrin (bCD), was assessed via wire myography. Expression of mRNA for phospholipase C (PLC) isoforms was conducted via quantitative RT-PCR (qRT-PCR). Data are given as mean±SEM alongside the number of independent experiments.

Results Heterologously expressed TMEM16A currents were enhanced by 2.3±0.3 fold (n=14) during pharmacological inhibition of NPC1 or by 2.6±1.0 fold (n=15) as a consequence of genetic deletion of the *Npc1* gene (knockout). These increases were prevented by treatment with b-cyclodextrin or re-introduction of *Npc1* gene in the knockout cells. The activation of cloned TMEM16A currents by NPC1 inhibition was independent on KCNE1, a proposed TMEM16A auxiliary subunit. Depletion of plasmalemmal PIP_2 or an inactivating mutation in the channel PIP_2 binding site (TMEM16A-R482A), prevented TMEM16A activation during NPC1 inhibition. Artery (aorta and mesenteric) rings obtained from *Npc1 null* mice showed increased contractility in response to phenylephrine, which was prevented by Ani9, a selective TMEM16A inhibitor and enhanced by increasing the depolarising Cl^- gradient. The underlying mechanism involves augmented plasmalemmal PIP_2 levels during NPC1 inhibition, assed via genetically-encoded PH-PLCd domains and FRET imaging. The plasmalemmal PIP_2 level was increased by 1.2 ±0.6 fold (n=67) during pharmacological inhibition of NPC1 and was rescued by treatment with b-cyclodextrin. This change in PIP_2 homeostasis was presumably caused by

reduction in the expression of phospholipase C during NPC1 inhibition, assessed using qRT-PCR.

Conclusions PIP_2-dependent changes in TMEM16A activity may form the basis of vascular overactivity during pathology caused by loss of NPC1 function and establish a role for the lysosome in the control of cell excitability and vascular tone.

1. Whitlock J et al. (2016). Pflugers Arch 468(3): 455-473. 2. Al-Hosni R et al. (2022). Trends Pharmacol Sci 43: 712–725. 3. Dinsdale R et al. (2021). Proc Natl Acad Sci U. S. A 118: e2023572118. 4. Chau T et al. (2017). Br J Pharmacol 174(18): 2894-2999. 5. Platt F et al. (2012). J Cell Biol 199(5): 723-734.

C12

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

Nabil Nicolas[1], Marie-Ange Renault[1], Etienne Roux[1]

[1]Univ. Bordeaux, INSERM, Biologie des maladies cardiovasculaires, U1034, F-33600 Pessac, France

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

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

All procedures were done accorded with current national and European legislation, and agreed by the local ethical committee. 14-week-old female C57BL/Ks mice, a genetic background predisposing to renal dysfunction, and deficient for leptin receptors, inducing obesity and type 2 diabetes, were used as HFpEF model. 8-week-old C57BL/6J mice, lacking HFpEF risk factors, were used as healthy controls with 50% sex ratio, since no difference were found between sex for the studied parameters. SS_{sys} and SS_{dia} were calculated from the vascular diameter and maximal blood velocity (MBV) measured on the right common coronary artery (RCCA) by ultrasound imaging on anesthetized mice. After sacrifice, RCCA EC were labelled for the nucleus (DAPI) and the Golgi apparatus (Golph4) and imaged by confocal microscopy. EC orientation was measured as the angle of the nucleus-Golgi vector with blood flow direction, and classified as dromic (0°-60°), lateral (60°-120°), or antidromic (120°-180°). EC polarity was defined as nucleus elongation (major/minor axis ratio). Volumic (per mm^3) vascular density (VD), segment number (SN), node number (NN), and total capillary length (TCL) of the left ventricle capillary network were calculated on processed light-sheet 3D microscopy images of lectin-labeled optically cleared hearts. Quantitative data are expressed as mean±standard deviation and compared using Student *t* test. EC orientation angle distributions were compared by chi2 test. Results were considered statistically significant for $p < 0,05$, either non-significant (ns).

Compared to healthy mice (n=14), HFpEF mice (n=8) showed no significant changes in systolic and diastolic MBV nor SS_{dia}, but significant SS_{sys} decrease (Table). In healthy (566 cells, 14 mice) vs HFpEF (243 cells, 7 mice) RCCA, EC orientation was 23 vs 33 % dromic, 22 vs 27 % lateral, and 55 vs 40 % antidromic, respectively, and the distribution statistically different (p=0.0006), whereas EC nucleus elongation ratio was 1.83±0.2 vs1.97±0.2 (ns). In healthy (n=14) vs HFpEF mice (n=7), VD was 46.6±9 vs 39.6±6% (p = 0.048), SN was 495,000±92,000 vs 402,000±68,000 (p = 0.019), NN was 265,000±49,000 vs 220,000±39,000 (p = 0.039), and TCL was 10±1.3 vs 8.3±1.3m (p = 0.008), respectively.

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

C13

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

Margaux Horn[1]

[1]*School of Medicine, Keele University, Keele, United Kingdom*

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

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

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

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

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

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

C14

"What is Physiology?" – interview insights straight from the physiologists' mouths

Harley Stevenson-Cocks[1], Michael Taggart[1], Charlie Biggin[1], Aine Browne[1], Joseph Cleghorn[1], Calum Earl[1], Beth Henshaw[1], Areej Mahmood[1], Elysia Marrs[1], Luisa Roa-Gil[1], Kavishi Sheth[1], Nakshatra Sivaraj[1], Rebecca Watson[1]

[1]Newcastle University, Newcastle upon Tyne, United Kingdom

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

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

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

C15

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

Marie Bowers[1], Cas Nicholas[4], Evie Bowers[1], Sara Ahmed[1], Stuart Nicolson[1], Ryley Solferini[1], Eve Anderson[1], Laura Kerr[1], Eirini Karavasili[1], Katherine Price[1], Iain Rowe[1,2]

[1]University of Glasgow, Glasgow, United Kingdom, [2]University of Aberdeen, Aberdeen, United Kingdom, [3]None, Swindon, United Kingdom, [4]University of Hull, Hull, United Kingdom

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

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

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

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

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

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

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

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

C16

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

Matthew Jones[1]

[1]Biomedical Research and Innovation Centre, School of Science, Engineering and Environment, University of Salford, Salford, United Kingdom

Introduction

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

Methods

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

Results

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

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

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

Conclusion

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

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

C17

What do students want from practical classes?

Matthew Mason[1], Kamilah Jooganah[2]

[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom

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

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

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

C18

A multifaceted approach to building more employable Biomedicine graduates.

Sara Namvar[1]

[1]*University of Salford, Salford, United Kingdom*

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

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

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

C19

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

Harry Witchel[1]

[1]Brighton and Sussex Medical School, Brighton, United Kingdom

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

(A) more demanding exam questions

(B) students who are less studious

(C) students who have weaker academic reserve

(D) weaker teaching that is unmatched to the exams, or

(E) an increase of taught material and a dearth of time on the exam.

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

C20

Developing authentic assessment by using a design sprint methodology

Ruth Norman[1], David Lewis[1], Charlotte Haigh[1]

[1]University of Leeds, Leeds, United Kingdom

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

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

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

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

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

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

C21

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

Lauri Ovaska, Derek Scott

undefined

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

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

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

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

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

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

C22

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

Laura Ginesi[1]

[1]*University of East Anglia, Norwich, United Kingdom*

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

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

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

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

Learning about core concepts in physiology (Michael & McFarland, 2020) seems to have the potential to create the keystone for a solid and meaningful lifelong learning opportunities that fill the gap between theory and practice learning. Encouraging this open-ended approach to

learning physiology served to form a framework for developing learning activities which helped to unpack students' misconceptions, partial understanding and confusion. By exploring core concepts through discussion with other students, the mind mapping activities appeared to help students to verify their understanding and ability to integrate new learning in physiology with their experience in professional settings.

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

C23

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

Stefan Naczk, Jessica Anderson, Laura Ginesi, Derek Scott

undefined

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

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

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

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

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

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

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

C24

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

Elisabeth Urbauer[1], Doriane Aguanno[1], Valentina Schüppel[1], Dirk Haller[1,2], Eva Rath[1]

[1]Technische Universität München, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany, [2]Technische Universität München, ZIEL Institute for Food & Health, Freising-Weihenstephan, Germany

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

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

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

C25

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

Judith Lorenz[3], Sandy Richter[2], Anna S. Kirstein[1], Florentien Kolbig[1], Michèle Nebe[1], Marco Schulze[4], Wieland Kiess[1], Ingo Spitzbarth[5], Nora Klöting[6], Gabriela-Diana Le Duc[7], Ulrike Baschant[8], Antje Garten[1]

[1]Pediatric Research Center, Hospital for Children and Adolescents, Leipzig University, Leipzig, Germany, [2]Pediatric Research Center, Hospital for Children and Adolescents, Leipzig University, Leipzig, Germany, [3]Pediatric Research Center, Hospital for Children and Adolescents, Leipzig University, Leipzig, Germany, [4]Saxon Incubator for Clinical Translation (SIKT), Leipzig University, Leipzig, Germany, [5]Faculty of Veterinary Medicine, Institute of Veterinary Pathology, Leipzig University, Leipzig, Germany, [6]Helmholtz Institute for Metabolism, Adiposity and Vascular Research (HIMAG), Helmholtz Center Munich at Leipzig University, Leipzig, Germany, [7]Institute of Human Genetics, Faculty of Medicine, Leipzig University, Leipzig, Germany, [8]Department of Medicine III, Technische Universität Dresden, Dresden, Germany

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

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

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

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

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

1. Filtz EA, Emery A, Lu H, Forster CL, Karasch C, Hallstrom TC. Rb1 and Pten Co-Deletion in Osteoblast Precursor Cells Causes Rapid Lipoma Formation in Mice. PLoS One. 2015;10(8):e0136729.

C26

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

Joana F. Sacramento[1], Silvia Vilares Conde[1], Dinis Sampaio-Pires[1], Adriana M. Capucho[1], Gonçalo M. Melo[1], Fatima O Martins[1]

[1]NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Portugal, Lisboa, Portugal

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

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

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

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

C27

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

Oluwaseun Anyiam[1], Bethan Phillips[1], Daniel Wilkinson[1], Kenneth Smith[1], Philip Atherton[1], Iskandar Idris[1]

[1]Centre of Metabolism Ageing and Physiology (COMAP), University of Nottingham, Derby, United Kingdom

Introduction

Type 2 diabetes (T2D) is characterised by chronic hyperglycaemia resulting from insulin resistance and pancreatic beta cell failure. Very-low calorie diets (VLCD) are recommended for management of T2D as they improve both of these defects[1,2]. However, the deleterious consequences of loss of lean body mass (LBM) with VLCD is becoming of increasing concern[3]. Similarly to VLCD, new anti-diabetic therapies, such as Glucagon-like peptide-1 receptor agonists (GLP1RA), promote weight loss and glycaemic improvements in T2D, and our recent data demonstrated that exogenous GLP-1 infusions enhanced postprandial muscle protein synthesis[4]. Investigating interactions between anti-diabetic interventions, such as GLP1RA, and VLCD, with body composition is timely, especially since individuals with T2D are vulnerable to accelerated age-related declines in muscle mass. We therefore investigated the effect of the GLP1RA Semaglutide, VLCD or a combination of the two therapies, upon body composition and muscle function outcomes.

Methods

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

Results

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

Conclusion

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

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

1. NHS England. Low calorie diets to treat obesity and Type 2 diabetes. https://www.england.nhs.uk/diabetes/treatment-care/low-calorie-diets/. Published 2020. Accessed August 27, 2022. 2. Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R. Reversal of type 2 diabetes: Normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. Diabetologia. 2011;54(10):2506-2514. 3. Ardavani A, Aziz H, Smith K, Atherton PJ, Phillips BE, Idris I. The Effects of Very Low Energy Diets and Low Energy Diets with Exercise Training on Skeletal Muscle Mass: A Narrative Review. Adv Ther. 2020;38(1):149-163. 4. Abdulla H, Phillips BE, Wilkinson DJ, et al. Glucagon-like peptide 1 infusions overcome anabolic resistance to feeding in older human muscle. Aging Cell. 2020;19(9):1-12.

C28

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

Sofia Bellalta[1,2], Torsten Plosch[3], Paola Casanello[4], Marijke Faas[5]

[1]Division of Medical Biology, Department of Pathology and Medical Biology, University Medical Center Groningen (UMCG)., Groningen, Netherlands, [2]PhD Program in Medical Sciences, School of Medicine, Pontificia Universidad Católica, Santiago, Chile, [3]Department of Obstetrics and Gynecology, UMCG, Groningen, Netherlands, [4]Deparments of Neonatology & Obstetrics, School of Medicine, Pontificia Universidad Católica, Santiago, Chile, [5]Division of Medical Biology, Department of Pathology and Medical Biology, University Medical Center Groningen (UMCG), Groningen, Netherlands

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

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

Methods: Umbilical cords were obtained from UMCG maternity ward with patients' consent (#2019.175). WJ-MSCs were isolated from newborns of normal-weight women (NW-MSC; Body Mass Index 18.5-24.5 kg/m²) and women with obesity (OB-MSC; Body Mass Index > 30 kg/m²) through the explant method. WJ-MSCs were characterized by surface markers, lineage commitment, clonogenic capacity (CFU-F) and cell growth rate. Basal and H_2O_2-challenged gene expression for superoxide dismutase 1/2 (SOD1/2), glutathione peroxidase (GPx1) and catalase (CAT) were quantified (qRT-PCR). FOXO1 protein expression was quantified in WJ-MSCs during induced-adipogenic commitment for 5 days (DMEM, insulin, IBMX, dexamethasone). Data was analyzed with Mann-Whitney test (mean ± SEM, n=7).

Results: Primary cultures of WJ-MSCs are a reliable source of MSC as shown by immunophenotype and differentiation assays. The OB-MSC presented a lower CFU-F and higher cell population doubling time, compared to NW-MSCs. OB-MSC presented a basal decrease in SOD1/2 and GPX gene expression ($p<0.05$), compared to NW-MSCs. The OB-MSC presented no response to H_2O_2 SOD2 gene expression, while NW-MSC responded with higher gene expression ($p<0.05$). OB-MSCs showed a trend to higher levels of FOXO1 protein ($p=0.0571$).

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

K. E. Boyle, Z. W. Patinkin, A. L. B. Shapiro, P. R. Baker, D. Dabelea, and J. E. Friedman, “Mesenchymal stem cells from infants born to obese mothers exhibit greater potential for adipogenesis: The healthy start babybump project,” Diabetes, vol. 65, no. 3, pp. 647–659, 2016, doi: 10.2337/db15-0849. L. Iaffaldano et al., “Altered Bioenergetic Profile in Umbilical Cord and Amniotic Mesenchymal Stem Cells,” vol. 27, no. 3, pp. 199–206, 2018, doi: 10.1089/scd.2017.0198. E. Jing, S. Gesta, and C. R. Kahn, “SIRT2 Regulates Adipocyte Differentiation through FoxO1 Acetylation/Deacetylation,” Cell Metab., vol. 6, no. 2, pp. 105–114, 2007, doi: 10.1016/j.cmet.2007.07.003.

C29

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

Matthew Biggart[1], Robert Tarran[2], Deborah Baines[1]

[1]St George's, University of London, London, United Kingdom, [2]University of North Carolina, Chapel Hill, United States

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

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

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

C30

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

Samuel Ellis[1], Claire Smith[1]

[1]UCL Great Ormond Street Institute of Child Health, London, United Kingdom, [2]UCL Centre for Clinical Microbiology, Royal Free Hospital, London, United Kingdom, [3]Great Ormond Street Hospital NHS Foundation Trust, London, United Kingdom

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

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

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

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

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

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

C31

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

Claire Pearson[1], Paolo Tammaro[1]

[1]*University of Oxford, Oxford, United Kingdom*

Background: Proton activated chloride (PAC) channels, encoded by the *PACC1* gene, mediate outwardly rectifying chloride currents activated by acidification of the extracellular environment (1, 2). PAC is expressed in a plethora of mammalian cell types, but the physiological mechanisms of PAC modulation are incompletely understood. Recently, phosphatidylinositol-4,5-bisphosphate (PIP_2) was reported to bind and inhibit PAC channel from the extracellular side (3). However, PIP_2 is predominantly an inner-leaflet lipid. Here, we explore whether physiological variations in inner-leaflet PIP_2 levels, such as those associated with activation of G_q-protein coupled receptors (G_qPCRs) may influence PAC activity.

Methods: Whole-cell patch-clamp recordings of PAC currents in human embryonic kidney 293T (HEK293T) cells were used in conjunction with heterologous expression of the acid sensing GqPCR ovarian cancer G-protein coupled receptor 1 (OGR1), the a_1-adrenergic receptor (a_1R) or the *Danio rerio* voltage sensitive protein phosphatase (DrVSP). In some experiments, DrVSP with a C302S mutation (DrVSP(C302S)) to abolish phosphatase activity was used as control. Data are given as mean ± SEM alongside the number of experiments (n). P-values < 0.05 were considered significant.

Results: A chloride current was recorded in HEK293T cells at extracellular pH (pH_e) below 5.5. At pH_e 5 and 7.4, the current was 100.7±8.6 pA/pF (n=18) and 4.5±0.5 pA/pF (n=18) when measured at + 95 mV, respectively. The pH_e giving the half-maximal activation (EC_{50}) was 5.2±0.0 (n=5). HEK293 cells in which the *PACC1* gene was deleted (2) presented negligible currents even at pH_e 5 (4.9±2.9 pA/pF (n=5)), and reintroduction of *PACC1* cDNA (transcript variant 2) restored PAC currents (838.6±119.7 pA/pF at pH 5).

The PAC current measured at 5 pH was not affected by heterologous expression of OGR1 or a_1R (stimulated with phenylephrine (1 mM)), suggesting that the PAC current may not be modulated by the G_qPCR signaling pathway in HEK293T cells. The role of second messengers associated with G_qPCR signalling, Ca^{2+} and PIP_2, was further investigated. PAC current amplitude was not affected when free Ca^{2+} in the intracellular recording solution ($[Ca^{2+}]_i$) was raised from 0 to 1 mM. Inclusion of the PIP_2 scavenger neomycin (1 mM) in the intracellular solution had no effect on PAC current magnitude. In cells transfected with DrVSP the current at a negative potential (-60 mV) did not differ from that measured in cells expressing DrVSP(C302S). However, at a supra-physiological hyperpolarizing potential (+100 mV), the steady-state current was reduced by a factor ~1.8 from 152.0±24.6 pA/pF (n=11) in DrVSP(C302S) to 86.3±9.1 pA/pF (n=14) with DrVSP.

Conclusions: G_qPCR and PIP_2 signaling did not produce significant modulation of the PAC current in HEK293T in a physiological range of membrane potentials. The data suggest that cellular responses to extracellular acidification that are mediated by OGR1 and PAC may involve different pathways. While further work will be required to establish the crosstalk of pH_e

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

1. Yang J et al., (2019). Science 364, 395-399. 2. Ullrich F et al., (2019). eLife 8, e49187. 3. Mihaljević L, et al., (2023). eLife 12, e83935.

C32

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

Tereza Masonou[1], Maximillian Woodall[1], Ayad Eddaoudi[1], Timothy D McHugh[2], Colin Butler[1], Marko Nikolic[1], Rosalind L Smyth[1], Claire M Smith[1]

[1]UCL GOSH Institute of Child Health, London, United Kingdom, [2]UCL Centre for Clinical Microbiology, London, United Kingdom

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

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

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

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

Huang, C. et al. (2020) ‘Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China’, The Lancet, 395(10223), pp. 497–506. doi: 10.1016/S0140-6736(20)30183-5.
Veras, F. P. et al. (2020) ‘SARS-CoV-2 triggered neutrophil extracellular traps (NETs) mediate

COVID-19 pathology', Journal of Experimental Medicine, 217(12). doi: 10.1101/2020.06.08.20125823. Woodall MNJ, Masonou T, Case KM, Smith CM. Human models for COVID-19 research. J Physiol. 2021 Sep;599(18):4255-4267. doi: 10.1113/JP281499. Epub 2021 Aug 17. PMID: 34287894; PMCID: PMC8447334.

C33

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

Theresa Leslie[1,2], Aurelien Tripp[3], George Poulogiannis[4], Christopher Huang[5,6], Samantha Salvage[5], Hugh Matthews[6], Antony Jackson[5], Sangeeta Chawla[1,2], William Brackenbury[1,2]

[1]Department of Biology, University of York, York, United Kingdom, [2]York Biomedical Research Institute, University of York, York, United Kingdom, [3]Signalling and Cancer Metabolism Team, Division of Cancer Biology, The Institute of Cancer Research, London, United Kingdom, [4]Signalling and Cancer Metabolism Team, Division of Cancer Biology, The Institute of Cancer Research, London, United Kingdom, [5]Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom, [6]Department of Physiology, University of Cambridge, Cambridge, United Kingdom

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

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

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

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

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

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

1. Lopez-Charcas O et al. (2021). iScience 24, 102270 2. Leslie TK et al. (2021). New Roles for Ion Channels and Transporters in Health and Disease, online symposium, 2-3 September 2021. 3. Brisson L et al. (2013). J Cell Sci 126, 4835-4842 4. Fraser SP et al. (2005). Clin Cancer Res 11, 5381-5389.

C34

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

Bonnie Blazer-Yost[3], Louise Hulme[2], Alexandra Hochstetler[1]

[1]Harvard University, Boston, United States, [2]Virgin Media, Leeds, United Kingdom, [3]Indiana University, Indianapolis, United States

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

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

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

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

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

C35

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

Damian Bailey[1]

[1]*University of South Wales, Pontypridd, United Kingdom*

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

Methods: Ten ultra-elite breath-hold divers (6 male/4 female) aged 33 (mean) ± 9 (SD) years old performed two maximal dry apneas after normoxic hyperventilation (NX: severe hypoxaemia-hypercapnia) and hyperoxic hyperventilation (HX: absence of hypoxaemia while exacerbating hypercapnia) with measurements obtained at eupnea and after apnea. Mean arterial (MAP) and internal jugular venous (IJVP) pressures were recorded directly. The latter served as a validated surrogate of intracranial pressure (ICP). Net transcerebral biomarker exchange was calculated as the product of global cerebral blood flow (gCBF, duplex ultrasound) and radial arterial to internal jugular venous concentration gradients of plasma ascorbate free radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy), plasma nitrite (NO2-, ozone-based chemiluminescence) and select panel of serum NVU proteins (ELISA, Single Molecule Array). Following confirmation of distribution normality (Shapiro-Wilk *W* tests), data were analysed using a combination of two (*State*: eupnea *vs*. apnea × *Site*: arterial *vs*. venous) and three (*Trial*: NX *vs*. HX × *State* × *Site*) factor repeated measures ANOVAs with post-hoc Bonferroni-corrected paired samples *t*-tests.

Results: Compared to HX, greater increases in MAP (+61 ± 9 vs. +47 ± 14 mmHg, P = 0.021) and IJVP (+12 ± 2 vs. +7 ± 5 mmHg, P = 0.005) were observed during apnea in NX whereas the increase in gCBF was lower (+83 ± 22 % vs. +206 ± 52 %, P = <0.001). Apnea in NX stimulated a greater net cerebral output (venous > arterial) of $A^{\bullet-}$ (-4049 ± 5035 vs. -609 ± 5273 AU/100g/min, P = 0.042) and lower uptake (arterial > venous) of NO2- (1787 ± 2029 vs. 3806 ± 2334 nM/100g/min, P = 0.036), highlighting the key contribution of hypoxaemia to OXNOS. This coincided with a greater net cerebral output of S100B, glial fibrillary acidic protein, ubiquitin carboxy-terminal hydrolase L1, neurofilament light-chain and total tau (all P < 0.05).

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

C36

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

Elizabeth Wrench[1], Jack Cunnington[1], Alexandra Dent[2], Abdul Shugaba[1], Louise Curran[1], Lucy Jackson-Jones[3], Lawrence D Hayes[4], Clifford L Shelton[1], Daren Subar[5], Robert M Lauder[1], Christopher J Gaffney[1]

[1]Lancaster Medical School, Lancaster University, Lancaster, United Kingdom, [2]University Hospitals Morecambe Bay Trust, Lancaster, United Kingdom, [3]Faculty of Biological and Life Sciences, Lancaster University, Lancaster, United Kingdom, [4]Institute of Clinical Exercise and Health Science, School of Health and Life Sciences, University of West of Scotland, Glasgow, United Kingdom, [5]East Lancashire NHS Hospitals Trust, Blackburn, United Kingdom

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

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

Methods: Participants (n=10) (age 23 ± 4, BMI = 26 ± 5 kg/m^2) attended the lab and cycled at 60% $\dot{V}O_2$ max for a time commensurate with expending 0kcal, 175kcal, 350kcal or 700kcal (randomised). Participants were fitted with a continuous glucose monitor (CGM) for the next 72 hours. The evening of these exercise visits, subjects consumed a control meal (772kcal, carbohydrate=66%, fat=18%, and protein=16%). Following a 12 hour overnight fast, subjects undertook an oral glucose tolerance test (OGTT). Bloods were taken from an arterialised dorsal hand vein at baseline (before ingestion of 75g dextrose in 300ml water), and every 30 minutes for two-hours. Indirect calorimetry was taken 20 mins before the test and during the last 20 mins of the OGTT.

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

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

C37

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

Hannah Blount[1], Alessandro Valenza[1], Jade Ward[1], Peter Worsley[2], Silvia Caggiari[2], Grant Simmons[3], Davide Filingeri[1]

[1]ThermosenseLab, Skin Sensing Research Group, The University of Southampton, Southampton, United Kingdom, [2]PressureLab, Skin Sensing Research Group, The University of Southampton, Southampton, United Kingdom, [3]Nike Sport Research Lab, Nike Inc., Portland, United States

Introduction:

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

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

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

Method:

Fifteen healthy females (24±7yr) with large differences in BrSA (from small to x-large, range=147.2-480.5cm^2) performed a 50-min submaximal run in a climatic chamber regulated at 33.0±0.8℃ and 53.4±2.0% RH. Sweat gland density (SGD; modified iodine technique [4]) and local sweat rates (LSR; absorbent patches [5]) were measured above and below the nipple, and at the bra triangle, during the final 5-min of exercise. Gastrointestinal (core) temperature and metabolic rate were monitored throughout the run. We used linear regression analyses to evaluate the relationship between: a) SGD and BrSA; and b) sweat output per gland (calculated as LSR/SGD) and BrSA. Furthermore, we assessed regional differences in SGD and sweat output per gland amongst the bra triangle, above and below the nipple, with a repeated-measures ANOVA.

Results:

SGD above (R^2=0.55, p<0.01, Fig. 1A) and below the nipple (R^2=0.63, p<0.01, Fig. 1B) decreased with increasing BrSA. This effect was not observed at the bra triangle (R^2=0.12, p=0.101, Fig. 1C). Sweat output per gland above the nipple increased with BrSA (R^2=0.29, p=0.02, Fig. 2A). This effect was not observed below the nipple (R^2=0.13, p=0.10, Fig. 2B) nor at the bra triangle (R^2=0.04, p=0.24, Fig. 2C).

SGD was lower at both breast sites (above nipple=35.6±6.0 glands/cm^2; below nipple=31.2±4.8 glands/cm^2, p<0.01) than at the bra triangle (86.8±5.3glands/cm^2, Fig. 3). Sweat output per gland above (343.4±39.6µg, p<0.01), but not below (416.4±62.5µg, p=0.89), the nipple was lower than at the bra triangle (690.6±76.0µg, Fig. 4).

Conclusion:

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

1. Kuno, Y., Human perspiration. Thomas, Spring-filed, 1956. 2. Szabo, G., The regional frequency and distribution of hair follicles in human skin in: The biology of hair growth. Montagna W, Ellis A. 1958, Academic Press Inc, New York. 3. Kondo, N., et al., Function of human eccrine sweat glands during dynamic exercise and passive heat stress. Journal of Applied Physiology, 2001. 90(5): p. 1877-1881. 4. Gagnon, D., et al., Modified iodine-paper technique for the standardized determination of sweat gland activation. Journal of applied physiology, 2012. 112(8): p. 1419-1425. 5. Smith, C.J. and G. Havenith, Body mapping of sweating patterns in male athletes in mild exercise-induced hyperthermia. European journal of applied physiology, 2011. 111(7): p. 1391-1404.

C38

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

Brenda Pena Carrillo[1], Oscar Duval[1], Emily Cope[1], Amber Kenny[1], Sati Gurel[1], Sam Philip[2], Frank Thies[1], Dimitra Blana[3], Brendan Gabriel[1,4]

[1]The Rowett Institute, University of Aberdeen, Aberdeen, United Kingdom, [2]Grampian Diabetes Research Unit, Diabetes Centre, Aberdeen Royal Infirmary, Aberdeen, United Kingdom, [3]Centre for Health Data Science, Institute of Applied Health Sciences, University of Aberdeen, Aberdeen, United Kingdom, [4]Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden

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

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

Gabriel BM & Zierath JR (2021). Zeitgebers of skeletal muscle and implications for metabolic health. The Journal of Physiology; 600(5):1027-1036. Gabriel, B. M., & Zierath, J. R. (2017). The Limits of Exercise Physiology: From Performance to Health. Cell metabolism, 25(5), 1000–1011. Savikj, M., Gabriel, B. M., Alm, P. S., Smith, J., Caidahl, K., Björnholm, M., Fritz, T., Krook, A., Zierath, J. R., & Wallberg-Henriksson, H. (2019). Afternoon exercise is more efficacious than morning exercise at improving blood glucose levels in individuals with type 2 diabetes: a randomised crossover trial. Diabetologia, 62(2), 233–237.

C39

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

Tiarna Stothers[1,2], Travis Gibbons[1,3], Emma Jones[1,2], Kate Thomas[2], Holly Campbell[2], Toby Mündel[4], Naoto Fujii[5], Tatsuro Amano[6], Narihiko Kondo[7], James Cotter[1]

[1]School of Physical Education, Sport & Exercise Science, University of Otago, Dunedin, New Zealand, [2]Department of Surgical Sciences, University of Otago, Dunedin, New Zealand, [3]Centre for Heart, Lung and Vascular Health, University of British Columbia-Okanagan, School of Health and Exercise Science, Kelowna, Canada, [4]School of Sport, Exercise and Nutrition, Massey University, Palmerston North, New Zealand, [5]Faculty of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan, [6]Laboratory for Exercise and Environmental Physiology, Faculty of Education, Niigata University, Niigata, Japan, [7]Graduate School of Human Development and Environment, Kobe University, Kobe, Japan

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

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

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

Results: Core temperature was not higher at baseline for females than males (37.18 vs 37.06°C; P=0.479; CL: -0.22, 0.45). Neither was thermal reserve smaller (2.07 vs 1.75°C; P=0.271; CL: -0.28, 0.93), nor rate of heating faster (1.25 vs 1.21 °C/h; P=0.841; CL: -0.37, 0.45). Only one participant reached the 40.00°C ethical end point. End tidal CO_2 pressure did not differ at baseline or decrease (5 vs 6 mm Hg) more for females than males during heat stress (Sex: P=0.081; Strain: P=0.001; Sex*Strain: P=0.634). Mean arterial blood pressure and its decrease (2 vs 6 mm Hg) were also similar between sexes (P=0.503; P=0.011; P=0.221). Perfusion of the internal carotid artery also remained similar (-1 vs -9%; P=0.879; P=0.181;

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

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

C40

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

Alexander Carswell[1], Kevin Howland[2], Glen Davison[3]

[1]Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, United Kingdom, [2]School of Biosciences, University of Kent, Canterbury, United Kingdom, [3]School of Sport and Exercise Sciences, University of Kent, Canterbury, United Kingdom

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

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

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

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

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

Grgic J et al. (2021). Eur J Nutr 60(3), 1181-1195. Nehlig A (2018). Pharmacol Rev 70(2), 384-411.

C41

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

Charlotte Merrick[1], Alessandro Valenza[1,2], Riley Wootten[1], Jasmin Dearden[1], Hannah Blount[1], Jade Ward[1], Charlotte Wildgoose[1], Antonino Bianco[2], Alex Buoite-Stella[3], Peter R Worsley[4], Davide Filingeri[1]

[1]ThermosenseLab, Skin Sensing Research Group, School of Health Sciences, The University of Southampton, Southampton, United Kingdom, [2]Sport and Exercise Sciences Research Unit, SPPEFF Department, University of Palermo, Palermo, Italy, [3]Department of Medicine, Surgery and Health Sciences University of Trieste, Trieste, Italy, [4]PressureLab, Skin Sensing Research Group, School of Health Sciences, The University of Southampton, Southampton, United Kingdom

Introduction

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

Methods

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

Results

We found a statistically significant main effect of age on cold-wetness sensing (p=0.031). Post-hoc analyses indicated that, irrespective of body region, children were more sensitive than older adults (mean difference: 20.4mm [95%CI: 1.3, 39.5], corresponding to 20% difference; p=0.033; Fig. 1A), and as sensitive as younger adults (mean difference: 8.5mm [95%CI: -8.7, 25.8], p=0.467). We also found a main effect of skin site on cold-wetness sensing (p=0.014). Post-hoc analyses indicated that, irrespective of age, the foot was more sensitive than the neck (mean difference: 11.1mm [95%CI: 2.2, 20.0], corresponding to 11% difference; p=0.011; Fig. 1A). We found no main effect of age on warm (p=0.842, Fig. 1B) or neutral wetness perception (p=0.158, Fig. 1C), yet we found a main effect of skin site on warm wetness sensing, with the foot being less sensitive than the neck (mean difference: 12.9mm [95%CI: 2.8, 23.0], corresponding to 13% difference; p=0.008; Fig. 1A).

Conclusions

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

References: [1] Vargas NT, Chapman CL, Ji W, Johnson BD, Gathercole R, Schlader ZJ. Increased skin wetness independently augments cool-seeking behaviour during passive heat stress. J Physiol. 2020 Jul;598(13):2775-2790. [2] Filingeri D, Fournet D, Hodder S, Havenith G. Why wet feels wet? A neurophysiological model of human cutaneous wetness sensitivity. J Neurophysiol. 2014 Sep 15;112(6):1457-69. [3] Typolt O, Filingeri D. Evidence for the involvement of peripheral cold-sensitive TRPM8 channels in human cutaneous hygrosensation. Am J Physiol Regul Integr Comp Physiol. 2020 Mar 1;318(3):R579-R589.

C42

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

Eleanor Jones[1], Yuxiao Guo[2], Ken Smith[3], Daniel Wilkinson[3], Bethan Phillips[3], Philip Atherton*[3], Mathew Piasecki*[3]

[1]Centre of Metabolism, Ageing and Physiology (COMAP), MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Derby, United Kingdom, [2]Centre of Metabolism, Ageing and Physiology (COMAP), MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham., Derby, United Kingdom, [3]Centre of Metabolism, Ageing and Physiology (COMAP), MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Derby, United Kingdom., Derby, United Kingdom

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

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

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

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

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

1. Mcleod JC, et al. Front Physiol. 2019;10. doi:10.3389/fphys.2019.00645 2. Keogh JWL, et al. Sport Med. 2019;49: 1199–1216. doi:10.1007/s40279-019-01141-6 3. Hortobagyi T, et al. Journals Gerontol Ser A Biol Sci Med Sci. 2001;56: B38–B47. doi:10.1093/gerona/56.1.B38 4. Ely IA, et al. Exp Physiol. 2022;107: 1061–1070. doi:10.1113/EP090367

C43

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

Abdulmajeed Altheyab[1,2], Nishadi Gamage[4], Bethan E Phillips[4], Mathew Piasecki[6]

[1]1. Centre of Metabolism, Ageing and Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research & National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham., Derby, United Kingdom, [2]2. Faculty of College of Applied Medical Sciences, King Saud bin Abdulaziz University for Health Science., Riyadh, Saudi Arabia, [3]1. Centre of Metabolism, Ageing and Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research & National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Derby, United Kingdom., Derby, United Kingdom, [4]1. Centre of Metabolism, Ageing and Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research & National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Derby, United Kingdom, [5]1. Centre of Metabolism, Ageing and Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research & National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Derby, United Kingdom, [6]1. Centre of Metabolism, Ageing and Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research & National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, University of Nottingham, Derby, United Kingdom

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

Methods: This study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (FMHS 390-1121). After providing written, informed consent to participate, 16 healthy participants (8 male, 8 female. 74±5 years, BMI 26±3 kg/m^2) underwent two assessment visits separated by 4-weeks fully-supervised unilateral knee extensor FAT. FAT occurred 3x/wk and consisted of 6 sinusoidal force-tracking contractions at 10, 25 and 40% of maximum voluntary contraction (MVC) in each session. Bilateral knee extensor strength was assessed via MVC and the coefficient of variation of force (force steadiness (FS)) was quantified at 25% MVC. Left and right handgrip strength (HGS) were measured with a handheld dynamometer, and mobility was assessed by the timed up-and-go (TUG). Data were analysed via two-way repeated measures ANOVA (leg/hand x time) and paired Students t-test (TUG). Statistical significance was accepted at $p<0.05$.

Results: There was no leg x time interaction for MVC (p=0.822), but there was a main effect of time (p=0.003) with MVC increasing in the trained (+15.2%, p=0.04) but not the untrained

(p=0.09) limb. There was a significant leg x time interaction (p=0.026) for FS, improving to a greater extent in the trained (+16.2%, p=0.0001) than the untrained (+8.9%, p=0.041) limb. There was no hand x time interaction for HGS (p=0.885), however there was main effect of time (p=0.001) with HGS improving in the right (+3.2% p=0.04) and left (+3.8%, p=0.028) hand. There was a significant improvement in TUG time following the FAT intervention (p=0.023).

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

1. Enoka RM, Farina D. Force steadiness: from motor units to voluntary actions. Physiology. 2021;36(2):114-30. 2. Ely IA, Jones EJ, Inns TB, Dooley S, Miller SBJ, Stashuk DW, et al. Training-induced improvements in knee extensor force accuracy are associated with reduced vastus lateralis motor unit firing variability. Experimental Physiology. 2022;107(9):1061-70. 3. Blackwell J, Doleman B, Boereboom C, Morton A, Williams S, Atherton P, et al. High-intensity interval training produces a significant improvement in fitness in less than 31 days before surgery for urological cancer: a randomised control trial. Prostate cancer and prostatic diseases. 2020;23(4):696-704. 4. Coupland AP, Thapar A, Qureshi MI, Jenkins H, Davies AH. The definition of stroke. Journal of the Royal Society of Medicine. 2017;110(1):9-12.

C44

The effect of exercise intensity on calcium metabolism

Scott Hannah[1], Conor McClean[2], Sonyia McFadden[2], Andrea McNeilly[2]

[1]University of Winchester, Winchester, United Kingdom, [2]Ulster University, Belfast, United Kingdom

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

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

Results: Exercise produced a biphasic intensity-dependent Ca^{2+} response to exercise ($F(12, 189.83) = 3.45$, $p < .001$). Exercise below GET did not alter Ca^{2+} when referenced to baseline (M_{diff} = -0.01–0.02 mmol·L^{-1}, SE = 0.01, $p > 0.05$), but exercise above GET (including above RCP) significantly increased Ca^{2+}, peaking at 10-minutes (M_{diff} = 0.04–0.07, SE = 0.01, $p < 0.001$). Plasma-volume adjusted PTH (PTH_{Adj}) was significantly decreased in the initial 10-minutes of exercise above GET/RCP (M_{diff} = -15.81 – -10.61 , SE = 3.30, $p < 0.001$). Ca^{2+} decreased throughout the remainder of exercise above GET and RCP, returning to baseline concentrations. PTH_{Adj} mirrored Ca^{2+}'s response: PTH_{Adj} increased from 10 minutes above GET and above RCP, but was only significantly greater than baseline following above GET exercise (M_{diff} = 30.93, SE= 3.38, $t(189.33) = 9.14$, $p < 0.001$). Introducing pH as a covariate in the Ca^{2+} model (b = -0.27, SE = 0.06, $t(189.30) = -4.11$, $p < 0.001$) removed significant interactions at 5 and 10-minutes between exercise below GET and above RCP. However, pH could not account for all Ca^{2+} variation, as the main effect of time ($F(7, 191.77) = 13.85$, $p < 0.001$), and its interaction with condition ($F(12, 190.17) = 4.37$, $p < 0.001$), remained significant. Pooled concentrations of PTH_{Adj} were negatively associated with Ca2+ ($R = -0.6$, $p < 0.001$).

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

Townsend R, Elliott-Sale KJ, Pinto AJ, Thomas C, Scott JPR, Currell K, Fraser WD & Sale C (2016). Parathyroid hormone secretion is controlled by both ionized calcium and phosphate during exercise and recovery in men. Journal of Clinical Endocrinology and Metabolism 101, 3231–3239. Kohrt WM, Wherry SJ, Wolfe P, Sherk VD, Wellington T, Swanson CM, Weaver CM & Boxer RS (2018). Maintenance of Serum Ionized Calcium During Exercise Attenuates Parathyroid Hormone and Bone Resorption Responses. Journal of Bone and Mineral Research 33, 1326–1334. Duckham RL, Peirce N, Meyer C, Summers GD, Cameron N & Brooke-Wavell K (2012). Risk factors for stress fracture in female endurance athletes: a cross-sectional study. BMJ open 2, 1–8. Scofield KL & Hecht S (2012). Bone health in endurance athletes: Runners, cyclists, and swimmers. Current Sports Medicine Reports 11, 328–334. Fogh-Andersen N, Bjerrum PJ & Siggaard-Andersen O (1993). Ionic binding, net charge, and Donnan effect of human serum albumin as a function of pH. Clinical Chemistry 39, 48–52.

C45

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

Cameron Hill[1], Michaeljohn Kalakoutis[1], Alice Arcidiacono[1], Flair Paradine Cullup[1], Yanhong Wang[1], Atsuki Fukutani[1], Narayanan Theyencheri[3], Elisabetta Brunello[1], Luca Fusi[1,4], Malcolm Irving[1]

[1]Randall Centre for Cell and Molecular Biophysics, King's College London, London, United Kingdom, [2]King's College London, London, United Kingdom, [3]European Synchrotron Radiation Facility, Grenoble, France, [4]Centre for Human and Applied Physiological Sciences, King's College London, London, United Kingdom

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

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

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

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

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

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

C46

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

Thomas Inns[1,2,3], Joseph Bass[1], Edward Hardy[1,4], Daniel Stashuk[5], Philip Atherton[1], Bethan Phillips[1], Mathew Piasecki[1]

[1]Centre Of Metabolism, Ageing & Physiology, MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research and NIHR Nottingham BRC, University of Nottingham, Derby, United Kingdom, [2]Sport, Health and Performance Enhancement Research Centre, Nottingham Trent University, Nottingham, United Kingdom, [3]School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom, [4]Department of Surgery and Anaesthetics, Royal Derby Hospital, Derby, United Kingdom, [5]Department of Systems Design Engineering, University of Waterloo, Waterloo, Canada

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

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

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

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

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

1. Wall BT, Dirks ML, Snijders T, Senden JMG, Dolmans J & Van Loon LJC (2014). Substantial skeletal muscle loss occurs during only 5 days of disuse. Acta Physiol 210, 600–611. DOI: 10.1111/apha.12190. 2. Belavý DL, Miokovic T, Armbrecht G, Richardson CA, Rittweger J & Felsenberg D (2009). Differential atrophy of the lower-limb musculature during prolonged bed-rest. Eur J Appl Physiol 107, 489–499. DOI: 10.1007/s00421-009-1136-0. 3. Bass JJ, Hardy EJO, Inns TB, Wilkinson DJ, Piasecki M, Morris RH, Spicer A, Sale C, Smith K, Atherton PJ & Phillips BE (2021). Atrophy Resistant vs. Atrophy Susceptible Skeletal Muscles: “aRaS” as a Novel Experimental Paradigm to Study the Mechanisms of Human Disuse Atrophy. Front Physiol 12, 1–11. DOI: 10.3389/fphys.2021.653060. 4. Heckman CJ, Johnson M, Mottram C & Schuster J (2008). Persistent inward currents in spinal motoneurons and their influence on human motoneuron firing patterns. Neuroscientist 14, 264–275. DOI: 10.1177/1073858408314986.

C47

Local and systemic mediators of skeletal muscle wasting in humans following acute trauma

Harrison Gallagher[1], Hanzla Naeem[2], Nathanael Wood[1], Hélène N. Daou[3], Marcelo G. Pereira[1], Lee D. Roberts[3], Anthony Howard[2], T. Scott Bowen[1]

[1]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom, [2]Academic Department of Trauma and Orthopaedic Surgery, Leeds General Infirmary, Leeds, United Kingdom, [3]Faculty of Medicine, University of Leeds, Leeds, United Kingdom

Introduction: Skeletal muscle wasting is an important clinical issue following acute traumatic injury, and can delay recovery and cause permanent functional disability. However, the fundamental mechanisms involved in trauma-induced muscle wasting remain poorly defined and therapeutic interventions are limited.

Objectives: To characterise local and systemic mediators of skeletal muscle wasting in patients following acute trauma.

Methods: Experiments were approved by a local NHS Research Ethics Committee and all participants provided written informed consent. *Vastus lateralis* biopsies and serum samples were taken from human male and female patients shortly after acute trauma in lower limbs (n=6; mean age 78.7±4.4 y) and compared to age-matched controls (n=6; mean age 72.6±6.3 y). Atrogenes and upstream regulators (MuRF1; MAFbx; IL6, TNFα, PGC-1α) mRNA expression was assessed in muscle samples via RT-qPCR. Serum profiling of inflammatory markers (e.g. IL6, TNFα, IL1β) was further performed via multiplex assays. To determine whether systemic factors induced by trauma directly affect muscle phenotype, differentiated primary human myotubes were treated *in vitro* with serum from controls or trauma patients (pooled; n=3 each) in the final 24 hours of differentiation. Cells were then fixed, stained for myogenin and imaged to determine minimum ferret diameter. Statistical significance was determined at P<0.05.

Results: There was an increase in skeletal muscle mRNA expression for E3 ligase MAFbx and inflammatory cytokine IL-6 (4.6 and 21.5-fold respectively; P<0.05) in trauma patients compared to controls. Expression of myogenic determination factor MyoD and regulator of mitochondrial biogenesis PGC-1α was lower in muscle of trauma patients vs controls (0.5 and 0.39-fold respectively; P<0.05). In serum, trauma patients showed increased concentrations of circulating pro-inflammatory cytokines IL-6 (14.5 vs. 0.3 pg/ml; P<0.05) and IL-16 (182.7 vs. 85.2 pg/ml; P<0.05) compared to controls. Primary myotube experiments revealed serum from trauma patients induced atrophy (32% decrease in diameter) compared to control serum-treated cells (P<0.001).

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

N/a

C48

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

Alessandro Valenza[3,4], Hannah Blount[3], Antonino Bianco[4], Peter R Worsley[5], Davide Filingeri[6]

[1]University of Palermo, Palermo, Italy, [2]University of Southampton, Southampton, United Kingdom, [3]ThermosenseLab, Skin Sensing Research Group, School of Health Sciences, The University of Southampton, Southampton, United Kingdom, [4]Sport and Exercise Sciences Research Unit, SPPEFF Department, University of Palermo, Palermo, Italy, [5]PressureLab, Skin Sensing Research Group, School of Health Sciences, The University of Southampton, Southampton, United Kingdom, [6]ThermosenseLab, Skin Sensing Research Group, School of Health Sciences, The University of Southampton, Southampton, United Kingdom

Introduction

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

Methods

Eleven younger (25±5y; 1.7±0.1m; 63.1±5.2Kg) and 11 older women (53±6y; 1.7±0.1m; 65.4±13.9Kg) performed a 40-min incremental cycling test (workload: 20 to 80W; 20-W increments at 10-min intervals) on a cycle-ergometer in a thermoneutral environment (22 ± 1.7°C; 36±4 RH). Throughout the test, a cooling probe (25cm^2) was secured to the participants' wrist, and participants freely adjusted the probe's temperature to offset thermal discomfort arising from exercise heat-stress. We continuously recorded the probe-wrist interface temperature ($T_{interface}$; micro-thermocouple) to quantify participants' cool-seeking behavior. We also measured participants' rate of metabolic heat production (H_{prod}; indirect calorimetry), changes in core temperature (T_{core}; gastrointestinal telemetry), and in mean skin temperature (T_{sk}) and wetness (*w*; thermo-hygro- sensors) throughout the exercise. Finally, we quantified participants' cold sensitivity at the wrist via quantitative sensory testing prior to exercise. We compared the onset (time) and amplitude (Δchange) of cooling, and its minimum temperature, between younger and older women, with unpaired t-tests. We modelled the relative contributions of H_{prod}, T_{core}, T_{sk} and *w* to changes in $T_{interface}$ for each participant via multiple regression analyses and compared older and younger women with a mixed-model ANOVA. We evaluated the association between wrist cold sensitivity and cooling amplitude via Pearson's correlation.

Results

We found no differences in cool-seeking behaviour's onset time (1.4min [95%CI -4.8, 7.7]; p=0.633), amplitude (2.2℃[-3.2, 7.7] p=0.406) and minimum cooling temperature (-0.3℃ [-5.5, 4.9]; p=0.908), between younger and older women (Fig. 1). We also found no association between wrist cold sensitivity and cooling amplitude in younger (0.11; p=0.737) and older women (-0.36; p=0.269). Multiple regression models indicated that changes in $T_{interface}$ were primarily described by changes in T_{core}, followed by *w*, T_{sk}, and H_{prod} (R^2=0.95±0.05; p<0.0001) in both younger and older women (Fig. 2). However, we observed a statistically significant decrease in the relative contribution of T_{core} to changes in $T_{interface}$ in the older women (-23% [5, 42]; p=0.006; Fig. 2).

Conclusions

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

[1] Ebi et al (2021) Hot weather and heat extremes: health risks. The Lancet, 398 (10301). [2] Schlader, Z. J. & Vargas, N. T. Regulation of Body Temperature by Autonomic and Behavioral Thermoeffectors. Exerc. Sport Sci. Rev. 47, 116–126 (2019). [3] Vargas, N.T., Schlader, Z.J., Jay, O. et al. Prioritize research on human behaviour during extreme heat. Nat Hum Behav (2023). https://doi.org/10.1038/s41562-023-01569-x. [4] Hutchins et al (2021) Female (Under) Representation in Exercise Thermoregulation Research. Sports Med, 7 (43).

C49

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

Danniella Hurt[1], Benjamin Stacey[1], Damien Laneelle[1,2,3], Lewis Fall[1], Ryan Sixtus[4], Chris Marley[1], Adnan Haq[1], Tom Owens[1], Leon Yandle[1], Chris Puhl[5], Herve Normand[3], Damian Bailey[1]

[1]Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Glamorgan, UK, Cardiff, United Kingdom, [2]Service de Médecine Vasculaire, Centre Hospitalo-Universitaire, Caen, Caen, France, [3]Normandie University, UNICAEN, INSERM, COMETE, GIP CYCERON, Caen, France, Caen, France, [4]School of Biosciences, University of Cardiff, Glamorgan, UK, Cardiff, United Kingdom, [5]elespazio Belgium S.R.L. for the European Space Agency, Noordwijk, Netherlands, Noordwijk, Netherlands

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

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

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

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

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

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

C50

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

Hui Tien Liu[2], Philip Atherton[3], Daniel Wilkinson[2], Matthew Brook[1]

[1]MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, Physiology, Pharmacology and Neuroscience, School of Life Sciences, University of Nottingham, UK., nottingham, United Kingdom, [2]Centre of Metabolism, Ageing & Physiology (COMAP); MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research; NIHR Nottingham Biomedical Research Centre (BRC), School of Medicine, University of Nottingham, UK., nottingham, United Kingdom, [3]Centre of Metabolism, Ageing & Physiology (COMAP); MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research; NIHR Nottingham Biomedical Research Centre (BRC), School of Medicine, University of Nottingham, UK., Nottingham, United Kingdom

Skeletal muscle aging is associated with increased risk of frailty, morbidity, and mortality and to date has few safe and efficient treatments. Although the mechanistic target of rapamycin complex 1 (mTORc1) signaling pathway in younger animals/humans, regulates cell metabolism positively, recent findings suggest mTOR becomes over-active in older age, and that this could be a cause of sarcopenia. In this study, we sought to map mTOR-related signaling as a function of age and fibre type.

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

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

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

C51

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

Samuel Lord[1], Yu-Chiang Lai[1], Harvey Johnston[2], Ryan Marshall[1], David Hughes[3], Sue Bodine[3], Rahul Samant[2]

[1]University of Birmingham, Birmingham, United Kingdom, [2]Babraham Institute, Cambridge, United Kingdom, [3]University of Iowa, Iowa, United States

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

C52

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

Eleanor Raffan[1,2], Natalie Wallis[1,2], Alyce McClellan[2], Alexander Mörseburg[1], Justine Chan[2], Sambhavi Kumar[2], Ellen Schofield[2], Katherine Kentistou[1], John Perry[1], Sadaf Farooqi[1], Stephen O'Rahilly[1], Giles Yeo[1], Rebecca Bounds[1]

[1]Wellcome-MRC Institute of Metabolic Science, Cambridge, United Kingdom, [2]Department of Physiology Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

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

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

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

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

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

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

C53

Mild hypoxia augments acute temperature sensing in the rat carotid body

Demitris Nathanael[1], Andrew Coney[1], Clare Ray[1], Prem Kumar[1], Andrew Holmes[1]

[1]*The University of Birmingham, Birmingham, United Kingdom*

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

All experiments and procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986. Chemoafferent activity was measured *in vitro* from CBs dissected from adult male Wistar rats (200-300g). CB tissue was surgically removed under non-recovery anaesthesia (isoflurane (3-5%) in O_2 at a flow rate of 1.5L min^{-1}), death via cervical dislocation. CB preparations were superfused with a physiological salt solution equilibrated at 37°C and at a normocapnic, normoxic PO_2 to establish a baseline discharge between 0.25-1.5Hz. The CB was then cooled to 32°C, followed by steady incremental warming to 40°C over approximately 5 minutes (n=24 spikes, 10 animals). This protocol was repeated in hyperoxia (n=6 spikes, 6 animals), mild hypoxia in normocapnia (n=8 spikes, 7 animals) and during the addition of 100 µM 2-APB, a non-selective modulator of TRP channels, in normoxic normocapnia (n=8 spikes, 5 animals). Discharge was recorded at 1°C increments between 32°C and 40°C, results expressed as mean ±SEM and significance ($P<0.05$) was established by linear regression analysis.

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

Overall, this data demonstrates that acute temperature sensing in the CB is PO_2 dependent, being abolished by hyperoxia and augmented by mild hypoxia. Thermal sensitivity during mild hypoxia is exaggerated above, rather than below, 37°C, supporting the notion of a primary role for the CB in heat-induced hyperventilation (Gibbons et al., 2022). The augmented response to temperature in mild hypoxia may be regulated by TRP channels but future studies warrant the use of more selective drugs to determine the exact mechanism of acute temperature sensing in the CB.

Alcayaga, J et al. (1993). Brain Research, 600(1), 103-111. Eyzaguirre, C et al. (1983). Brain Research, 279(1-2), 282-285. Gibbons, T et al. (2022). Journal of Physiology-London, 600(15), 3603-3624. Kumar, P., & Bin-Jaliah, I. (2007). Respiratory Physiology & Neurobiology, 157(1), 12-21. McQueen, D. S., & Eyzaguirre, C. (1974). Journal of Neurophysiology, 37(6), 1287-1296.

C54

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

Arslan Shakeel[1], Alexander Johns[1], Holly Wilkinson[1]

[1]Hull York Medical School, Hull, United Kingdom

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

Human skin samples were collected post-surgery, with full informed and written patient consent, from Castle Hill Hospital with no other inclusion criteria. Skin was washed and 2mm wounds were created using a biopsy punch. A 6mm biopsy punch was then used to cut out a 6mm explant around each wound, with the 2mm wounds lying centrally within the explants. Metformin and Rapamycin were diluted in dimethyl sulfoxide (DMSO) to a working concentration of 62.5nM and 12.5nM respectively with 2µL of treatment applied topically to wounds. 0.1% DMSO was used as a control treatment. Wounds were incubated in a humidified incubator at 32-37°C and 5% CO_2 for 2 days. Wound explants were fixed and immunofluorescence staining was conducted with a primary Keratin-14 antibody followed by a secondary antibody (AlexaFluor488). Wounds were counter-stained with DAPI and images were acquired using confocal microscopy. Images were analysed using ImageJ. The edge of the wound and non-healed area was drawn around using the freehand tool and the percentage of the original wound area that had healed was calculated for each wound. Significance testing was conducted with ANOVA when there was 3 or more groups, or an unpaired T test for pairwise comparisons.

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

1. Tan SY, Mei Wong JL, Sim YJ, Wong SS, Mohamed Elhassan SA, Tan SH, et al. Type 1 and 2 diabetes mellitus: A review on current treatment approach and gene therapy as potential intervention. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019;13(1):364-72.
2. Vileikyte L. Diabetic foot ulcers: a quality of life issue. Diabetes/Metabolism Research and

Reviews. 2001;17(4):246-9. 3. Alexiadou K, Doupis J. Management of Diabetic Foot Ulcers. Diabetes Therapy. 2012;3(1):4. 4. Resnik SR, Egger A, Abdo Abujamra B, Jozic I. Clinical Implications of Cellular Senescence on Wound Healing. Current Dermatology Reports. 2020;9(4):286-97.

C55

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

Shaimaa A. Gad[1,2], Helene Daou[1], Amanda D.V. MacCannell[1], Nicole T. Watt[1], Laeticia Lichtenstein[1], David J Beech[1], T. Scott Bowen[3], Lee D. Roberts[1]

[1]Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, United Kingdom, [2]Faculty of Medicine, Mansoura University, Mansoura, Egypt, [3]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

Background: Exercise provides both a protective and therapeutic approach to target systemic metabolic dysfunction. In part, the health benefits of exercise are mediated by exerkines as endocrine signals released from skeletal muscle during physical activity. Exerkines include micro-RNAs, proteins, lipids, and metabolites. The bioactive metabolite endocrine signals have been termed metabokines. β-aminoisobutyric acid (BAIBA) is a non-protein-beta-amino-acid, that functions as a Pgc1α (a transcriptional co-regulator; peroxisome proliferator-activated receptor-γ co-activator-1α) and exercise-regulated muscle-derived metabokine. BAIBA modulates crosstalk between skeletal muscle, liver, and fat by inducing white adipocyte browning and hepatic fatty acid β-oxidation (FAβ-O). The liver regulates functional processes including homeostasis of systemic lipid and glucose levels through de-novo lipogenesis (DNL), FAβ-O, and gluconeogenesis. Liver exhibits a high degree of metabolic flexibility (the ability to adapt to excess or restricted substrate to maintain homeostasis). The signalling and phenotypic effects of BAIBA on hepatic tissue remain poorly characterized. Here the role of BAIBA in regulating beneficial effects on liver metabolism is investigated. We hypothesise that BAIBA can improve hepatic metabolic and mitochondrial function.

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

Results: Hepatic tissue from BAIBA-treated mice was characterized by significantly greater gene expression of *Cpt1a* (carnitine palmitoyl transferase-1a; 66% higher, p=0.015), *Pparα* (peroxisome proliferator-activated receptor-alpha; 97% greater, exact-p=0.035), and a decrease in *Acacα* expression (acetyl-CoA carboxylase-alpha; –61%, p=0.013) with a trend to 44% increase in *Pgc1α*. There was a trend towards lower expression of genes for DNL (as fatty acid synthase and stearoyl-CoA desaturase-1) as well as a trend for enhanced glucose-6-phosphatase and phosphoenolpyruvate carboxykinas-1 representing carbohydrates metabolic activity within the liver of BAIBA-treated mice. In treated group, a significant elevation in hepatic CS activity (28.1% more active, exact-p=0.029) (mitochondrial density) was observed. High-resolution respirometry analysis showed significant functional enhancement of both

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

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

C56

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

Rashmi Sivasengh[1], Iris Pruñonosa Cervera[2], Brendan M. Gabriel[1,4], Nicholas Morton[2,3]

[1]Aberdeen Cardiovascular & Diabetes Centre, The Rowett Institute, University of Aberdeen, Aberdeen, United Kingdom, [2]Molecular Metabolism Group, Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom, [3]School of Science and Technology, Nottingham Trent University, Nottingham, United Kingdom, [4]Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institute, Stockholm, Sweden

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

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

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

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

C57

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

Amanda MacCannell[1], Amy Moran[1], Anna Whitehead[1], T. Simon Futers[1], Sulayman Lyons[2], Grant McClelland[3], Lee Roberts[1]

[1]School of Medicine, University of Leeds, Leeds, UK., Leeds, United Kingdom, [2]Department of Nutritional Sciences, University of Toronto, Toronto, Canada, [3]Department of Biology, McMaster University, Hamilton, Canada

Background: White adipose tissue (WAT) stores excess energy and acts as an endocrine organ releasing hormone-like factors known as adipokines that regulate whole-body energy balance. Brown adipose tissue (BAT) regulates energy expenditure through futile cycling of the electron transport chain (ETC) by the action of uncoupling protein 1 (UCP1) to generate heat. BAT's heat producing capacity may be a novel approach to treating obesity. Adult humans possess metabolically active BAT, but obesity reduces BAT's thermogenic activity and mass causing BAT to resemble WAT in a process termed "whitening". Leucine-rich-α2-glycoprotein 1 (*Lrg1*) is an adipose-associated secretory protein and potential adipokine, with plasma levels positively correlated to body mass index in humans. However, the role of LRG1 in the regulation of BAT and sWAT energy metabolism and function has not yet been identified.

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

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

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

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

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

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

C58

Autocrine and paracrine effects of leptin on adipogenesis

Mariami Jasaszwili[1], Lasse Fuchs[1], Sandy Richter[1], Anna Kirstein[1,2], Linnaeus Bundalian[3], Akhil Velluva[4], Felipe Engelberger[5], Georg Künze[5], Jens Meiler[5,6], Rami Abou Jamra[3], Johannes Lemke[3], Wieland Kiess[1], Diana Le Duc[3], Antje Garten[1]

[1]Pediatric Research Center, University Hospital for Children and Adolescents, Leipzig University, Leipzig, Germany, [2]Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark, [3]Institute of Human Genetics, University Medical Center Leipzig, Leipzig, Germany, [4]Rudolf Schönheimer Institute of Biochemistry, Medical Faculty, Leipzig University, Leipzig, Germany, [5]Institute for Drug Development, Medical Faculty, Leipzig University, Leipzig, Germany, [6]Department of Chemistry, Center for Structural Biology, Vanderbilt University, Nashville, United States

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

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

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

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

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

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

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

[1] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994 Dec 1;372(6505):425-32. doi: 10.1038/372425a0. Erratum in: Nature 1995 Mar 30;374(6521):479. PMID: 7984236. [2] Francisco V, Pino J, Campos-Cabaleiro V, Ruiz-Fernández C, Mera A, Gonzalez-Gay MA, Gómez R, Gualillo O. Obesity, Fat Mass and Immune System: Role for Leptin. Front Physiol. 2018 Jun 1;9:640. doi: 10.3389/fphys.2018.00640. PMID: 29910742. [3] Kässner F, Kirstein A, Händel N, Schmid GL, Landgraf K, Berthold A, Tannert A, Schaefer M, Wabitsch M, Kiess W, Körner A, Garten A. A new human adipocyte model with PTEN haploinsufficiency. Adipocyte. 2020 Dec;9(1):290-301. doi: 10.1080/21623945.2020.1785083. PMID: 32579864.

C59

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

Edmund Battey[1], Emma Frank[1], Essi Havula[2], Daniel Fazakerley[3], Lykke Sylow[1]

[1]Department of Biomedical Science, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, [2]Stem Cells and Metabolism Research Program, Faculty of Medicine University of Helsinki, Helsinki, Finland, [3]Department of Clinical Biochemistry, Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom

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

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

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

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

C60

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

Ellen Besa[1], Ruth Phiri[1], Gwen Nayame[4], Andreck Tembo[4], Douglas Heimburger[3], Paul Kelly[1,2]

[1]Tropical Gastroenterology and Nutrition Group, Lusaka, Zambia, [2]Blizard Institute, Centre for Neuroscience, Surgery and Trauma, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom, [3]Vanderbilt Institute of Global Health, Vanderbilt University School of Medicine, Nashville, United States, [4]Lusaka Children's Hospital, University Teaching Hospitals, Lusaka, Zambia

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

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

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

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

C61

On the regulation of arterial blood pressure by intracranial baroreceptor mechanism

Pippa Chapman[1], Alla Korsak[1], Daniel Kellett[1], Nephtali Marina[1], Alexander Gourine[1]

[1]University College London, London, United Kingdom

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

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

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

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

Marina, N., Christie, I.N., Korsak, A., Doronin, M., Brazhe, A., Hosford, P.S., Wells, J.A., Sheikhbahaei, S., Humoud, I., Paton, J.F. and Lythgoe, M.F., 2020. Astrocytes monitor cerebral perfusion and control systemic circulation to maintain brain blood flow. Nature Communications, 11(1), 131.

C62

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

Teresa Filipponi[1], Thomas S. Owens[1], Christopher J. Marley[1], Thomas A. Calverley[1], Benjamin S. Stacey[1], Lewis Fall[1], Hayato Tsukamoto[2], Angelo Ianetelli[1], Bruce Davies[1], Gareth L. Jones[1], Damian M. Bailey[1]

[1]Neurovascular Research Laboratory, University of South Wales, Pontypridd, United Kingdom, [2]Research Organization of Science and Engineering, Ritsumeikan University, Kyoto, Japan

Background:

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

Methods:

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

Results:

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

Conclusions:

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

1. Owens, T.S., Calverley, T.A., Stacey, B.S., et al. 2021. Concussion history in rugby union players is associated with depressed cerebrovascular reactivity and cognition. Scandinavian Journal of Medicine & Science in Sports, 31(12), pp. 2291-2299. 2. Venturelli, M., Pedrinolla, A., Boscolo Galazzo, I., et al. 2018. Impact of Nitric Oxide Bioavailability on the Progressive Cerebral and Peripheral Circulatory Impairments During Aging and Alzheimer's. Frontiers in Physiology, 9 (169), pp. 1-12. 3. Scarmeas, N., Anastasiou, C. A., Yannakoulia, M. 2018 Nutrition and prevention of cognitive impairment. The Lancet, 17(11), pp. 1006-1015. 4. Smith, A.D., Refsum, H., Bottiglieri, T., et al. 2018 Homocysteine and Dementia: An International Consensus Statement, Journal of Alzheimer's disease, 62(2), pp. 561-570. 5. Stanhewicz, A.E., Kenney, W.L. 2017 Role of folic acid in nitric oxide bioavailability and vascular endothelial function. Nutr Rev. 75(1), pp. 61-70.

C63

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

Aelfwin Stone[1], Joern Steinert[1]

[1]*University of Nottingham, Nottingham, United Kingdom*

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

Drosophila were kept at standard conditions (12hr light-dark-cycle, 25°C). TPI mutant expressing *Drosophila* (wstd[1] and M80T point mutations[2,3]) were used as a disease model *vs* w^{1118} (wild-type control).

Electrophysiological recordings in two-electrode voltage-clamp were taken from muscle 6/7 in segments A2/3 of third instar larvae fillets in HL-3 buffer (1.5mM Ca^{2+}). Evoked and spontaneous excitatory junctional currents (e/sEJCs) were recorded, alongside 60Hz train stimulations and recovery protocols to investigate synaptic depletion and subsequent recovery.

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

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

Average sEJC amplitudes were -0.81±0.14nA for w^{1118}, -0.68±0.08nA for wstd[1], and -0.92±0.05nA for M80T ($p>0.05$), at a frequency of 1.29 ±0.18Hz for w^{1118}, 0.83±0.08Hz for wstd[1], and 1.01±0.20Hz for M80T ($p>0.05$, n=14, 13, 10). Average eEJC amplitudes were -95.9±5.8nA for w^{1118}, -99.1±3.5nA for wstd[1], and -88.1±8.9nA for M80T ($p>0.05$, n=13, 13, 10).

Synaptic depletion at 60Hz stimulation (950ms) reduced amplitudes to 41±7% of initial values in wstd[1] and 57±7% in M80T *vs* 56±4% in w^{1118}, exponential fits to decaying amplitudes revealed tau values of 148±28ms for wstd[1] and 390±127ms for M80T *vs* 256±18ms for w^{1118} ($p<0.05$, n=10, 4, 9). Subsequent eEJC recovery times were altered in TPI mutants, tau: 9.2±0.8s for wstd[1] and 8.2±4.4s for M80T, *vs* 5.8±0.6s for w^{1118} ($p<0.05$, n=10, 2, 9).

Calcium dependency of evoked release (0.25-3mM) was unaltered in wstd[1] larvae compared to w^{1118}. Confocal imaging studies did not show significant differences in NMJ morphology, bouton counts: 27.6±3.3 in w^{1118} and 33.0±3.8 in wstd[1], active zone counts: 262±37 for w^{1118} and 224±20 for wstd[1], active zone areas: 134±20pixels in w^{1118} and 95±12pixels in wstd[1], and total bouton areas: 751±51pixels in w^{1118} and 711±131pixels in wstd[1] (n=7, 10, $p>0.05$). TPI mutant

flies showed reduced longevity, median values of 40 days in wstd[1] and 42 days in M80T *vs* 60 days in w^{1118} ($p<0.05$, n=90, 90, 72).

The data suggests that the TPI-mutant phenotype is in part due to altered synaptic vesicle dynamics, possibly associated with vesicle pool organisation or endo/exocytosis, thus expanding our knowledge of TPI involvement in synaptopathology[3]. Supressed TPI activity also enhances protein glycation and redox stress, which may potentially be responsible for our findings. Future studies will examine the physiological impact of redox stress, focusing on the link between NO-mediated post-translational modifications and TPI function.

1. Pacher et al, (2007), Physiol Rev, 87, 315-424. 2. Gnerer et al, (2006), PNAS, 103, 14987-14993. 3. Celotto et al, (2006), Genetics, 174, 1237-1246

C64

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

Nils Korte[1,3], Anna Barkaway[1], Felipe Freitas[1], Huma Sethi[2], David Attwell[1]

[1]UCL, London, United Kingdom, [2]Neurosurgery, Queen Square, UCLH, London, United Kingdom, [3]Harvard, Boston, United States

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

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

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

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

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

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

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

C65

Thalamic deep brain stimulation relieves hypercapnic induced air hunger.

Tom Chapman[14], Emmanuel Debrah[12], Sarah Farrell[11], Shakeeb Moosavi[13], Alexander Green[9]

[1]Oxford Brookes university, Oxford, United Kingdom, [2]Nuffield department of clinical neurosciences, Oxford, United Kingdom, [3]Nuffield department of surgical neurosciences, Oxford, United Kingdom, [4]Oxford brookes University, Oxford, United Kingdom, [5]University of Oxford, Oxford, United Kingdom, [6]Oxford University, Oxford, United Kingdom, [7]Newcastle University, Newcastle, United Kingdom, [8]Nuffield department of surgical sciences, University of Oxford, Oxford, United Kingdom, [9]Nuffield department of surgical sciences, University of Oxford, Oxford, United Kingdom, [10]Nuffield department of surgical sciences, University of Oxford, oxford, United Kingdom, [11]Nuffield department of surgical sciences, University of Oxford, oxford, United Kingdom, [12]Nuffield department of clinical neurosciences, University of Oxford, Oxford, United Kingdom, [13]Oxford Brookes University, Department of biological and medical sciences, Oxford, United Kingdom, [14]Oxford Brookes University, Department of biological and medical sciences, Oxford, United Kingdom

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

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

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

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

Green, A. L., Debrah, E., Roy, H. A., Rebelo, P. and Moosavi, S. H. (2019) 'Letter to the editor: Thalamic deep brain stimulation may relieve breathlessness in COPD', Brain Stimul, 12(3), pp.

827-828. Ries, A. L. (2005) 'Minimally clinically important difference for the UCSD Shortness of Breath Questionnaire, Borg Scale, and Visual Analog Scale', Copd, 2(1), pp. 105-10.

C66

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

Vaughan Macefield[1], Rebecca Wong[3], Gianni Sesa-Ashton[2], Brendan McCarthy[2], Sudipta Datta[2], Luke Henderson[4], Tye Dawood[2]

[1]Department of Neuroscience, Monash University, Melbourne, Australia, [2]Baker Heart and Diabetes Institute, Melbourne, Australia, [3]Baker Department of Cardiometabolic Health, Melbourne University, Melbourne, Australia, [4]School of Medical Sciences (Neuroscience), Brain and Mind Centre, The University of Sydney, Sydney, Australia

Introduction: The dorsolateral prefrontal cortex (dlPFC) is primarily involved in higher-order executive functions, and little is known of its role in control of the autonomic nervous system. Our brain imaging studies have revealed links between the dlPFC and the generation of muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA) in humans (Macefield et al., 2013; Macefield & Henderson, 2016, 2019). We recently showed that sinusoidal electrical stimulation of the dlPFC causes a cyclic modulation of MSNA, heart rate and blood pressure, but had no effect on respiration (Sesa-Ashton et al., 2022). Here we assessed whether stimulation of the dlPFC can also modulate sympathetic outflow to skin. **Methods:** Spontaneous bursts of SSNA were recorded from cutaneous fascicles of the right common peroneal nerve via a tungsten microelectrode in 21 healthy participants. Negative-going sympathetic spikes were extracted from the neurogram. Low-frequency sinusoidal stimulation (-2 to 2mA, 0.08 Hz, 100 cycles) was applied to the right dlPFC (EEG electrode site F4, n=21) or left dlPFC (F3, n=12) and the nasion via surface electrodes. The modulation index (peak-trough/peak) was calculated for each stimulation paradigm by constructing cross-correlation histograms between the times of occurrence of the sympathetic spikes and the peak of the sinusoidal stimulus. **Results:** Sinusoidal stimulation of either the right or left dlPFC caused significant cyclic modulation of SSNA (Mann-Whitney, $p<0.01$), but there was no side-to-side difference. Stimulation also caused cyclic modulation of skin blood flow and sweat release. **Conclusions:** We have shown that sinusoidal stimulation of the dlPFC causes modulation of sympathetic outflow to skin as well as muscle in humans, as directly recorded via intraneural microelectrodes, as well as modulation of their effector-organ responses. This supports an important role for the dlPFC in the control of the sympathetic nervous system, which likely contributes to the ability of mental stress to bring about increases in MSNA, heart rate and blood pressure, and emotions to bring about increases in SSNA, cutaneous vasoconstriction and sweat release.

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