PCB001

Comparative Microarray Profiling of Exosomal miRNAs in the Serum of Acute Ischemic Stroke Patients and Healthy Controls

Muhammad Ammar Zahid[1], Ghulam Jeelani Pir[2], Aijaz Parray[2], Abdelali Agouni[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, Doha, Qatar, [2]The Neuroscience Institute, Academic Health System, Hamad Medical Corporation (HMC), Doha, Qatar

Circulating exosomal miRNAs have demonstrated potential as both biomarkers and therapeutics for various diseases. However, their expression in acute ischemic stroke (AIS) patients has not been extensively investigated (Xu et al., 2022). Therefore, this study sought to compare the circulating exosomal miRNA profiles of AIS patients to those of healthy controls to identify differentially expressed miRNAs. The study adhered to the guidelines set by the Declaration of Helsinki and was approved by Hamad Medical Corporation Institutional Review Board.

Five AIS patients were recruited within 24 hours of the attacks' onset and matched with five healthy controls. Exosomal RNA was extracted using the exoRNeasy kit (Qiagen), and miRNA expression profiles were analyzed using the Affymetrix GeneChip miRNA arrays. Signals were then normalized using the robust multi-array average method. To identify present or absent genes the Detection Above BackGround algorithm was applied. An empirical Bayes moderated t-test (limma package), was applied to detect differences in gene expression between patients and controls (Ritchie et al., 2015). *P*-values were adjusted for multiple testing using the method of Benjamini and Hochberg, and a statistical significance level of $p<0.05$ and log2 fold-change of 1 were employed (Benjamini & Hochberg, 1995). Targets of the differentially expressed miRNAs were identified using miRTargetLink 2.0 (Kern et al., 2021). ClusterProfiler package in R was used with different functional annotation databases such as KEGG and GO to perform enrichment analysis (Wu et al., 2021). Protein-protein interaction (PPI) networks were generated using STRING and hub genes were identified.

We observed differential expression of five miRNAs in patients with AIS compared to controls. Specifically, we found upregulation of hsa-let-7b-5p, hsa-miR-16-5p, and hsa-miR-320c, and downregulation of hsa-miR-548a-3p and hsa-miR-6808-3p. To gain insight into the potential biological implications of these miRNAs, we performed functional and pathway enrichment analyses of their target genes. Our findings suggest that the PI3K/AKT signaling pathway, as well as miRNAs involved in cancer, were the most enriched KEGG pathways. The top enriched GO biological process terms were the regulation of transcription by RNA polymerase II, negative regulation of apoptosis, and regulation of proliferation and protein phosphorylation. A PPI network was constructed to investigate the interactions between the proteins encoded by the target genes of these miRNAs. Our analysis revealed a significant network of protein associations ($P = 1.0 \times 10^{-16}$) with 241 edges between 101 nodes, compared to the expected 86 edges if the associations were random. Furthermore, we identified hub proteins based on the network's connectivity degree, which included TP53, HRAS, KRAS, NRAS, IGF1-R, VEGF-A, MTOR, CCND1, CCNA2, and CDC25A.

This study is the first investigation of the expression profile of exosomal miRNAs in AIS in the Middle East. However, additional research and validation in a larger cohort of patients are

necessary to further elucidate the specific roles of these exosomal miRNAs in AIS, including their potential as predictive, diagnostic, or prognostic biomarkers. Ultimately, this research has the potential to identify novel prognosis markers for AIS, which could significantly impact the diagnosis, treatment, and management of this disease.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological), 57(1), 289–300. Kern, F., Aparicio-Puerta, E., Li, Y., Fehlmann, T., Kehl, T., Wagner, V., Ray, K., Ludwig, N., Lenhof, H.-P., Meese, E., & Keller, A. (2021). miRTargetLink 2.0—Interactive miRNA target gene and target pathway networks. Nucleic Acids Research, 49(W1), W409–W416. https://doi.org/10.1093/nar/gkab297 Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research, 43(7), e47. https://doi.org/10.1093/nar/gkv007 Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. The Innovation, 2(3). https://doi.org/10.1016/j.xinn.2021.100141

PCB002

Electrophysiological effects of extracellular histones in isolated cardiomyocytes

Moza Al-Owais[1], Z Yang[1], H Kirton[1], E White[1], D Steele[1]

[1]School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK, Leeds, United Kingdom

Histones are highly basic proteins present in eukaryotic cell nuclei. DNA wraps around histones creating structural units called nucleosomes, the building blocks of chromatin[1]. Previous work[2] has shown that following sepsis or blunt trauma, circulating histones released from damaged cells cause secondary damage to organs, including the heart. Here we investigated the mechanism of H3 histone toxicity in isolated ventricular myocytes. Wistar rats (150–200 g) were sacrificed in accordance with the UK Home Office Guidance on the Operation of Animals (Scientific Procedures) Act of 1986. H3 histone (5 µg/ml) induced membrane damage and cell necrosis. Following 10 min exposure, topographical images obtained using scanning ion conductance microscopy' (SICM) showed fine dark lines consistent with 'micro-tears'. The effects of H3 on membrane potential were assessed using whole cell current clamp recording. Under control conditions, the mean (± SEM) resting membrane potential (E_m) was -79.1 ±0.65 (n=87). H3 (µg/ml) application caused a dose and time dependent depolarisation of E_m, spontaneous action potentials and the development of early afterdepolarisations (EADs, **Table 1**). Low levels of H3 (0.1-0.01 µg/ml) increased the action potential duration measured 90% of maximal amplitude ($APD_{0.9}$; **Table 1**). These data show that H3 induces membrane damage, leading to myocyte depolarisation and pro-arrhythmic changes in the action potential. Such effects may contribute to cardiac dysfunction in pathological conditions that result in a rise in circulating histones.

1. Kleine TJ, et al. Am J Physiol 1997;273:C1925-C1936. 2. Abrams ST, et al. Am J Respir Crit Care Med 2013;187:160-9.

PCB003

In silico Investigation of Pro-arrhythmogenic Effects of KCNE2 Mutations in Human Atrial Fibrillation

Ghadah Alrabghi[1,2], Henggui Zhang[1]

[1]Biological Physics Group, University of Manchester, Manchester, United Kingdom,
[2]Department of Physics, Faculty of Science, University of Jeddah, Jeddah, Saudi Arabia

Functional analysis has shown that gain-of-function mutations (I57T and M23L) in the slow delayed rectifier potassium current (I_{Ks}), carried by the *KCNE2* channel, are associated with early-onset lone atrial fibrillation (AF) (Nielsen et al., 2014). These mutations affect not only I_{Ks} but also the rapid delayed rectifier current (I_{Kr}) and the transient outward potassium current (I_{to}). Using biophysically detailed computer models, this study aimed to investigate the underlying mechanisms by which the two mutations (I57T and M23L) facilitate and promote AF. In the simulations, the MCZ (Colman et al., 2013) model of the human atrial cell was modified to incorporate experimental data on changes of I_{Ks}, I_{Kr}, and I_{to} induced by *KCNE2* I57T and M23L mutations. The cell models were then incorporated into homogeneous multicellular one- (1D) and two-dimensional (2D) models of atrial tissue, as well as a 3D realistic model of the human atria. Functional effects of the two mutations on atrial electrical activities were quantified on the action potential (AP) profile and the AP duration (APD) restitution at the single-cell level; and on the conduction velocity (CV), the effective refractory period (ERP), and the wavelength (WL) restitutions at the tissue level. The widths of the temporal vulnerability window (VW) to re-entry were measured. Dynamical behaviours of re-entrant excitation waves (lifespan (LS), tip trajectory patterns, and dominant frequency (DF)) in 2D and 3D models were investigated. It was shown that both mutations shortened APD and flattened its restitution curve. At the 1D level, they abbreviated both ERP and WL restitutions and displaced the CV curve to the left, facilitating the conduction of atrial excitations at high rates. Although they reduced the temporal VW widths, *KCNE2* I57T and M23L mutations increased the lifespan and stabilization of the re-entrant excitation waves at the 2D level, which was corroborated by 3D simulations. Collectively, these simulation results revealed the pro-arrhythmic effects of the *KCNE2* I57T and M23L mutations, which are attributable to the shortened APD, ERP, and WL, and altered CV, which, in combination, facilitate the maintenance of re-entrant excitation waves leading to AF.

1- Nielsen JB, Bentzen BH, Olesen MS, David JP, Olesen SP, Haunsø S, et al. Gain-of-function mutations in potassium channel subunit KCNE2 associated with early-onset lone atrial fibrillation. Biomark Med. 2014;8(4):557–70 2- Colman MA, Aslanidi OV, Kharche S, Boyett MR, Garratt C, Hancox JC, et al. Pro-arrhythmogenic effects of atrial fibrillation-induced electrical remodelling: insights from the three-dimensional virtual human 8/25/22 4:47:00 PMatria. J Physiol. 2013 Sep 1;591(17):4249–72.

PCB004

Machine-learning analysis of near-infrared spectroscopy to improve clinical decision making for hypoxic-ischemic encephalopathy in term infants

Minoo Ashoori[1,2], John M O'Toole[1,3], Aisling A Garvey[1,3,4], Vicki Livingstone[1,3], Brian Walsh[1,3,4], Andreea M Pavel[1,3,4], Mary Anne Ryan[1,3,4], Geraldine B Boylan[1,3], Deirdre M Murray[1,3], Michael Moore[5], Lavinia Panaite[1,3,4], Fiona B McDonald[1,2], Eugene M Dempsey[1,3,4]

[1]INFANT Research Centre, University College Cork, Cork, Ireland, [2]Department of Physiology, University College Cork, Cork, Ireland, [3]Department of Paediatrics & Child Health, University College Cork, Cork, Ireland, [4]Department of Neonatology, Cork University Maternity Hospital, Cork, Ireland, [5]Department of Radiology, Cork University Hospital, Cork, Ireland

Background: Hypoxic-ischemic encephalopathy (HIE) is a severe brain injury that occurs in neonates due to perinatal oxygen deprivation, often leading to adverse neurodevelopmental outcomes or death. The therapeutic window for HIE is within the first 6 hours of life but implementing therapeutic hypothermia up to 12 hours after birth has been shown to be effective in reducing the severity of brain injury. To ensure the timely and effective recognition of HIE during this critical therapeutic window, it is essential to use objective methods for diagnosis. Near-infrared spectroscopy (NIRS) provides a non-invasive continuous regional measurement of cerebral oxygenation.

Objective: We have sought to assess the potential clinical utility of NIRS as an additional tool in the diagnosis of HIE.

Methods: We analysed 53 infants with all grades of HIE (>36 weeks GA) enrolled in the Multimodal Assessment of Newborns at Risk of Neonatal Hypoxic Ischaemic Encephalopathy (Monitor) trial. All infants had continuous cerebral oxygenation monitoring for at least 2 hours in their first 12 hours after birth. HIE was graded (mild, moderate, severe) based on assessment using the modified Sarnat score at 1 hour of life. The NIRS signals recorded in the first 12 hours of life were pre-processed, and quantitative features were extracted. Furthermore, prolonged relative desaturations (PRDs; data-driven desaturations lasting 2-15 minutes) were identified and removed from NIRS signals, termed filtered NIRS. The quantitative features were combined in a machine-learning model using a leave-one-out cross-validation approach to determine the likelihood of requiring hypothermia treatment, distinguishing between mild vs moderate and severe HIE. We used logistic regression models to control for the potentially confounding effects of clinical features on the NIRS machine-learning model. We controlled for Apgar score (5min) and mode of delivery for the NIRS and filtered NIRS models for detecting mild HIE. In all models, the significance level was set at $p < 0.05$.

Results: Logistic regression analysis revealed that features extracted from NIRS were significant predictors of requiring hypothermia in this population ($\beta = 0.61$, $p = 0.01$). Furthermore, features extracted from filtered NIRS were found to be significant predictors of mild HIE ($\beta = 0.72$, $p < 0.001$). The predictability of the Apgar score when assessed independently was significant ($\beta = -0.11$, $p < 0.001$), while the mode of delivery did not demonstrate a significant impact. The regression model, which included filtered NIRS, Apgar score, and delivery mode, accounted for 50.4% of the variance in the outcome variable (R-squared = 0.504), with the root mean squared error (RMSE) of 0.36. This model performed better than both the logistic model based on filtered NIRS and Apgar score (R-squared = 0.48,

RMSE = 0.37) and the model based on Apgar score and delivery mode (R-squared = 0.41, RMSE = 0.40).

Conclusion: Utilizing machine-learning methods to analyse NIRS in the first 12 hours of life, allows for early objective identification of infants at risk of adverse short-term outcomes and may aid in the stratification of infants for intervention in the effective therapeutic window.

PCB005

The role of Sodium fluoride on electrolytes and blood pressure in male Wistar rats

Olusola Ayegboyin[1], Abayomi Ige[2], Elsie Adewoye[3]

[1]University of Ibadan, Ibadan, Nigeria, [2]University of Ibadan, Ibadan, Nigeria, [3]University of Ibadan, Ibadan, Nigeria

This study investigated the role of sodium fluoride on electrolytes and blood pressure in male Wistar rats. Sodium fluoride and the damage done to human health have been of public concern in recent years. Electrolyte imbalance poses threat and can predispose the body to life-threatening cardiovascular conditions including hypertension. Hypertension has been identified as one of the most significant risk factors for morbidity and mortality worldwide. Ten male adult Wistar rats (150-180g) divided into two groups (n=5) were used for this study. Group 1 was the control group and received distilled water orally for 21 days, group 2 received 10mg/kg sodium fluoride *p.o.* through oral gauge for 21 days. Twenty-four hours after the last treatment with sodium fluoride, blood pressure indices; systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were determined non-invasively in awake animals by tail plethysmography using an automated blood pressure monitor (CODA S1, Kent Scientific Corporation, CT). The average of no less than nine readings was recorded for each animal at rest during the blood pressure measurements after the acclimatization period. About 3 mL of blood was collected by retro-orbital venous puncture using plain capillary tubes into plain bottles and left to clot. The clotted blood was then centrifuged at 4,000 rpm for 10 min. Clear serum was separated using a Pasteur pipette into another plain tube and then stored at 4^0C. Data collected were expressed as mean ± SEM. Statistical significance was set at $P<0.05$ using Student's t-test analysis. The results from this study showed that sodium fluoride caused a significant increase $P<0.001$ in systolic blood pressure, diastolic blood pressure, mean arterial pressure, and $P< 0.01$ in heart rate compared to the control group that received distilled water. Also, the administration of sodium fluoride caused a significant increase $P<0.05$ in serum sodium and calcium when compared to the control group. The oral administration of sodium fluoride caused a significant decrease $P<0.05$ in potassium and phosphorus when compared to control group. There was no significant difference in chloride level in sodium fluoride-treated rats compared to the control. This study showed that sodium fluoride has hypertensive effects on blood pressure as seen in the significant increase in systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate. The results from this study also showed that sodium fluoride elevate serum electrolytes that have the potency to lead to increasing blood pressure as seen in sodium and calcium while reducing the electrolytes that have beneficial effects on lowering blood pressure such as potassium and phosphates.

PCB007

Screening for small molecules that rescue the defective trafficking of mutant KCNQ1 channels

Hongwei Cheng[1], Grace Salsbury[2], David N Sheppard[1], Stephen C Harmer[1]

[1]School of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, United Kingdom, [2]William Harvey Heart Centre, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

Introduction: The congenital long QT syndrome (cLQTS) increases the risk of arrhythmia and is an important cause of sudden death in the young. The ion channel subunits KCNQ1 (Kv7.1) and KCNE1 assemble in cardiac myocytes to produce the repolarising slow delayed rectifier potassium current I_{Ks}. Loss-of-function (LOF) mutations in the *KCNQ1* gene cause cLQTS type 1 (LQTS1) (Wang *et al*., 1996; Sanguinetti *et al*., 1996). Missense LQTS1 mutations cause LOF by altering channel gating (Class III), promoting defective channel trafficking (Class II) or through a combination of mechanisms. Recent studies highlight that Class II mechanisms underlie disease pathogenesis for a substantial proportion of LQTS1 mutations (Huang *et al*., 2018) but current therapeutic managements for LQTS1 patients do not target this defect. Therefore, the aim of this study was to identify small molecules that rescue defective mutant KCNQ1 channel trafficking.

Methods: *Trafficking assays: LI-COR*-based On/In-Cell Western assays were used to quantify channel trafficking as described in Royal *et al*., (2017). The 'On-Cell' assay quantifies cell surface expression (CSE). *Cell lines:* Two HEK-293 cell lines were generated which stably express the trafficking deficient KCNQ1 mutant channel G325R (VSV-KCNE1-G325R) and the wild-type channel (VSV-KCNE1-KCNQ1). *Compound Screening:* 26 compounds were tested in three phases. Compounds were applied for 24 hours at 37 °C unless otherwise indicated. DMSO was the vehicle control. Data are presented as fold-matched control (fold) or normalised arbitrary fluorescent units (NAFUs) ±SEM. Statistical analysis was performed using one-way ANOVA with Dunnett's multiple comparison test or multiple unpaired *t*-test.

Results: In Phase 1, clinically approved CFTR channel modulators were screened because they may exhibit cross-channel activity (Mehta *et al*., 2018). Four modulators (including VX-809 and VX-661) were tested but none altered the CSE of VSV-KCNE1-G325R (P>0.05, n=3). In Phase 2, seven compounds that act as proteostasis regulators were screened. Of these, the proteasome inhibitor MG-132 (1 μM) and Thapsigargin (10 μM) promoted increases in VSV-KCNE1-G325R CSE (3.24±0.26 fold (P<0.0001, n=12) and 1.42±0.04 fold (P<0.01, n=3), respectively). However, both compounds exhibited cell toxicity. In Phase 3, 15 KCNQ1 channel modulators (blockers and activators) were screened. Of these, the activator R-L3 (L-364,373) (100 μM) induced a small but significant increase in CSE (1.44±0.15 fold, P<0.05, n=3) and the activator Docosahexaenoic acid (DHA) at 100 μM significantly increased VSV-KCNE1-G325R CSE by 2.27±0.26 fold (P<0.01, n=3). Furthermore, upon extended treatment (for 48 hours), 5 and 10 μM DHA promoted large increases in VSV-KCNE1-G325R CSE (5.57±0.94 and 8.24±0.66 fold; NAFUs: 0.0768±0.0152 and 0.1123±0.0118 vs. DMSO 0.0135±0.0006, both P<0.001, n=5). By contrast, 10 μM DHA for 48 hours did not alter wild-type (VSV-KCNE1-KCNQ1) channel trafficking (P>0.05, n=5).

Conclusions: These data highlight that the channel activators DHA and R-L3 may be able to promote trafficking rescue. However, the underlying mechanisms are unknown and whether these compounds can correct other Class II mutants within a cardiomyocyte cellular setting warrants investigation.

1. Wang Q et al. (1996). Nat Genet 12, 17-23. 2. Sanguinetti MC et al. (1996). Nature 384, 80-83. 3. Huang H et al. (2018). Sci Adv 4, eaar2631. 4. Royal AA et al. (2017). PLoS One 12, e0186293. 5. Mehta A et al. (2018). Eur Heart J 39, 1446-1455.

PCB008

Embryonic origins of vascular dysfunction in developmental hypoxia: the role of miR-21-5p

Anna Cochrane[1], Aaliyah Razi[1], Youguo Niu[1], Sage Ford[1], Rachael Crew[1], German Arenas[3], Bernardo Krause[2], Dino Giussani[1]

[1]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom, [2]Instituto de Ciencias de la Salud, Universidad de O'Higgins, Chile, Santiago, Chile, [3]Instituto de Ciencias de la Ingenieria, Universidad de O'Higgins, Chile, Santiago, Chile

Background: Chronic fetal hypoxia is a common complication during pregnancy, which reduces fetal growth, promotes endothelial dysfunction and can trigger a developmental origin of cardiovascular disease (Giussani, D.A. (2021) *Circulation* 144(17):1429-1443). Underlying mechanisms remain unknown, preventing identification of targets for intervention. Epigenetics may underpin these alterations, however the role of microRNAs in affecting cardiovascular risk in the hypoxic fetus has not been investigated. MicroRNA-21-5p is of particular interest, as it is a modulator of endothelial function that is downregulated by hypoxia (Peñaloza et al. (2020) *Biochem. Pharmacol.* 182:114288). Therefore, this study tested the hypothesis that treatment with synthetic microRNA-21-5p (AgoMiR-21) rescues peripheral endothelial dysfunction in the hypoxic chicken embryo. This model system permits isolation of the direct effects of therapy on the developing vasculature independent of effects on the maternal and/or placental physiology.

Aims: To determine the effects of treatment of the chronically hypoxic chicken embryo with synthetic microRNA-21-5p (AgoMiR-21) on embryonic growth and endothelial function.

Methods: This research was carried out under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Board. Fertilised Bovans Brown eggs were incubated under normoxia (21% O_2) or chronic hypoxia (14% O_2) from day 1 to day 19 (hatching occurs at 21 days). Chicken embryos were treated topically onto the chorio-allantoic membrane via a small hole in the air cell with AgoMiR-21 (1µg in 100µl sterile H_2O/embryo/day) or vehicle (100µl sterile H_2O) on days 13, 15 and 17 of incubation. On day 19, following biometry, embryos were killed via cervical transection, and cranio-tibial arteries were isolated for analysis of vascular reactivity by *in vitro* wire myography.

Results: Chronic hypoxia promoted asymmetric growth restriction, shown by a reduction in embryo weight (Fig. 1A, n=13-29, P<0.0001) and an increase in relative brain weight (Fig. 1B, n=10-29, P=0.0077). Hypoxic embryos also showed an impairment in endothelium-dependent vasodilatation (Fig. 1C-E, n=9-13, P=0.0039). Treatment of hypoxic embryos with AgoMiR-21 had no effect on growth (Fig. 1A&B), but it partially rescued endothelium-dependent vasodilatation in hypoxic embryos (Fig. 1C-E, n=9-13, P=0.0051). Treatment of normoxic embryos with AgoMiR-21 had no effects.

Conclusions: AgoMiR-21 is a promising candidate for preventative therapy against developmental origins of vascular dysfunction in offspring of hypoxic pregnancy.

PCB009

Effect of Vitamin D Supplementation on Doxorubicin-Induced Cardiotoxicity in Rats

Faten Diab, Noha Abdel-Aziz Nassef, Manal Abd-El Hamid, Yasmin Khaled

undefined

Background: Doxorubicin, a chemotherapeutic agent, that has great efficacy in treatment of many solid tumors, bone sarcomas, and cancers of the breast, ovary, and other tumors. Unfortunately, the clinical use of this valuable anticancer drug is limited due to its life-threatening cardiotoxic effect[1]. Vitamin D is recently known for its cardioprotective effects[2]. **The aim of this study:** To investigate role of vitamin D on the doxorubicin-induced cardiac dysfunction. **Materials and Methods:** 70 female Albino-rats were divided into 4 groups: control group (C: n=21); doxorubicin-treated group (Dox: n=18): given i.p. injection of 2.5 mg/kg twice per week (cumulative dose:15 mg/kg) over 3 weeks; vitamin D-supplemented group (Vit D: n= 16): given vitamin D by oral gavage in a dose of 500 IU/kg daily, 5 days a week, also for 3 weeks; and combined Doxorubicin-treated+vitamin D-supplemented group (Dox+Vit D: n=15): rats received the same used doses for the same duration. After 3 weeks, all rats were subjected to EGG recording, determination of plasma levels of brain natriuretic peptide (BNP), cardiac troponin I (cTnI), vitamin D concentration (vit D) and total calcium level (Ca). Hearts were excised and perfused in Langendorff preparation to record intrinsic in vitro activity of the heart under basal conditions according to the technique of Langendorff. Malondialdehyde (MDA), total antioxidant capacity (TAC) and heat shock protein 20 (HSP 20) were assessed in the cardiac tissue. Also, cardiac tissue histopathological studies were performed.

Results: Dox-treated rats showed significant depression in peak tension (PT) and myocardial flow rate (MFR) together with significant prolongation in time to peak tension (TPT), half relaxation time (HRT) and contraction time (CT). These changes were accompanied by significant elevation of plasma brain naturetic peptide (BNP), cardiac troponin I (cTnI) and in cardiac tissue malondialdehyde (MDA) and a significant decrease in plasma vit D, total calcium, and cardiac tissue total antioxidant capacity (TAC) and heat shock protein20. Histopathological examination of the Dox treated rats revealed markedly distorted muscle fibers with indistinct cell borders, bright eosinophilic cytoplasm, intra-cytoplasmic vacuoles and small pyknotic nuclei or absent nuclei, together with interstitial edema & aggregates of inflammatory cells and thick irregular collagen fibers in between the muscle fibers. This functional, biochemical, and histopathological data reflects development of doxorubicin-induced cardiomyopathy.

Concomitant supplementation of vitamin D to doxorubicin treated rats resulted in significant increases in PT, MFR, plasma vitamin D, total calcium as well as cardiac TAC and HSP20; while the MDA, plasma BNP and cTnI were significantly decreased, all compared to the Dox-treated rats. These findings were associated with regaining the normal collagen fiber distribution between cardiac muscle fibers with resolution of interstitial edema.

Conclusion: Vitamin D supplementation can partially mitigate cardiac dysfunction induced by chronic doxorubicin by improving the cardiac antioxidant state and heat shock protein 20 level.

1- Leong SL, Chaiyakunapruk N and Lee SW. (2017): Candidate Gene Association Studies of Anthracycline-induced Cardiotoxicity: A Systematic Review and Meta-analysis. Scientific

reports; 7(1): 39. 2- Gardner DG, Chen S and Glenn DJ. (2013): Vitamin D and the heart. Am J Physiol-Regul Integr Comp Physiol; 305(9): R969-7

PCB010

GPER1 increases expression of vascular alpha 2C-adrenoceptors and mediates cold-induced vasoconstriction

Manal Fardoun[1], Khodr Issa[2], Ali Eid[3]

[1]American University of Beirut, Faculty of Medicine, Beirut, Lebanon, [2]University of Lille, Proteomics, Inflammatory Response, Mass Spectrometry, INSERM U-1192, Lille, France, Lille, France, [3]Department of Basic Medical Sciences, College of Medicine, QU Health, Qatar University, Doha, Qatar

Background: Raynaud's phenomenon (RP), which results from exaggerated cold-induced vasoconstriction, is more prevalent in females than males, implicating 17b estradiol (estrogen; E2) in its etio-pathology. We previously reported that estrogen acts through the cAMP/Epac/Rap/JNK pathway to induce the expression of vascular alpha 2C-adrenoceptors (α2C-AR), the sole mediator of cold-induced vasoconstriction. An impermeable form of E2, namely E2:BSA, mimics estrogen's effects, suggesting a role for the membrane estrogen receptor (GPER1) in E2-induced α2C-AR expression.

Objective: Therefore, we hypothesized that GPER1 mediates E2-induced upregulation of α2C-AR through cAMP/Epac/JNK/AP-1 pathway.

Methods: In-cell ELISA, Luciferase assay, cAMP assays were used to measure kinase activity, transcription and intracellular accumulation of cAMP, respectively. Ethical approval was obtained for isolating cells from dermal arterioles (post-circumcision clinical "waste").

Results: Here, we show that G15, a selective GPER1 antagonist (1 μM), diminished E2 (10^{-10} M)-induced transcription of α2C-AR in primary arteriolar smooth muscle cells that we extracted from human dermal arterioles (n=3; $p<0.05$). G-1, a selective GPER1 agonist, (10 μM) was sufficient to induce α2C-AR transcription, increase cAMP levels and induce JNK activation (n=3; $p<0.05$ for all). Pretreatment with ESI09 (10 μM; an Epac inhibitor) abolished both G-1-induced α2C-AR upregulation (n=3; $p<0.05$). and JNK activation (n=3; $p<0.01$). Moreover, pretreatment with SP600125 (3 μM; a JNK specific inhibitor) but not H89 (2 μM; a PKA specific inhibitor) suppressed G-1-induced α2C-AR upregulation (n=3 for both; $p<0.05$ or $p>0.05$, respectively). Similarly, overexpression of Epac dominant negative mutant (Epac-DN) attenuated G-1-induced expression of α2C-AR (n=3; $p<0.05$). This inhibitory effect of Epac-DN was overridden by the co-transfection of constitutively active JNK mutant (n=3; $p<0.05$). Furthermore, G-1 caused a concentration-dependent increase in the transcriptional activity of AP-1-driven reporter construct (n=3; $p<0.01$). Mutation of this AP-1 site in the α2C-AR promoter significantly reduced its G1-induced transcription (n=3; $p<0.01$).

Conclusion: Collectively, these results show that GPER1 acts through the cAMP/EPAC/JNK/AP-1 signaling to induce expression of vascular α2C-AR. These findings unravel a new mediator of cold-induced vasoconstriction, namely GPER1, and present it as a potential target in the management of RP in estrogen-replete females.

PCB011

Effect of home-based dynamic intermittent pneumatic compression therapy on vascular and functional health outcomes in chronic stroke: A randomized controlled trial

James Faulkner[1], Eloise Paine[1], Nick Hudson[1], Scott Hannah[1], Amy Dennis-Jones[2], Louis Martinelli[2], Helen Hobbs[2]

[1]University of Winchester, Winchester, United Kingdom, [2]Hobbs Rehabilitation, Winchester, United Kingdom

Introduction: Stroke is the second leading cause of disability worldwide (Feigin et al., 2022). Although individuals undergo traditional physiotherapy, intermittent pneumatic compression (IPC) therapy may benefit stroke patients as increases in venous return may allow people to engage with more physical activity and more intensive training sessions (Shroeder et al., 2019) that may result in better health, mobility and ultimately quality of life (Park and Kim, 2019).

Aim: The purpose of this study was to assess the effect of using a home-based IPC device on vascular health and functional outcomes in individuals with chronic stroke using a randomized controlled study.

Methods: Research was conducted following institutional human ethics committee approval, while the study was registered with Clinical Trials.gov Protocol Registration and Results System (NCT05276453; https://clinicaltrials.gov/ct2/show/NCT05276453). Thirty-one stroke survivors (64.3 ± 14.3y; 4.3 ± 2.7y since stroke) took part in this study and completed pre- and post-intervention assessments which consisted of measures of vascular health (pulse wave analysis, carotid-femoral pulse wave velocity) and functional capacity (six-minute walk test, timed-up-and-go, 10m walk test). On completion of the initial (pre) assessment, individuals were randomly assigned to either a daily, 12-week, home-based IPC condition, or to a usual care control (CON) group. Outcomes were assessed using analysis of covariance, controlling for any baseline differences.

Results: A Time by Condition interaction was observed for peripheral systolic blood pressure ($p < 0.05$, $np^2 = 0.140$), with significantly greater reductions reported between pre- and post-intervention for IPC (147.4 ± 18.1 to 139.5 ± 15.6 mmHg, respectively) than CON (139.1 ± 17.5 to 137.7 ± 16.4 mmHg, respectively) . Similar findings were observed for central systolic blood pressure and the six-minute walk test (both $p < 0.05$). For the six-minute walk test, participants significantly increased their walking distance between pre- and post-intervention assessments for IPC (158 ± 73 to 181 ± 109m, respectively) but not CON (170 ± 87 to 174 ± 117m, respectively) ($np^2 = 0.248$). Average weekly physical activity levels significantly increased, and time spent sitting significantly decreased for IPC compared to CON (both $p < 0.05$).

Conclusions: The observed improvements in blood pressure and six-minute walk test distance, in combination with an increase in physical activity and reduced sedentary behaviours, are important positive findings when considering the use of IPC training for “at home” rehabilitation therapy for chronic stroke survivors.

References: Feigin, V.L., et al., World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. Int J Stroke, 2022. 17(1): p. 18-29. Park, J. and T.H. Kim, The effects of balance and gait function on quality of life of stroke patients. NeuroRehabilitation, 2019. 44(1): p. 37-41.

Schroeder, J., et al., Feasibility and effectiveness of repetitive gait training early after stroke: a systematic review and meta-analysis. 2019. 51(2): p. 78-88.

PCB013

The molecular basis of control of vascular tone by the therapeutic drug niclosamide

Rachel Kaye[1], Rumaitha Al Hosni[1], Paolo Tammaro[1]

[1]University of Oxford, Oxford, United Kingdom

TMEM16A Ca^{2+}-gated Cl^- channels (CaCCs) are highly expressed in vascular smooth muscle cells and contractile pericytes, where they provide a key depolarising mechanism. Thus, the TMEM16A channel has been proposed as a drug target for diseases of altered vessel tone including stroke, vascular dementia and (systemic, pulmonary) hypertension (1, 2). However, no therapeutic drugs interacting with the channel have yet reached clinical practice. The FDA-approved anthelmintic drug niclosamide inhibits the TMEM16A channel (3), however the effect of niclosamide on vascular smooth muscle and contractile pericytes is not fully defined. Here, we examine the effect of niclosamide on the tone of isolated rat aorta and the diameter of cerebral cortical capillaries and examine the underlying mechanism.

Niclosamide (1 mM) led to a reduction in both phenylephrine- and KCl-induced contraction of rat aortic rings, assessed through wire myography. The force generated by aortic rings in response to phenylephrine (10 μM) or KCl (100 mM) was reduced in the presence of niclosamide by 82.0±5.5% (n = 7) and 94.8±3.6% (n = 5), respectively. Niclosamide also impaired the constriction of pericytes in rat cortical brain slices by 41.6±11.3% (n = 6) after exposure to endothelin-1 (10 nM), assessed through differential interference contrast imaging.

Heterologous TMEM16A current in HEK293T cells and native CaCC currents in isolated rat aortic smooth muscle cells (SMCs) were similarly modulated by niclosamide; when measured at +100 mV, the heterologous TMEM16A and native CaCC currents were reduced by 27.8±3.9% (n = 10) and 18.7±3.7% (n = 6) in the presence of niclosamide (1 mM). However, at negative potentials (ranging from -100 to -40 mV) niclosamide activated these currents by ~2.9 folds and 3.1-folds, respectively. The potentiation of the CaCC currents at negative potentials could not explain the niclosamide-mediated vasorelaxation, since CaCC currents are depolarising, and promote smooth muscle contraction. In SMCs, niclosamide (1 mM) significantly inhibited voltage gated Ca^{2+} currents and potentiated a hyperpolarising current; these effects are likely determinants of niclosamide-induced relaxation.

This study elucidated the effects of niclosamide on a range of ionic currents and excluded the TMEM16A channel as a mediator of niclosamide-induced relaxation in arterial smooth muscle and contractile pericytes. Since niclosamide has been proposed for drug repurposing for a variety of indications, knowledge of its molecular targets will increase our understanding of the therapeutic and possible side effects of this drug. This work also offers insight into the relative contribution of a range of ionic currents to the physiological control of vascular tone.

1. Korte N et al. (2022). J Clin Invest 132(9):e154118 2. Al-Hosni R et al. (2022). Trends Pharmacol Sci 43: 712–725. 3. Cabrita I et al. (2019). JCI Insight 4(15):e128414

PCB014

NATRIURESIS INDUCED BY ACUTE INTRAVENOUS SALINE INFUSION IN MICE IS MEDIATED BY TUMOR NECROSIS FACTOR-ALPHA (TNFα) - EVIDENCE FOR PHYSIOLOGICAL ROLE OF THIS CYTOKINE IN KIDNEY.

Dewan Majid[2], Alexander Castillo[1]

[1]Department of Physiology, Tulane University School of Medicine, New Orleans,, New Orleans,, United States, [2]Department of Physiology, Tulane University School of Medicine., New Orleans, United States

Although TNFα is considered to play its role in many pathological conditions, the physiological role of this cytokine in regulating function of many organs including the kidney is increasingly recognized in recent days. Chronic high salt (HS) intake in diet induces an immune response that activates the mononuclear phagocyte system (MPS) cells to release TNFα that appears in the circulation in its soluble form (sTNFα). It has been shown that HS (4% NaCl) diet alone for 2 weeks increased MPS cell infiltration in the renal tissue in mice and this is associated with increases in the circulating sTNFα level in the plasma (Singh et al-2013). It was also demonstrated that 1% salt added to the drinking water for 3 days in mice increases the urinary excretion rate of sTNFα indicating that its release in the kidney from the infiltrated MPS cells occur even at the early stage of HS intake (Hao et al-2013). We have previously demonstrated that intravenous infusion of recombinant TNFα in mice induces natriuretic response by inhibiting tubular sodium reabsorption (Shahid et al-2008; Majid-2011). We hypothesize that the intravenous saline infusion can also induce the production of sTNFα from activated MPS cells due to an increase in salt content in immune tissues and such increase in sTNF influences saline induced natriuretic response in the kidney. To examine this hypothesis, we measured the changes in sTNFα levels in plasma and urinary excretion rate ($U_{TNF\alpha}V$) during intravenous infusion of isotonic saline (0.9% NaCl), first at euvolemic conditions (3 µL/min for 60 min; Baseline period) and then at an enhanced infusion rate (12 µL/min for 90 min; saline volume infusion period) in anesthetized mice (n=5). The concentration of sTNFα in plasma and urine samples were determined using ELISA kit (Ebioscience, Woburn, MA) for measuring this cytokine. Baseline level of plasma sTNFα was undetectable, however, the level was increased to 3.7±1.3 pg/mL during saline volume infusion period. Baseline $U_{TNF\alpha}V$ level was 0.01±0.002 pg/min/g of kidney wt, which was increased to 0.11±0.03 pg/min/g ($P<0.05$) during volume infusion period. In another group of mice (n=5) pretreated with a TNFα inhibitor, etanercept (0.5 mg/kg intraperitoneally once daily for 3 days prior to the experiment day), it was observed that this increase in $U_{TNF\alpha}V$ during saline volume infusion period was markedly attenuated (0.003±0.002 to 0.006±0.004 pg/min/g; P=n.s.). Interestingly, the diuretic and natriuretic responses to enhanced saline infusion were markedly attenuated in these etanercept pretreated mice without any significant changes in renal blood flow or glomerular filtration rate. The usual natriuretic response (0.5±0.2 to 4.8 ±1.1 µmol/min/g; $P<0.05$) to enhanced saline infusion observed in control mice was seen markedly attenuated (0.4±0.1 to 0.8±0.3 µmol/min/g; P=n.s.) in etanercept pretreated mice. These findings demonstrate for the first time that an intravenous saline volume infusion resulted an increase in sTNFα level in plasma and in urine. These results strongly suggest a physiological natriuretic role for sTNFα in regulating renal excretory function during acute saline volume infusion.

PCB015

Back to the future – mathematical models to capture ion channel kinetics using short high-information voltage clamp protocols

Gary Mirams[1]

[1]*Centre for Mathematical Medicine & Biology, School of Mathematical Sciences, University of Nottingham, Nottingham, United Kingdom*

Introduction: since the work of Hodgkin & Huxley, mathematical models of ion channel gating have been used to understand and predict the effects of ion currents in action potential formation. Today we still tend to use the same approach to voltage-clamp protocol design that Hodgkin & Huxley used: designs that enable model parameter values to be estimated manually from graph paper.

Aims/Objectives: to use short high-information voltage clamp protocols in partnership with computational modelling to characterise ion currents. To allow a model (Figure 1A) to be fitted and tested, with this process repeated after performing multiple experimental interventions in the same cell. Here we show results of using short protocols to capture the kinetics of I_{Kr} / K_V11.1 / hERG currents in a range of settings.

Method: we apply either a short sinusoidal voltage clamp protocol (Figure 1B, [1]) or a square wave 'staircase' version [2]to CHO cells stably expressing hERG1a at room temperature or physiological temperature in manual and automated patch settings. We then use computational optimisation to fit a simple mathematical model for hERG to the resulting currents, and use it to predict the results of conventional voltage clamp protocols and physiological action potentials.

Results: the short protocols result in highly predictive mathematical models: Figure 1C shows a prediction from a model fitted to the sinusoidal protocol shown in Figure 1B against experimental data. The parameter values within these models then capture our knowledge of channel gating more accurately than a series of Current-Voltage or Time Constant-voltage curves, with the benefit they can be re-used to predict currents in new situations/voltage-clamp protocols that were not examined in the original experiment. There are also opportunities to use mathematical models to account for patch clamp artefacts [3] to consolidate information from different patch clamp recordings more reliably.

Conclusions: this approach offers the opportunity to intervene and reassess currents multiple times in one experiment, and to generate a mathematical model that quantitatively captures our understanding about channel gating. For instance, we can alter temperature to examine the temperature dependence at the level of individual rate and voltage-dependence parameters within a model, rather than at the level of processes such as 'activation' or 'recovery from inactivation' [4]. We can also build models of mutant channels, and/or characterise changes in currents in the presence of drug compounds that alter channel gating [5].

[1] K. A. Beattie et al., "Sinusoidal voltage protocols for rapid characterisation of ion channel kinetics," J Physiol, vol. 596, no. 10, pp. 1813–1828, May 2018, doi: 10.1113/JP275733. [2] C. L. Lei, M. Clerx, D. J. Gavaghan, L. Polonchuk, G. R. Mirams, and K. Wang, "Rapid Characterization of hERG Channel Kinetics I: Using an Automated High-Throughput System," Biophys J, vol. 117, no. 12, pp. 2438–2454, Dec. 2019, doi: 10.1016/j.bpj.2019.07.029. [3] C. L.

Lei, M. Clerx, D. G. Whittaker, D. J. Gavaghan, T. P. de Boer, and G. R. Mirams, “Accounting for variability in ion current recordings using a mathematical model of artefacts in voltage-clamp experiments,” Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, vol. 378, no. 2173, p. 20190348, Jun. 2020, doi: 10.1098/rsta.2019.0348. [4] C. L. Lei et al., “Rapid Characterization of hERG Channel Kinetics II: Temperature Dependence,” Biophys J, vol. 117, no. 12, pp. 2455–2470, Dec. 2019, doi: 10.1016/j.bpj.2019.07.030. [5] J. M. Kemp et al., “Electrophysiological characterization of the hERG R56Q LQTS variant and targeted rescue by the activator RPR260243,” Journal of General Physiology, vol. 153, no. 10, Oct. 2021, doi: 10.1085/jgp.202112923.

PCB016

Glutaredoxin1-overexpression attenuates chronic angiotensin-II induced hypertension and changes cardiac dynamics

Yashika Relan[1], Jennifer Kerr[1], Agathe Lermant[1], Alison McNeilly[1], Colin Murdoch[1]

[1]University of Dundee, Dundee, United Kingdom

Angiotensin-II (ANGII) induces hypertension and cardiac hypertrophy, leading to heart failure in humans. ANGII is a potent inductor of oxidative stress, which changes proteins function via oxidative post-translational modifications (oxPTM). Glutaredoxin-1 (Grx) catalyses the removal of an oxPTM, S-glutathionylation, and has been shown to be important in peripheral artery. However, the role of Grx in ANGII induced cardiac failure is unknown. This project aims to investigate the effect of ANGII infusion in mice that overexpress Grx.

Grx transgenic (TG) and wild-type (WT) littermates (C57Bl6/j, Male, 24.7-30.5g) were implanted with osmotic pumps (Alzet) containing either saline or ANGII (1.1mg/kg/day; s.c, n=4-8.). After 2 weeks, mice were anesthetised with isoflurane (2%) and a pressure-volume catheter (Transonics, 1.2F, 4.5mm) was inserted retrogradely into the left ventricle (LV) via the aorta in a closed chest preparation. To obtain aortic blood pressure (BP) and LV pressure-volume loops. All values are mean±SEM, compared by 1-way ANOVA.

In WT mice (n=4-8), ANGII increased systolic BP(SBP) (Saline: 95.7±4.0 mmHg vs ANGII: 116.0±5.6mmHg, $p<0.05$) and diastolic BP(DBP) (Saline: 65.2±3.9mmHg vs ANGII: 82.9±4.0mmHg, $p<.05$). However, no significant increase in SBP and DBP were observed in TG mice (SBP Saline: 96.8±2.3mmHg vs ANGII: 109.4±6.1mmHg, p=ns) and DBP (Saline: 64.26±2.1 mmHg vs ANGII: 74.58±4.9 mmHg, p=ns) remained constant. Heart rates were not significantly different between the four groups ranging from 560 to 588bpm.

Although there was an increase in BP in WT ANGII mice, left ventricle end-diastolic/systolic pressure (EDP/ESP) and end systolic volume(ESV) remained unchanged between the four groups. However, ANGII significantly lowered end diastolic volume (EDV) in TG compared to WT hearts (WT:42.2±2.7uL vs TG:22.4±2.0uL, $p<0.05$). Hence, stroke volume(SV) was also lowered in TG compared to WT (WT:24.4±3.0uL vs TG:16.4±1.6uL, $p<0.05$). Yet, these changes had no overall effect on LV cardiac output (ANGII, WT: 13947±1353 mL/min vs. TG: 2191.0±286.2mL/min, p=ns) or stroke work (ANGII, WT: 2191±286 mmHg*uL, vs. 2191.0±286.2mmHg*uL, p=ns). Interestingly, ANGII significantly lowered LV contractile state (Powermax) in response to ANGII in TG compared to WT mice (WT:343467±782502 mmHg*uL/s vs TG:161963±29044 mmHg*ul/s, $p<0.05$).

In the control groups, there was no difference in cardiac function between saline treated WT and TG mice. With stroke work (WT: 1719±109 mmHg*uL vs TG: 1341±402mmHg*uL, p=ns), cardiac output (WT: 12004±783 mL/min vs TG: 9227±2237mL/min, p=ns) and stroke volume (Saline: 20.5±0.8uL vs 16.4±1.6uL , p=ns) remained unchanged between WT and TG mice.

In summary chronic ANGII infusion did not increase blood pressure in mice overexpressing Grx, but lowered pre-load with subsequent lower stroke volume and cardiac contractility. Future studies will find which redox sensitive proteins undergo reversal oxPTM to elicit these functional changes, leading to identification of novel therapeutic targets.

PCB017

CARDIOPROTECTIVE EFFECT OF CO-ENZYME Q10 ON DOXORUBICIN INDUCED CARDIOTOXICITY IN ADULT MALE ALBINO WISTAR RAT

Enivwenaye Nabofa[1], EKEMINIMFON-ABASI EMMANUEL Okon[1]

[1]Babcock University, Ilishan-Remo, Nigeria

Introduction: Doxorubicin (DOX) is a widely used chemotherapeutic agent but it is limited by its cardio-toxic side effect. Coenzyme Q10 (CoQ10) which could be considered as a vitamin is one of the most significant lipid antioxidants, which prevents the generation of free radicals and modifications of proteins, lipids, and DNA. However, it is not known whether CoQ10 is capable of preventing or ameliorating DOX induced cardiotoxicity.

Aim: The present study was thus designed to ascertain the cardioprotective effects of CoQ10 on DOX induced cardiotoxicity.

Method: All procedures were undertaken in accordance with regulations as set out by the Babcock University Research and Ethical Committee (BUREC). Adult male Wister rats weighing 180-200 g were randomly divided into five groups (n=7). Animal experimentation lasted for 14 days. Group 1 animals served as control and were untreated. Groups 3-5 animals were treated with dexamethasone (3 mg/Kg), CoQ10 (1 and 10 mg/kg) respectively, for 14 days. Group 2-5 animals were given a single dose of DOXO (15 mg/Kg) on day 11 of the study. Cardiovascular, biochemical, histological and molecular parameters were determined at the end of the study.

Results: DOXO altered cardiovascular function in rats evidenced by cardiac arrhythmia, hyperlipidemia and increased serum creatine kinase-myocardial band (CK-MB) levels which was associated with increased cardiac oxidative stress markers. CoQ10 especially at the higher dose ameliorated the DOXO induced cardiovascular dysfunction which was associated with decrease cardiac expressions of TNF-α and GSK3B genes.

Conclusion: CoQ10 especially at 10 mg/Kg mitigated DOXO induced cardiac dysfunction which involved the modulation of TNF-α / GSK3B signaling pathway.

PCB018

Analysis of seasonal changes in cardiac conduction system

Il-young Oh[1], Youngjin Cho[1], Ji Hyun Lee[1]

[1]Seoul National University Bundang Hospital, Seonganm, Korea, Republic of

Most studies report there is a ‘winter peaks’ in cardiovascular-related events. It is known that temperature affects the velocity of electrical conduction in the heart. This study was aimed to investigate the change of intervals in an electrocardiogram (RR, PR, QTc interval and QRS duration) according to the season.

We analyzed 121,286 electrocardiograms from 60 to less than 100 beats per minute among people performed for health surveillance at Seoul National University Bundang Hospital for the past 10 years (2009-2018). The mean of each interval including QTc interval using Bazzett's formula, were compared according to the month and sex. The mean temperatures in Seoul during the above period was obtained from the Korea Meteorological Administration database.

During the 10-year period during which ECGs were obtained, Seoul, located in the northern hemisphere, had the lowest average temperature in January and the highest in August. The RR interval changed similarly to the change of temperature, so heart rate (obtained by dividing 60000 by the RR interval) was the fastest in January and shortest in August. The PR interval and QRS duration were consistently observed even with temperature changes and maintained constant levels throughout the four seasons. The QTc interval showed an inverse correlation with temperature, being longest in January and shortest in August.

In winter, when the temperature was low, the heart rate was fast and the QTc interval was long, and in the summer when the temperature was high, the heart rate was slow and the QTc interval was short. This may be one of the reasons for the high frequency of cardiovascular diseases in winter.

PCB019

Cardiac Energy Metabolism in Crotonaldehyde (Beta-methyl Acrolein) Exposed Male Wistar Rats

Olufemi Oluranti[1,2], Abayomi Ige[2], Bernard Adele[2], Elsie Adewoye[2]

[1]Bowen University, Iwo, Iwo, Nigeria, [2]University of Ibadan, Ibadan, Nigeria

Introduction: Alterations in cardiac energy metabolic pathways arising from exposure to environmental pollutants have been linked to the development of cardiac dysfunction. Crotonaldehyde (CRO), a hazardous environmental pollutant, has been reported to be cardio-toxic.

Aim: However, dearth of information exists on the effect of CRO exposure on cardiac energy metabolism with respect to its cardio-toxicity. This study was therefore designed to investigate cardiac energy metabolism in male Wistar rats exposed to CRO.

Method: 36 male Wistar rats (150-170g; n=9) were grouped into 4 (I-IV): Control (10mL/kg normal-saline), CRO (0.75, 1.5, and 2.5mg/kg, p.o) for 28 days. Blood samples were obtained and evaluated for plasma Creatinine Kinase-Myocardial band (CK-Mb), cardiac troponin-I (cTnI), glucose, triglyceride, Free Fatty Acid (FFA) and insulin levels by spectrophotometry. Cardiac triglyceride, FFA, pyruvate, glucose-6-phosphate, cardiac hexokinase, pyruvate dehydrogenase (PDH) and nuclear factor erythroid-2 related-factor (Nrf2) activities were determined using ELISA. Cardiac malondialdehyde (MDA), hydrogen peroxide (H_2O_2), reduced glutathione (GSH) level, superoxide dismutase (SOD), and catalase activities were measured by spectrophotometry. Cardiac glucose transporter-4 (GLUT4), Peroxisome Proliferator-activated Receptor-alpha (PPARα), Carnitine Palmitoyl Transferase-1β (CPT1β) and AMP-activated Protein Kinase (AMPK) activities were determined immunohistochemically. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$. This study complied with ethical standard and was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/18/007).

Results: Levels of CK-Mb, cTnI, glucose increased significantly while plasma and cardiac triglyceride, FFA, and insulin reduced significantly in all CRO-treated groups compared with control. Cardiac Pyruvate level, hexokinase and PDH activities increased significantly in all CRO-treated groups compared with control. Cardiac levels of MDA and H_2O_2 increased significantly while GSH and Nrf2 reduced; SOD and CAT activities decreased following CRO exposure compared with control. Cardiac GLUT4 (15.31 and 28.86 %) and PPARα (73.25, 102.79 %) expression increased in groups II and III, while CPT1β expression decreased in group II (66.46 %) but increased in groups III and IV (52.80 and 33.45 %) compared with control.

Conclusion: Crotonaldehyde exposure exerts cardio-toxic effects by altering cardiac energy metabolism resulting in reduced free fatty acid, increased glucose uptake and utilization; down-regulation of nuclear factor erythroid-2 related-factor and up-regulation of peroxisome proliferator-activated receptor-alpha.

Keywords: Crotonaldehyde, energy metabolism, cardio-toxicity, redox imbalance

PCB020

Losartan treatment improves the cardiovascular profile in rats with right-sided insular experimental stroke

Liliane Santos Machado[1], Itamar Jesus[1], Marcos Eliezeck[1], Ana Caroline Ventris-Godoy[1], Maria José Campagnole-Santos[1], Silvia Guatimosim[1], Marco A Peliky Fontes[1]

[1]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

Intro: Stroke, a cerebrovascular disease, is the second leading cause of death in the world. Patients who survive stroke present physiological alterations and prognostic depends on the affected area. In this regard, stroke in the insular cortex (IC) results in marked sympathetic-mediated increase in baseline heart rate, cardiac molecular changes and arrythmias. Therefore, investigating strategies that can alleviate post-insular stroke consequences becomes extremely relevant. We have recently described an experimental model of right side insular hemorrhagic stroke in rats that reproduces some physiological alterations observed in humans, including marked increases in cardiac sympathetic output (Marins FR et al. 2020, 2021). Evidence indicates that the renin-angiotensin system (RAS) peptides interact with the sympathetic nervous system (Fontes MAP et al., 2016). **Aims:** Here, we evaluated the effect of angiotensin II (Ang II) AT_1 receptor blocker, losartan, on cardiovascular changes generated from experimental hemorrhagic stroke at the IC of rats. **Methods:** Experiments were conducted in accordance with the U.S. NIH Guide for the Care and Use of Laboratory Animals and approved by CEUA UFMG; protocol 112/2019). Wistar rats were: 1) anesthetized (ketamine 80mg/kg - xylazine 7 mg/kg); 2) submitted to unilateral nanoinjection of blood into the IC (Stroke IC; 200 nL; n=6) or saline into the IC (Sal IC; NaCl 0.9%, 200 nL; n=6; control), and 3) prepared for recording of mean arterial pressure (MAP) and heart rate (HR). Just after experimental stroke, separated groups of rats were submitted to three days of treatment (single daily intraperitoneal dose) of losartan (Los i.p.; 10 mg/kg) or saline (Sal i.p.; NaCl 0.9%, 0.1ml /100g). **Results:** Stroke IC rats showed elevated baseline HR when compared with the Sal IC group (422 ± 10 bpm vs 365 ± 6 $P<0.01$). Baseline MAP was similar between groups. In Stroke IC rats, treatment with Los i.p. restored HR to baseline levels and decreased baseline blood pressure values when compared with Stroke IC rats treated with Sal i.p. (HR: Los i.p. 358 ± 7 vs Sal i.p. 428 ± 10 bpm $P<0.01$; MAP: Los i.p. 99 ± 2 vs Sal i.p. 111 ± 2 mmHg $P<0.01$). The effects produced by Los in Stroke IC rats were prevented by concomitant treatment with Ang-(1-7) antagonist, A-779 (200 µg/kg SC). In agreement with this observation losartan treatment also increased the cardiac expression of Ang-(1-7)/Mas, receptor (0.93 a.u. Sal i.p. vs 1.6 a.u. Los i.p. $P=0.03$). **Conclusions:** The present data suggests that AT_1 receptors blockade may reduce the impact of cardiac sympathetic activity exacerbation observed after insular stroke as well as promote a protective reduction in baseline blood pressure values. At least part of these effects may involve activation of Ang-(1-7)/Mas receptors. The present study indicates that immediate treatment with losartan may help to minimize cardiovascular risk in the acute phase after insular stroke. Support: FAPEMIG APQ-01128-21; CNPq.

Marins FR, Limborço-Filho M, Iddings JA, Xavier CH, Biancardi VC, Stern JE, Ramiro Diaz J, Oppenheimer SM, Filosa JA, Peliky Fontes MA. Tachycardia evoked from insular stroke in rats is dependent on glutamatergic neurotransmission in the dorsomedial hypothalamus. European Journal of Neurology. 2021 Nov;28(11):3640-9. Fontes MA, Lima AM, dos Santos RA. Brain angiotensin-(1–7)/Mas axis: a new target to reduce the cardiovascular risk to emotional stress. Neuropeptides. 2016 Apr 1;56:9-17. Marins FR, Limborço-Filho M, D'Abreu BF, de Almeida PW,

Gavioli M, Xavier CH, Oppenheimer SM, Guatimosim S, Fontes MA. Autonomic and cardiovascular consequences resulting from experimental hemorrhagic stroke in the left or right intermediate insular cortex in rats. Autonomic Neuroscience. 2020 Sep 1;227:102695.

PCB021

Baroreflex dependent cardiovascular autonomic reactivity is deranged in COVID-19 survivors

Ayan Roy[1], Dinu Chandran[1], Ashok Jaryal[1], Manish Soneja[1], Ambuj Roy[1], KK Deepak[1]

[1]All India Institute of Medical Sciences, New Delhi, India

Title: Baroreflex dependent cardiovascular autonomic reactivity is deranged in COVID-19 survivors

Roy Ayan[1], Chandran S Dinu[1], Jaryal Ashok[1], Soneja Manish[2], Roy Ambuj[3], Deepak KK[1]

1. Department of Physiology, AIIMS, New Delhi
2. Department of Medicine, AIIMS, New Delhi
3. Department of Cardiology, AIIMS, New Delhi

Presenting Author:

Name: Ayan Roy

Email: drayanroy.jan21@aiims.edu

Corresponding Author:

Name: Dinu S Chandran

Email: dinu.chandran@aiims.edu

Abstract Body:

Introduction: COVID-19 has been reported to produce multi-system disorder during the acute as well as the post-acute 'long COVID' states. Emerging literature suggests long-term effects of COVID-19 on the autonomic nervous system manifesting as orthostatic intolerance or hypotension in COVID-19 survivors the mechanistic basis of which are currently not delineated.

Aims & Objectives: The study aimed at assessing the cardiovascular autonomic functions in COVID-19 survivors and compare it with age and sex-matched data from a historic (pre-pandemic) healthy control group.

Materials and methods: Ewing battery of cardiovascular autonomic reactivity tests along with cold pressor test (CPT) was conducted at least one month after clinical recovery from acute COVID-19 on 23 survivors free of any comorbidities (Age: 28 ± 4 years; 9 females). The responses were compared with that of 23 age and sex-matched pre-covid era healthy historic controls. Ewing battery of tests included head-up tilt test (HUT), deep breathing test (DBT), Valsalva maneuver and hand grip test (HGT).

Results: Resting systolic and diastolic BP and heart rates were comparable between the COVID-19 survivor group and the control group. COVID-19 survivor group had significantly lower Valsalva ratio (1.55 ± 0.25 vs 1.86 ± 0.46; p = 0.005) and displayed greater fall in systolic blood pressure during head up tilt test (-10.1 ± 6.7 *vs* -1 ± 8.5 mmHg; p = 0.0003) in comparison to the control group. Three out of the 23 COVID-19 survivors were diagnosed with orthostatic hypotension. Autonomic reflex responses to DBT, HGT and CPT were found to be comparable between the two groups.

Conclusion: COVID-19 survivors show abnormal responses in baroreflex dependent cardiovascular autonomic reactivity tests. This possibly indicates COVID-19 associated baroreceptor reflex dysfunction as a medium to long term autonomic sequelae and might explain the clinical presentation of orthostatic intolerance and hypotension in COVID-19 survivors.

Zanoli L et al. (2022). Circ Res. 130(9):1276-1285.

PCB022

Chronic hypoxia increases noradrenaline transporter activity in the rat left atrium

James Saleeb-Mousa[1], Andrew M Coney[1], Manish Kalla[3], Keith L Brain[1], Andrew P Holmes[1]

[1]School of Biomedical Sciences, Institute of Clinical Sciences, University of Birmingham, United Kingdom, Birmingham, United Kingdom, [2]School of Biomedical Sciences, Institute of Clinical Sciences, University of Birmingham, United Kingdom, Birmingham, United Kingdom, [3]University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom

Atrial fibrillation (AF) is the most common cardiac arrhythmia with a prevalence of 2-4% in adults (Hindricks et al., 2021). Emerging evidence indicates that autonomic dysfunction is a key driver of atrial arrhythmogenesis. Conditions associated with chronic hypoxia (CH), such as chronic obstructive pulmonary disease and heart failure, are independent risk factors for AF (Grymonprez et al., 2018). In addition to sympathetic activation secondary to peripheral hypoxia sensing, it is possible that CH causes remodelling of left atrial sympathetic neurones, pre-disposing to arrhythmia. At sympathetic terminals, efficacy of noradrenergic outflow is governed by multiple regulatory processes, including the noradrenaline transporter (NAT). As yet, little is known about the regional differences in NAT function in the left atrium and whether this is modified by CH. Here, we have studied NAT kinetics in the left atrial appendage (LAA), left atrial posterior wall (LAPW) and pulmonary veins (PVs) from animals exposed to normoxia (N) and CH.

A recently developed method for dynamic analysis of single-terminal NAT rate (Cao et al. 2020) was employed in atrial tissue obtained from adult male Wistar rats (200-300g). All experiments and procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986. Hearts were excised under non-recovery, terminal inhalation isoflurane (3-5% in O_2, flow rate 1.5L min^{-1}, death by cervical dislocation) and immediately transferred to a superfusion chamber. Atria were dissected free and separated into regions of interest. Tissues were subsequently loaded with a low-concentration solution (1:100 dilution) of Noradrenaline Transporter Assay (NTUA, Molecular Devices), a fluorescent substrate for the NAT, and imaged with confocal microscopy to identify sympathetic terminals. Tissues were then superfused with a 1:20 solution of NTUA, and image stacks were recorded over 15 minutes to monitor uptake rates. Images were analysed using FIJI (v1.53t) to allow quantification of single-terminal NAT activity. Experiments were performed on N (n=6-11; F_iO_2=0.21) and CH (n=3-5; FiO_2=0.12, 9-10 days) animals. Values are expressed as mean ± SEM. Significance was taken as $p<0.05$, two-way ANOVA with Tukey post-hoc analysis.

Analysis of fluorescence traces revealed a qualitatively similar uptake profile in all 3 regions regardless of CH exposure, demonstrating a linear 6-minute rising slope followed by a plateau phase. CH caused a marked increase in the magnitude of the rising slope in the LAA (N 6.52 ± 0.50% $minute^{-1}$ vs CH 10.39 ± 0.66% $minute^{-1}$, p=0.001) and LAPW (N 5.93 ± 0.47% $minute^{-1}$ vs CH 8.81 ± 0.90% $minute^{-1}$, p=0.02), alongside 15-minute plateau fluorescence peaks (LAA N 68.0 ± 4.7% vs CH 102.3 ± 6.7%, p=0.0004; LAPW N 51.3 ± 4.7% vs CH 87.9 ± 6.0%, p=0.003). NAT kinetics were unaffected by CH in the PV ($p>0.05$).

Our data demonstrate that exposure to CH augments NAT activity in the LAA and LAPW but not in the PV. This could be an adaptive response to a chronic rise in atrial sympathetic nerve firing

frequency. Further investigation is required to reveal the mechanism underpinning this upregulation and to what extent this may be of relevance to CH-related atrial arrhythmogenesis.

Grymonprez M et al. (2018). Int J Cardiol 276, 118-124. Hindricks G et al. (2021). Eur Heart J 42, 373-498.

PCB023

VEGF receptor roles in human cardiac progenitor cell contributions to new vascular formation

Andrew Smith[2], Hanqing Zhao[2], Sreenivasan Ponnambalam[1]

[1]School of Molecular and Cellular Biology, University of Leeds, Leeds, United Kingdom, [2]School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom, [3]School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom

Introduction. Human cardiac progenitor cells (CPCs) have been identified as an endogenous myocardial cell population, capable of aiding myocardial tissue maintenance, including the potential to differentiate into endothelial and vascular smooth muscle cells. Vascular endothelial growth factor (VEGF) ligands have been identified playing critical roles in angiogenesis. VEGF receptors have three major subtypes (VEGFRs 1, 2 and 3); previous data identified CPC VEGFR expression and pro-angiogenic growth factor secretion.

Aims. This study examined whether human CPCs utilise VEGFR signalling to potentiate angiogenesis, both directly by CPC differentiation and indirectly through pro-angiogenic paracrine signalling.

Methods. Human adult myocardial tissue was collected during cardiac surgery and c-Kit-positive (c-Kit$^+$), CD45-negative (CD45$^-$) CPCs were isolated by immunomagnetic bead sorting; five unique CPC lines were generated from individual donor samples. c-Kit$^+$/ CD45$^-$ CPCs were then characterised *in vitro* by clonogenicity assays, immunocytochemistry and RT-qPCR. Human CPC lines were sorted by FACS into three lineages: endothelial (CD31$^+$), smooth muscle (CD91$^+$/CD140b$^+$/CD31$^-$) or uncommitted (CD91$^-$/CD140b$^-$/CD31$^-$) groups. VEGFR and marker (SDF1; TGF-β) expression in CPC sub-populations were quantified (qPCR; Western blot; immunocytochemistry). VEGF-A stimulation and effects on signal transduction were examined (Western blot; immunocytochemistry). Statistics: ANOVA plus Tukey's test, significance: $p<0.05$; data are mean±SEM. This work was approved by the Faculty Research Ethics Committee at the University of Leeds and by the Wales Research Ethics Committee for NHS clinical tissue samples (NREC 17/WA/0314).

Results. Human CPC lines were isolated (n=5) and the stem cell phenotype confirmed by analyses of differentiation (immunocytochemistry) and self-renewal (clonogenicity: 50-90% of single-cell clones generated clonal colonies; RT-qPCR: 'stemness' genes confirmed in all 5 lines, n=3 technical replicates). CPCs from a representative line were then FACS-sorted into populations, separated by markers of: endothelial linage CD31$^+$ (1.99% of total cells), smooth muscle lineage CD91$^+$/CD140b$^+$/CD31$^-$ (13.77%) and CD91$^-$/CD140b$^-$/CD31$^-$ (31.28%) cells. Gene expression analyses identified mRNA for VEGFRs 1, 2 and 3 in all sub-populations (n=3). However only VEGFR1 protein expression was confirmed in all three sub-populations, not VEGFR2 or VEGFR3 (n=3). For growth factors previously identified as being secreted by CPCs (SDF, TGF-β, VEGFs, FGF-2), we identified high gene expression levels in human CPCs, with expression seen in all sub-populations (n=3). Application of CPC secretome, from each sub-population, to human endothelial cells on Matrigel *in vitro* did not show a clear increase in tube junction formation or tube segment length (n=6).

Conclusions. We isolated and analysed human CPCs, in bulk lines and sub-populations, identifying VEGFR1 gene and protein expression, but not VEGFR2 nor VEGFR3. We are building on this work to identify signalling pathways in human CPCs linked to VEGF-A stimulation, and further assessing impacts of VEFG-A stimulation on CPC secretome and associated potential to drive angiogenesis.

PCB024

Investigation of human tissue-specific proteome enrichment.

Huei Teng Ng[1], Florence Burte[1], Julie Taggart[1], Paul Ayuk[2], Stephen Robson[3], Michael Taggart[1]

[1]Biosciences Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom,
[2]Department of Obstetrics, Royal Victoria Infirmary, Newcastle Upon Tyne, United Kingdom,
[3]Population and Health Sciences Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom

Tissues throughout the human body exert finely-tuned specific functions even when there is considerable overlap in the cellular constituents. Even when the major cellular component of tissues is the same – for example in the case of smooth muscle-rich tissues - discrete phenotypes are evident. Yet, the molecular expression profiles underpinning such specialised functionality is often unclear. This presents a challenge for introducing new medicinal therapies for pathophysiologies: can one target what makes tissue functionality unique without producing unwanted effects on the features that are similar.

Such a scenario can be considered for the human uteroplacental unit. Ideally, one would like to target complications of pregnancy like pre-eclampsia (hypertension and proteinuria) or spontaneous preterm birth (early activation of parturition) by targeting particular types of smooth muscle tissues (e.g. arteries versus myometrium) without adversely affecting others. This situation is further complicated by the need to consider maternal and fetoplacental circumstances. Three smooth muscle-rich tissues of importance here are myometrium, maternal uterine (myometrial) arteries and placental arteries. Indeed, each display subtly distinct phenotypes suggesting that distinct molecular signatures may underlie these differences. As proteins determine cell/tissue structure and function, the aim of this work was to compare the proteome-wide expression profiles of these three tissues.

Paired uterine biopsies and placentas were obtained, following written informed consent (LREC 10/H0906/71), from healthy pregnant women undergoing elective Caesarean section at term (39-40 weeks gestation, n=9). Myometrial strips(M), myometrial arteries(MA) and placental chorionic plate arteries(PA) were each isolated, and cleaned of surrounding material, by careful microdissection, snap frozen in liquid N_2 and stored at -80^{o}C until further use. Frozen samples were homogenised (5% SDS in 50mM TEAB, pH 8.5), digested (1:20(w:w) trypsin:protein ratio, 47^{o}C for 2hours) and peptides analysed in triplicate via liquid chromatography mass spectrometry(LC-MS) using SWATH[1].

5895 proteins were quantified (using ≥5 unique fragment ion intensities per peptide, log(2) transformed and median-corrected) and 2832 differentially expressed across the three tissue types (ANOVA with multiple corrections FDR<0.01). Hierarchical k-mean clustering indicated five main groupings of relative protein changes across the tissue types. Paired t-test (FDR<0.05) identified differentially expressed proteins between tissues as follows: M vs MA: 1277 proteins; M vs PA: 3376 proteins; MA vs PA: 2486 proteins. Multiple pathway enrichment analyses indicated that the three tissues possess distinct proteomic profiles with PA being the most distinguishable from the other two. Notable biological pathway differences across these tissues are proteins related to ribosomal structure, oxidative phosphorylation, cytoskeleton and extracellular matrix(ECM). One cluster was indicated by notably higher relative protein abundances from placental arteries than each of the other two tissue types. For example,

identified in this cluster ECM laminin isoforms LAMB2 and LAMC1 had higher abundances in PA (log(2) fold-change: 4.94±0.57; 6.10±0.48 respectively), followed by MA (3.81±1.04; 5.33 ±0.36) and M (2.13±0.74; 4.81±0.44).

In summary, distinctive proteomic profiles are evident between human placental arteries, myometrial arteries and myometrium. This opens the possibility of uncovering the molecular signatures, and biological pathways, that serve to furnish different smooth muscle-rich tissues with specialised physiological (and pathophysiological) phenotypes.

[1] Ludwig, C, Gillet, L, Rosenberg, G, Amon, S, Collins, B, Aebersold, R 2018, Data-independent acquisition-based SWATH-MS for quantitative proteomics: a tutorial, Mol Syst Biol, 14(8):e8126.

PCB025

BIOMARKERS OF HEART FAILURE WITH A PRESERVED EJECTION FRACTION IN BLACK SOUTH AFRICAN PATIENTS

Marilet van Hoogland-van Heerden[1], Pindile Mntla[2], Harold Majane[1]

[1]Sefako Makgatho Health Sciences University, Pretoria, South Africa, [2]Dr George Mukhari Academic Hospital, Pretoria, South Africa

***M van Hoogland-van Heerden[1]**, P Mntla[2], OHI Majane[1]

Department of Physiology, Sefako Makgatho Health Sciences University, South Africa[1]

Department of Cardiology, Dr George Mukhari Academic Hospital, South Africa[2]

BACKGROUND AND OBJECTIVES: Almost 50% of all heart failure (HF) cases have a preserved ejection fraction (EF), which is often observed in the elderly (≥ 75 years). However, the mean age of HF presentation is considerably lower (40-55 years) in sub-Saharan Africa. Heart failure with a preserved ejection fraction (HFpEF) is therefore unlikely to be due to arterial stiffness. The risk factors associated with HFpEF might also be different in a middle-age population group when compared to the elderly. The aim of this study is to investigate two biochemical markers, N-terminal pro b-type natriuretic peptide (NT-proBNP) and galectin-3, that predict HFpEF and specifically markers that predict or are associated with HFpEF in a black population in South Africa.

METHODS: The nature of the study was a case-control investigation. Sixty-six participants with HFpEF and 213 participants without HF from African descent and older than 18 years of age were enrolled. All participants gave informed consent and completed a standardised questionnaire. Echocardiographic, anthropometric, central haemodynamic measurements, pulse wave velocity (PWV) and biomarker analysis using commercially available enzyme-linked immunosorbent assay (ELISA) kits were done.

RESULTS: The mean age of HFpEF in black South African patients was 54.88±13.51 years. PWV was significantly increased in participants with HFpEF (9.97±2.78 m/s) when compared to participants without this pathology (6.11±2.18 m/s) with a p-value of $p<0.0001$, however there were no significant associations between central haemodynamic parameters, NT-proBNP ($p = 0.9746$) and galectin-3 ($p = 0.2166$). Lastly, NT-proBNP, but not galectin-3, was significantly associated with left ventricular hypertrophy (LVH) ($p = 0.0002$) and left atrial (LA) diameter ($p = 0.0005$).

CONCLUSION: HFpEF is more prevalent in a middle-aged black South African sample with increased arterial stiffness when compared to European and American populations. NT-proBNP, but not galectin-3, is independently associated with LVH and LA diameter and hence could be used for the diagnosis of HFpEF in this community sample.

PCB026

Technical skills for basic scientists- Hands on skills in basic science courses is appreciated and promotes employability/life skills which otherwise are difficult to demonstrate to employers

Liam Bagley[2,3], Natasha Hadgraft[3]

[1]Manchester Metropolitan University, Manchester, United Kingdom, [2]Department of Anaesthesia , Manchester NHS Foundation Trust, Manchester, Manchester, United Kingdom, [3]Department of Life Science, Manchester Metropolitan University, Manchester, United Kingdom

Students studying physiology undergraduate programmes often report aspirations of careers in clinical practice and clinical investigation (Steele *et al.*, 2020), which are heavily skills-based and theoretically grounded. Previous evidence suggests that simulation-based learning improves perception of life skills, motivation and self-efficacy in nursing students (Roh & Kim, 2015). However, evidence is limited in student perception of confidence and understanding gained from simulation-based learning in the study of basic sciences. This study aims to assess student perception of clinical based skills training and simulations in students studying degrees in the field of Physiology.

This study was approved by the Science and Engineering Research Ethics Committee, Manchester Metropolitan University. Anonymous surveys were conducted with students enrolled on the unit “Cardiovascular Science”, a level 6, 15 credit undergraduate unit as part of the degree programmes BSc (Hons) Human Physiology or BSc (Hons) Sports Science and Human Physiology during 2020-21. Students were surveyed following clinical sessions, one after a skills session to practice Basic Life Support (BLS), another using high-fidelity patient simulators to diagnose signs of myocardial infarction (Sim). Surveys were conducted in Microsoft Forms. Each session survey assessed enjoyment, understanding/confidence, transferrable skills and problem solving, all assessed using a scale from 1 to 5 (1=negative, 5=positive). Students also surveyed on completion of all teaching (n=7), assessing perception of understanding, confidence, overall study experience and whether skills practiced add to students’ overall physiology skills, assessed on a scale of 1 to 3 (1=negative, 3=positive). Students were optionally asked for free text comment on all surveys. Free text was analysed qualitatively for themes. Quantitative data is presented as Mean±SD of responses.

Combined, (n=21 responses, BLS; n=12, Sim; n=9) both clinical skills sessions were enjoyed (Combined rating: 4.8/5±0.4) with students also reporting confidence and understanding in the theoretical basis of the skills practiced (Combined rating: 4.6/5±0.6). Students also felt these experiences provided transferable clinical skills relevant to employability (Combined rating: 4.7/5±0.6) and developed ability to solve problems (Combined rating: 4.4/5±0.7). Optional free text comments received were also universally positive and provide indication that students feel the sessions developed employability skills relevant to future career development (Table 1).

In the post unit survey (n=7, all self-reported attending at least one clinical skill session), all students surveyed reported that the addition of clinical skills sessions aided understanding of theory (3.0/3±0.0). Students reported confidence in applying theory to practical scenarios (2.7/3±0.5) and skills sessions positively added to overall study experience (2.9/3±0.4). Overall, students felt the skills practiced also positively added to overall physiological skills (2.7/3±0.5). Optional free text comments indicates students find the sessions useful as “…really informative

putting theory into practice", "Practical application of theory", "Interactive learning" and "Add more practical's".

These findings suggest students feel perceived confidence and improved understanding in the theoretical concepts when "traditional" lecture, lab and tutorial activities are supplemented by specific clinical skills not normally associated with the teaching and learning of theoretical concepts in physiological sciences.

References: Roh YS & Kim SS. (2015). Integrating Problem-Based Learning and Simulation: Effects on Student Motivation and Life Skills. CIN: Computers, Informatics, Nursing 33, 278-284. Steele KJ, VanRyn VS, Stanescu CI, Rogers J & Wehrwein EA. (2020). Start with the end in mind: using student career aspirations and employment data to inform curriculum design for physiology undergraduate degree programs. Advances in Physiology Education 44, 697-701.

PCB028

Teaching and learning about physiology of death and dying

Laura Ginesi, Derek Scott

undefined

Death, loss and grief are fundamental and profound aspects of the human experience that have long been a long and contested construct (Blakemore and Jennett, 2001). Nevertheless, death tends to be a topic of conversation that is avoided – even feared - because most people have never been with a dying person or know what to expect (Mannix, 2018).

On inspection, we and our students have found that the taboo related to death and dying extends to most physiology textbooks - the basic reference texts for student healthcare professionals (Brown, 2022). Searches using key words like “death”, “dying” and “mortality” yield few or no results in their indexes, while “apoptosis” and “necrosis” yield detailed information about cellular death. Similarly, searches of curriculum documentation using the same terms yielded expectations of student knowledge and understanding of “end of life care”, “palliative care” and death certification. The data show clearly that there is a void in explanation of physiological mechanisms that underpin the changes that the human body normally goes through at the end of life.

We are our bodies. Just as conception, pregnancy and birth mark the beginning of a person’s life, dying is a physiological process with recognisable stages of progression leading to the final stage when vital activities of living cease. The trajectory of each unique death depends on whether death is brought about by old age, malnutrition, dehydration, major trauma or terminal injury like suicide or drowning. However, textbook explanations of clinical signs such as Cheyne-Stokes breathing, Cushing’s triad or “death rattle” do little to explain what a dying human body is actually experiencing.

We designed a workshop based around selected video clips from James Bond movies and core physiological concepts (Michael and McFarland, 2020) to enable students to learn about and discuss fundamental mechanisms that normally precede a person’s death. Trigger warnings were used in advance and at the start to help to prepare students ahead of the sessions, reduce anxiety and promote feelings of safety within the groups.

The workshop was structured around activities designed to help students to recognise the physiological changes that are characteristic of loss of essential characteristics of cellular homeostasis which in turn leads to failure of body organs as the person approaches the end of

life. Breakout discussions enabled students to share their ideas about death as an integral part of the human life cycle as well as some of the social aspects related to own culture.

Feedback from the pilot sessions has been remarkably positive.

Although there is little published literature addressing the effectiveness of learning about death and dying, some studies about psychological training have shown promising results (Siverdale and Katz, 2005; Weaver, Balkan and Decker, 2022). We share our thoughts about why sound understanding of core physiological concepts relevant for death and dying has the potential to reduce anxiety and help student healthcare professionals to be better equipped to communicate appropriately with dying and bereaved people.

Blakemore, C.; Jennett, S. Eds. (2001) The Oxford Companion to the Body. Oxford University Press. Brown, K (2022) We need to talk about death. Physiology News Issue 128 (Winter) Mannix, K. (2018) With the End in Mind: Dying, Death, and Wisdom in an Age of Denial. Little, Brown Spark Silverdale, N., Katz, J. (2005) The impact of a distance learning death and dying course: An analysis of student self-reported changes. Nurse Education Today (2005) 25, 509–518 Weaver, R., Bolkan, C., Decker, A. (2022) High death anxiety and ambiguous loss: Lessons learned from teaching through the COVID-19 pandemic, Gerontology & Geriatrics Education, 43:1, 43-54. Michael, J., Mcfarland, J. (2020) Another look at the core concepts of physiology: Revisions and resources. Adv. Physiol. Educ., 44, 752-762

PCB029

Encouraging students' development towards employability using a digital micro-activity

Joseph Bass[1], Aimee Whitton[1], Gwen Hughes[1]

[1]University of Nottingham, Nottingham, United Kingdom

Providing student employability support and guidance forms an integral part of education. Much academic and University Careers and Employability Services (CES) effort focusses on students' skills and competency development for future vocations. Numerous strategies are employed to promote interaction with centralised University services and 'career development learning' (see Bridgstock et al., 2019). Students on our Medical Physiology and Therapeutics degree programme mainly focussed on employability as they approached final year studies. We aimed to encourage CES engagement, careers awareness and preparation for employability from year one using a simple activity.

Technology has offered new opportunities to enhance student engagement (JISC, 2022). We introduced a rapid, user-friendly 'micro-engagement' digital monitoring system for the students to track their interactions with careers support and their personal development towards employment. Simply based on a Microsoft excel spreadsheet template, the activity generated a visual 'map'. Piloted from entry level and giving clear instructions, students were asked to self-rate their CES interaction and exploration of different careers around subject topics and areas that initially had piqued their interest. They were asked to gauge their engagement on 6 aspects using a scale of 0 to 6 that reflected minimal to maximal interaction.

Completion of the activity created a personalised visual map for each student with the shape of each map being dependent on the self-ratings. The 'signatures' reflect developmental needs and are anticipated to expand each year and provide a continuing, progressive record of active participation and CES proactivity in preparation for employment. The expectation is that exploration of career interests and gaining CES awareness in first year generates smaller area 'maps' compared to subsequent years which demands self- evaluation, preparation for career applications, and a more practice approach towards career applications.

The activity has been progressively introduced into the curriculum via three 'backbone' core skills modules that focus on study and academic skill development at each stage of our three-year Hons programme. Students are requested to include their maps in summative skills portfolios to incentivise completion, to provide a subtle reminder about the need for personal and professional development, and to also signpost the University CES support. Each year group has been asked to complete the activity using similar questions for familiarity, ease of use, and to enable simple comparison between their maps as they progress through the course.

To date, > 80% of year one students have completed the activity with informal positive commentary suggesting this is a useful way for them to reflect about their future employability, boost their awareness of professional services and consider their career development needs. Introducing this activity may also be a helpful aid for personal tutors to discuss and support their student's individual progress towards career aspirations. It also bolsters students' preferences for academic input regarding careers and employability support (AGCAS, 2022).

Bridgstock, R., Grant-Imaru, M., & McAlpine, A. (2019). Integrating career development learning into the curriculum: Collaboration with the careers service for employability. Journal of Teaching and Learning for Graduate Employability, 10 (1), 56–72. JISC (2022) Enhancing student engagement using technological solutions. JISC Reports https://www.jisc.ac.uk/reports/enhancing-student-engagement-using-technological-solutions) The Association of Graduate Careers Advisory Services (AGCAS) (2022). The opportunities and challenges of student engagement in careers and employability provision https://www.agcas.org.uk/write/MediaUploads/Resources/Research%20and%20knowledge/Symp_22_Student_Engagement_Report_Final.pdf

PCB030

The Use of Digital Storytelling to Compensate for Flexible Physiology Learning

Noha Lasheen[3], Maram Fawzy[1], Mostafa Ibrahim[1,2]

[1]Undergraduate Medical Student, Faculty of Medicine, Galala University, Suez, Egypt, [2]Faculty of Medicine, Galala University, Suez, Egypt, [3]Associate Professor of Physiology, Faculty of Medicine, Galala University, Suez, Egypt

This study aimed at using digital case scenarios created by students to improve

the process of learning Physiology. Additionally, it allowed students to visualize and understand clinical scenarios and the physiological reasons behind them while assessing how much they stand to gain from the experience. This study is a project

to implement FAIMER, ASU MENA-FRI Institute, Cairo, Egypt. In a foundation course for first-year medical students, the instructor utilized a variety of instructional methods including lecture, small group discussion, individual assignments, and reflection. The instructor had experience with prior use of a digital storytelling project, (Medical Workshop in IUPS, August 2017, Rio, Brazil). This study obtained

IRB approval from the Faculty of Medicine, Ain Shams Medical Ethics committee. The results reported by the students themselves revealed that the project helped them improve their skills in problem-solving, teamwork, active learning, communication,

planning, and time management. In addition, it also increased their confidence in their abilities to learn, face unexpected challenges, and achieve goals, while considering new life opportunities, those which became an option when the students searched by themselves and learned more about the different angles of medicine. This study concluded that compared to the traditional lecture format that focuses on memorizing definitions and theoretical structures, digital storytelling can be regarded as an innovative teaching tool and a unique medical education method that allowed students to participate more in the learning process. This article proposes an active learning method in undergraduate medical education.

PCB031

ANTI-OXIDANT AND ANTI-INFLAMMATORY EFFECTS OF MELATONIN ON OBESE WISTAR RATS

Tahir Abdussalam[1], Luqman Olayaki[1]

[1]Department of Physiology, University of Ilorin, Ilorin, Nigeria

Obesity, a metabolic disorder is reaching an epidemic proportion and 20% increase in a normal body weight has been shown to be related to 20% increase in mortality rate. However, unsuccessful battle against this condition has led to the quest of finding a novel therapeutic agent (Timper and Brüning, 2017). Orlistat, a gastrointestinal lipase inhibitor has been recommended for the treatment of obesity. However, the gastrointestinal side effects have discouraged most patient from its continuous usage. Hence, there is a need for a more potent drug with less side effect (Jain et al., 2011). Melatonin, which are widely used alternative medicine have been reported to be effective in lowering the body weight with very little side effects. Therefore, the present study investigated the effects of melatonin in obesity model of male Wistar rats weighing between 110 – 130 g. It was hypothesized that melatonin is not an anti-obesitogenic therapy on obese rat model. Fifty (50) rats of ten (10) animals per group were divided into the following: control (untreated); high fat diet (HFD); high fat diet recovery (hfd); hfd + melatonin (4 mg/kg); and hfd + orlistat (30 mg/kg). Obesity was induced by exposing the rats to high fat diet for 16 weeks and confirmation was done using Lee index, which was determined by the formula: $4\sqrt{}$body weight (g) / nose-anal length (cm). Rats with an index higher than 0.30 were considered obese and were used for the study (Adeyemi et al., 2020). Treatment started and lasted for 28 days after which the rats were anesthetized by intramuscular injection of 50 mg/kg of ketamine. Melatonin and orlistat were administered at 4 and 30 mg/kg b.w., p.o. respectively. Animals were cared for and used according to the University of Ilorin ethics guidelines. Diagnostic kits for the determination of the biomarkers were obtained from Abcam PLC, Cambridge, UK and the assays were performed according to the manufacturer's instruction. Data were analyzed using analysis of variance and LSD *post hoc* test at 0.05 level of significance. The results showed that the induced obesity was accompanied with significant increases in plasma glucose, but significant decreases in plasma insulin. Relative to the obese control, treatments with melatonin caused significant elevations in total antioxidant capacity (TAC), catalase, superoxide dismutase (SOD) and gluthathione peroxidase (GPx), however, a significant decrease in interleukin 6 (IL-6) and tumor necrotic factor (TNF-a). Hence, it was concluded that melatonin could be beneficial in the management of obesity.

Adeyemi et al. (2020). Biomedicine & Pharmacotherapy 126, 110110. Jain et al. (2011). Indian Journal of Endocrinology and Metabolism 15(2):99-104. Timper, K. and Brüning, J.C. (2017). Disease Model and Mechanisms, 10(6), pp. 679-689.

PCB032

Effect of pulp extract of Azanza garckeana fruits on hematological and hormonal indices

E. Sylvia Okungbowa[1], Idris. B. Momodu[1], Fred O. Agoreyo[2], Blessing O. Agoreyo[3]

[1]Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B. 1154,, Benin City, Edo State, Nigeria, [2]Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, P.M.B. 1154,, Benin City, Edo State., Nigeria, [3]Department of Biochemistry, Faculty of Life Sciences, University of Benin, P.M.B. 1154,, Benin City, Edo State., Nigeria

Introduction

Azanza garckeana is a tropical wild fruit plant that is found in Africa. It produces edible fruits that are used as food or herbal medicine.

Aim

In some African countries such as Nigeria, *Azanza garckeana* fruit products are used traditionally for the treatment of infertility, sexually transmitted diseases and for libido enhancement. The aim of this study was to investigate the effect of the extract of *Azanza garckeana* fruit pulp on haematological indices and reproductive hormones in male and female Wistar albino rats.

Method

This study was approved by the ethical committee of Faculty of Life Sciences, University of Benin, Nigeria. Forty-eight (48) Wistar albino rats comprising 24 males and 24 females were grouped into two control groups and six treatment groups. The treatment groups were administered 50, 300 and 2000 mg/kg body weight of aqueous-methanol pulp extract of *Azanza garckeana* fruits.

Red Blood Cells (RBC), White Blood Cells (WBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Lymphocytes (LYM) and Mean Corpuscular Volume (MCV) of these animals were assessed. Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin, Estradiol, Testosterone and Progesterone in the rats were determined.

Results

HGB & HCT were seen to significantly increase ($p<0.05$) in 300 mg/kg fruit pulp treated female rats. Prolactin was seen to be significantly increased ($p<0.05$) in 2000 mg/kg fruit pulp treated female, while progesterone was seen to increase ($p<0.05$) in 300mg/kg and 2000 mg/kg fruit pulp treated male rats.

Conclusion

Results from this preclinical study, suggest that *Azanza garckeana* fruit extract may impact haematological and hormonal indices in Wistar rats, clinical studies would be done to confirm these trends in humans.

PCB033

Thyroid function is related to body mass index in post-menopausal women.

MAUREEN EBOMOYI[1], Clement Ekhator[1]

[1]Department of Physiology, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria

Thyroid diseases increase with age, predominantly affecting women, thus they occur most often in post-menopausal and elderly women.1 There is no consensus on universal screening for thyroid dysfunction in post-menopausal and elderly women worldwide, 2 therefore this study was on thyroid function tests: serum TSH, thyroxine, (T_4), and tri-iodothyronine (T_3) in relation to body mass index (BMI) in post-menopausal women. It was a prospective cross-sectional study of post- menopausal women attending clinics at a Catholic hospital in Benin City, Nigeria. 40 pre-menopausal (control) and 40 post-menopausal healthy women (mean age 31.73±11.45 and 57.06±5.57 years respectively) were assessed. Informed consent was obtained, a pre- tested structured questionnaire containing their bio-data, gynaecology history and past medical history were obtained, and ethical approval was also obtained. At the end, blood samples were obtained following standard laboratory procedures.3 Results were expressed as mean±SD, analysed using SPSS version 20, and subjected to 't-test' and ANOVA. $P< 0.05$ was considered statistically significant. Post-menopausal women presented significantly higher ($p<0.05$) BMI than the control (27.60±4.32 kg/m^2 vs. 25.42±4.16 kg/m^2). Serum TSH levels were significantly higher in post-menopausal than in pre-menopausal women (2.04±0.56 mIU/L vs 0.74±0.11 mIU/L.) Despite the higher TSH, T_3 and T_4 were significantly lower in post-menopausal women (1.02±0.16 ng/ml and 6.07ng/ml) than in pre-menopausal women (4.91±0.43 ng/ml and 10.13±0.25 ng/ml). These findings suggest that there might be reduced T_3 and T_4 receptors in post-menopausal women. The observed increase in BMI in post-menopausal women might be as a result of a fall in BMR, due to the decreased levels of T_3 and T_4.4

1. Gietka-Czernel M (2017). Prz Menopauzalny 16(2), 33-37. 2. Garber JR, Cobin RH and Gharib H (2012). Thyr 22, 1200-1235. 3. World Medical Association (2013). JAMA 310(20), 2191-2194. 4. Ebomoyi MI and Ekhator CN (2019). Med J 6(1), 19-23.

PCB034

Hypoglycemic and antioxidant potential of Phyllanthus amarus leaf extract in Type 2 diabetic Drosophilia melanogaster flies.

Churchill Inneh[1], James Osakue[1]

[1]*University of Benin, Benin City, Nigeria*

INTRODUCTION. High sucrose diet has been reported to produce type 2 diabetic phenotypes in *Drosophila melanogaster*. Type 2 diabetes mellitus is a chronic metabolic disorder that is characterized by elevated blood glucose levels (hyperglycemia) caused by loss of insulin action (insulin resistance) (American Diabetes Association, 2014). Current management of this condition has been by the use of oral hypoglycemic drugs. However these drugs have their own side effects such as liver disorders, kidney toxicity, flatulence, abdominal pain, diarrhea (Lee et al. 2014) which has lead to the search for natural compounds with hypoglycemic potential. This study was carried out to investigate the hypoglycemic and antioxidant potential of *phyllanthus amarus* leaf extract in high sucrose diet induced-type 2 diabetes in *Drosophila melanogaster*.

METHODS. Flies were divided into four (4) groups containing 50 flies per group. Group I served as control and they were reared on normal corn meal diet while group II were fed with30% high sucrose via their diet. Group III flies were fed with 2.5mg of *phyllanthus amarus* (*P. amarus)* leaf extract. Group IV flies were co-treated with30% high sucrose and 2.5mg of *P. amarus* via their diet for seven (7) days. Each experiment was carried out in five (5) replicates (n-5). At the end of the experimental period, the flies were homogenized and the supernatants were used to estimate glucose concentration and also assay for catalase (CAT), glutathione (GSH) and hydrogen peroxide (H_20_2). A 15 days survival study was also carried out to investigate the effect of high sucrose diet on the survival rate of flies and the possible protective effect of *P. amarus.*

RESULTS. There was a significant increase in glucose concentration, hydrogen peroxide (H_20_2) and a significant reduction in catalase, glutathione (GSH) and the survival rate of flies fed with high sucrose diet. However, in flies co-treated with *P. amarus* and high sucrose diet, there was a significant reduction in glucose concentration , hydrogen peroxide (H_20_2) and a significant improvement in catalase, GSH and the survival rate of flies.

CONCLUSION. This study has shown that *P. amarus* possesses hypoglycemic and antioxidant potential and could be of therapeutic benefits in the management of type 2 diabetes mellitus.

REFERENCES American Diabetes Association. (2014). Diagnosis and classification of diabetes mellitus. Diabetes Care, 37(1), 581–590. Lee, K. H., Ha, K. S., Jo, S. H. , Lee, C., Kim, Y. C., Chung, K. H. and Kwon YI (2014). Effect of long-term dietary arginyl-fructose (AF) on hyperglycemia and HbA1c in diabetic db/db mice. Int J Mol Sci 15(5):8352–8359.

PCB035

Effect of ethyl acetate extract of Amaranthus hybridus on blood glucose, insulin, oxidative stress, and lipid profile in streptozotocin (STZ)-induced diabetic rats.

Grace Umoren[1], Gabriel Ajayi[2], Saka Bamiro[1], Fehintoluwa Femi-Olabisi[2], Odunayo Olumide[1], Samson Aina[1], Adebusola Shakunle[1], Isaac Ikuejamoye[1]

[1]Lagos State University College of Medicine, Lagos, Nigeria, [2]Mountain Top University, Ogun, Nigeria

Introduction

Diabetes Mellitus (DM) has become a global endemic with severe consequences such as cardiovascular diseases and end-organ damage. Considering the reported use of ethyl acetate extract of *Amaranthus hybridus* (EAH) as a treatment option in managing DM. It is therefore important to evaluate the outcomes of this plant on some consequences.

Aims /Objectives

This study is designed to evaluate the effect of ethyl acetate extract of *Amaranthus hybridus* (EAH) on blood glucose, insulin, lipid profile and oxidative stress in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Thirty male rats weighing 200g to 250g were randomly allotted into five groups (n=6). Group A (Control), B (DM), C; DM +300mg/kg body weight (BW) of Metformin (DMMET), D; DM+300mg/kgBW of EAH (DMEAH), E; 300 mg/kg body weight of EAH (EAH). Diabetes Mellitus was induced with 60mg/KgBW of STZ intraperitoneally. Dose of EAH and MET were administered daily via oral gavage for 14 days. Blood glucose was checked using a glucometer before and on the 7th and 14th day of treatment. On the 14th day, the rats were kept in diethylether fume chamber, blood samples were taken from the heart after cervical dislocation. Serum centrifuged from blood was used to estimate the superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), total cholesterol; low and high-density lipoprotein, triglyceride and insulin. Data were expressed as Mean ± SEM and compared using one-way ANOVA. Data was considered statistically significant when $P < 0.05$.

Results

Blood glucose level on day 7 was lower ($P < 0.05$) in Control (100.83±3.66mg/dl), DMMET (272.33±45.70 mg/dl) and EAH (152.50±48.27mg/dl) when compared with DM (435.83±34.46mg/dl). On day 14, it was lower ($p<0.05$) in Control (104.50±3.63mg/dl), DMMET (190.5±43.3266mg/dl), DMEAH (152.5±4866mg/dl) and EAH (75.33±2.3366mg/dl) when compared with DM (460.67±30.556mg/dl). Insulin was higher ($p < 0.05$) in Control (5.1±0.53 µiU/ml), DMEAH (1.55±0.09 µiU/ml), and EAH (3.23±0.26 µiU/ml) when compared with DM (0.81±0.03 µiU/ml). SOD was higher ($p<0.05$) in Control (2.94±0.04 µmol/ml), DMMET (2.67±0.07 µmol/ml), DMEAH (2.76±0.06 µmol/ml) and EAH (3.28±0.07 µmol/ml) when compared with DM (1.9±0.04 µmol/ml). CAT was also higher ($p < 0.05$) in Control (6.97±0.13 µmol/ml), DMMET (6.58±0.08 µmol/ml), DMEAT (6.77±0.06 µmol/ml) and EAH (7.25±0.11 µmol/ml) when compared with DM (5.65±0.14 µmol/ml). MDA was significantly higher in DM

(2.69±0.04 µmol/ml) when compared with control and EAH (1.34±0.27; 1.04±0.30 µmol/ml). Total cholesterol was higher($p<0.05$) in DM (2.08±0.16mg/dl) than in control (1.44±0.07mg/dl) and EAH (1.41±0.09mg/dl). The level of LDL was significantly lower in control (0.06±0.02mg/dl), DMMET (0.33±0.03mg/dl), DMEAH (0.33±0.02mgldl) and EAH (0.09±.02mg/dl) when compared with DM (0.52±0.05mg/dl). Triglyceride was significantly lower in control (0.72±0.11mg/dl), DMMET (1.1±0.05mg/dl), DMEAH (1.04±0.03mgldl) and EAH (0.82±0.08mg/dl) when compared with DM (3.42±1.44mg/dl). HDL was higher ($p< 0.05$) in groups control (1.19±0.04mg/dl), DMMET (0.99±0.26mg/dl), DMEAH (0.76±0.01mg/dl) and EAH (1.13±0.04mg/dl) when compared with group DM (0.49±0.03mg/dl).

Conclusion

The result demonstrated that ethyl acetate extract of *Amaranthus hybridus* improved blood glucose, Lipid profile and antioxidant enzymes in STZ-induced diabetic rats.

1. Akubugwo, I. & Obasi, Nwogo & Chinyere, Godwin & Ugbogu, Eziuche Amadike. (2007). Nutritional and chemical value of Amaranthus hybridus L. leaves from Afikpo, Nigeria. African Journal of Biotechnology (ISSN: 1684-5315) Vol 6 Num 24. 6. 10.5897/AJB2007.000-2452
Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia.2008;51(2):216–226 2. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216–226

PCB036

The role of electrogenic and electroneutral monocarboxylate transport in airway clearance.

Anita Guequen[1,2], Bárbara Tapia[1,2], Sandra Villanueva[1,3], Carlos Flores[1,3]

[1]Centro de Estudios Científicos, Valdivia, Chile, [2]Universidad Austral de Chile, Valdivia, Chile, [3]Universidad San Sebastián, Valdivia, Chile

Introduction: The properties of airway surface liquid (ASL) are controlled by the coordinated activity of ion channels and transporters that mediate transepithelial absorption and secretion of ions. ASL homeostasis is dramatically altered in cystic fibrosis (CF) due to CFTR dysfunction that produces ASL dehydration and acidification, resulting in mucus-stasis, reduced airway clearance and favoring bacterial infections and inflammation. Monocarboxylates can be transported by an electrogenic Na^+-coupled system that corresponds to SMCTs (SLC5 family) and an electroneutral H^+-coupled system that correspond to MCTs, (SLC16 family). Previous work demonstrated the presence of MCT2 on the apical surface and MCT4 on the basolateral surface in human bronchial epithelium. But there is no evidence of SMCTs activity in this tissue. In here, we describe for first time that SMCT1/SCL5A8 is functionally expressed in mouse tracheal epithelium. We hypothesized that the activation of monocarboxylate transport might down regulate airway clearance as it is known that increased Na^+ absorption reduce ASL, while increased H^+ secretion acidify ASL. Then we tested if mucociliary clearance is affected by MCT and SMCT activity in mouse airways.

Methods: Short-circuit current in mouse tracheas was measured in Ussing chambers, expression of transporters by qRT-PCR, particle track speed (PTS) and mucus transport velocity (MTV) by videomicroscopy. Localization of MCT2 and MCT4 was evaluated by immunofluorescence. Animals (C57BL6/J) were housed at CECs-Animal facility under controlled temperature and humidity with free access to water and food. All protocols were approved by the IACUC (#CECs-2022-04), in accordance with relevant guidelines and regulations.

Results: 10mM apical L-lactate and D-lactate induced a negative current (I_{SC} ~ -35 $\mu A \cdot cm^{-2}$; n=4-5 for each group) that was dependent on apical Na^+. qRT-PCR assay determined that SLC5A8/SMCT1 was highly expressed in airway epithelium compared to SLC5A12/SMCT2. In addition, L-lactate, which is transported by SMCT and MCT induced an increase in PTS (2.1±0.1 control vs 3.28±0.4 $\mu m \cdot s^{-1}$ n=3 each group; $p<0.05$ rank sum test) and inhibited by 1 µM of the MCT1/2 inhibitor AR-C155858 (1.9±0.1 $\mu m \cdot s^{-1}$). On the other hand, D-lactate that is exclusively transported by SMCT1 did not affected PTS (2.03±0.1 $\mu m \cdot s^{-1}$; $p>0.05$ compared to controls; n=3 each group), indicating that SMCT1-dependent Na^+ absorption didn't modify airway clearance. Furthermore, analysis of MTV determined that L-lactate but not D-lactate increased the velocity of mucus (1.05±0.1 control vs 1.29±0.1 $\mu m \cdot s^{-1}$ for L-lactate and 1.07±0.02 $\mu m \cdot s^{-1}$ for D-lactate; n=3 each group; $p<0.05$ rank sum test) and the increase of L-lactate was prevented by 1 µM of AR-C155858 (0.98±0.1 $\mu m \cdot s^{-1}$). Surprisingly, immunofluorescence determined that MCT2 and MCT4 are localized in basolateral membrane. Thus, our results indicate that the effect on PTS and MTV induced by L-lactate possibly is mediated by MCT1. Preliminary data indicated that in presence of MCT1 inhibitor (AZD3965) this effect on MTV was diminished.

Discussion**:** Monocarboxylate uptake by MCTs removes H^+ from ASL, alkalizing the airway surface and improving airway clearance. The use of MCTs transportable substrates might help alleviate mucostasis in muco-obstructive diseases and will be tested in animal models of these diseases.

PCB037

Characterisation of the respiratory epithelium within Primary Ciliary Dyskinesia patients compared to healthy controls.

Elizabeth Haughey[1], Fengxia Ding[1], Chris O'Callaghan[1]

[1]*University College London, London, United Kingdom*

Background: The human respiratory epithelium is lined with hundreds of millions of motile cilia that beat in a coordinated fashion to clear mucus, pathogens, and pollutants from the airways. Several diseases cause abnormal ciliary function, including severe asthma, COPD, cystic fibrosis and primary ciliary dyskinesia (PCD) (1). PCD is an autosomal recessive disorder that affects around 1:8000 new-born babies. Mutations in ciliary genes lead to protein defects within the cilia that cause them to beat in a dyskinetic fashion or become static. PCD patients demonstrate reduced mucociliary clearance, recurrent respiratory tract infections and progressive severe lung damage. However, our understanding of the epithelial biology of PCD patients remains incomplete (2).

Aims:

- To determine if the cellular composition of ciliated epithelial cell cultures from PCD patients is similar to that of matched healthy controls.
- To determine if baseline cytokine and chemokine release from PCD and healthy control cultures differ.

Methods: Nasal brush biopsies from PCD patients with static cilia were cultured at air-liquid interface (ALI) to a ciliated phenotype (3). To characterise the cellular composition, we optimised a flow cytometry panel adapted from Bonser et.al, to quantify three major epithelial cell types: basal, ciliated and goblet cells (4). We used a histopathology approach, sectioning the ALI cultures to visualise epithelial populations using H&E (Figure 1). Finally, supernatants were analysed for cytokine changes using ELISA. Static PCD nasal brushings were age/sex matched with healthy controls. The mean and S.E.M was calculated, and statistical significance determined by firstly testing the normality of data using Shapiro Wilks test, followed by two-tailed T-test (flow cytometry) or non-parametric Mann Whitney test (ELISA).

Results: We confirmed the epithelial morphology of ALI is maintained during histology processing and H&E-staining (n=6 Healthy & n=PCD, 4 sections/donor). Flow cytometry showed PCD patients had fewer basal cells (n = 6 healthy, 7 PCD; p =0.0009) and an increased number of ciliated cells (p =0.0221) compared to age/sex match controls. Initial studies suggest a trend for increased IL-8 (n=3) and IL-6 (n=6) levels in PCD supernatants however increased donor number is required for statistical significance.

Summary: Flow cytometry showed a difference in the cellular composition of PCD ALI cultures, with increased numbers of ciliated cells and reduced numbers of basal cells. Preliminary ELISA results suggest static PCD may be associated with increased epithelial inflammation. Further characterisation is underway, increasing study numbers and processing cultured cells for RNAseq and mass spec to investigate apical secretions. Overall, this work will improve our basic understanding of PCD epithelial biology and identify potential PCD-specific therapeutic targets.

References: 1. Tilley AE, Walters MS, Shaykhiev R, Crystal RG. Cilia Dysfunction in Lung Disease. Annual Review Physiology. 2015 Feb; 77:379. 2. Mirra V, Werner C, Santamaria F. Primary ciliary dyskinesia: An update on clinical aspects, genetics, diagnosis, and future treatment strategies. Vol. 5, Frontiers in Pediatrics. Frontiers Media S.A.; 2017. 3. Do D, Lee H, Petris A, Hynds RE, O'callaghan C. Ciliated Epithelial Cell Differentiation at Air-Liquid Interface Using Commercially Available Culture Media. Methods in Molecular Biology. 2020;Sept:275–91. 4. Bonser LR, Koh KD, Johansson K, Choksi SP, Cheng D, Liu L, et al. Flow-cytometric analysis and purification of airway epithelial-cell subsets. Am J Respir Cell Mol Biol [Internet]. 2021 Mar 1 ;64(3):308–17. Available from: https://doi.org/10.1101/2020.04.

PCB038

Insight into the mechanism of D-glucose accelerated exchange in GLUT1 from molecular dynamics simulations

Richard Naftalin[3], Saul Gonzalez-Resines[2], Carmen Domene[1]

[1]1Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK2, Bath, United Kingdom, [2]1Department of Chemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK2, Bath, United Kingdom, [3]BHF Centre of Research Excellence, School of Medicine and Life Sciences, King's College London, London, United Kingdom

Atomistic molecular dynamics simulations demonstrate that when multiple β-D-glucose molecules are present within the GLUT1 transporter, simultaneous position exchanges frequently occur between adjacent ligands. These exchanges take place in the internal cavities and at both external and internal solution interfaces of the protein. They involve rotation of adjacent ligand positions along the central pore axis of the transporter with variable duration in the nanoscale (4 – 100 ns). Exchanges occurring at the extracellular protein interfaces involve fast displacements (2 – 10 ns) of D-glucose H-bonded to the protein interface by other D-glucose molecules present in solution. These examples of simultaneous D-glucose exchanges demonstrate that *accelerated exchange* is consistent with a multisite model for D-glucose transport within GLUTs where multiple D-glucose molecules move independently and stochastically within the transporter's tunnels, cavities, and the central pore.

Higher frequency of D-glucose exchange is observed in the membrane gel state, corresponding with D-glucose transport in human erythrocytes at low temperatures. The presence of multiple D-glucose molecules both within the transporter and in bathing solutions increases D-glucose penetration depths from the solutions into transporter intramembranous zones, particularly in the gel state.

That exchange frequency between adjacent ligands depends on the local D-glucose density within the transport pathway explains why accelerated exchange occurs more frequently in conditions where bottlenecks at the openings of the transport pathway are prolonged, at low temperatures (1), thereby augmenting ligand aggregation in the adjacent upstream regions.

Lacko, L., Wittke, B. and Geck, P. (1973) 'The temperature dependence of the exchange transport of D-glucose in human erythrocytes.', Journal of cellular physiology, 82(2), pp. 213–8.

PCB039

Developing a neutrophil trans-epithelial endothelial migration model of the human air-blood barrier to identify biomarkers for respiratory virus infection.

Machaela Palor[1], Thomas Benoist[1], Robert Hynds[1], Claire M Smith[1]

[1]UCL GOS ICH, London, United Kingdom

BACKGROUND: Respiratory syncytial virus (RSV) is a major cause of bronchiolitis and pneumonia in young infants, with an estimated 33 million cases occurring globally each year (1). Despite the high prevalence of RSV, there are currently no licensed vaccines or effective anti-viral treatments available and so supportive care remains the mainstay of therapy. A distinguishing feature of RSV bronchiolitis is the recruitment of an inflammatory infiltrate known as neutrophils into the airways of infants (2). Neutrophil-mediated factors such as neutrophil elastase (NE) and interleukin 8 (IL-8) in the blood and airways of infants hospitalised with RSV infection are thought to corelate with disease severity, although we currently do not know why (3,4). Clinical studies have also shown that neutrophils found in the systemic circulation of infants with RSV bronchiolitis contain RSV mRNA (5).

METHODS: Our lab has developed a novel trans-epithelial endothelial model of the human air-blood barrier to study neutrophil behaviour and function during RSV infection using animal-free components. To do this, primary paediatric airway epithelial cells (AECs) were grown at air-liquid interface (ALI) and co-cultured with human endothelial cells (ECs) before being infected with RSV expressing green fluorescent protein (GFP) (**Figure 1A**). After 24h, human neutrophils, obtained from a healthy donor, were added to the basolateral side. After 1h different sub-populations including basolateral, adherent and apical (migrated) neutrophils were recovered for subsequent analyses by flow cytometry.

RESULTS: Our data show that RSV infection led to a shift in the number of basolateral neutrophils to apical neutrophils, indicating movement across the EC/AEC barrier (**Figure 1B**). Exposure to RSV infected AECs led to increased expression of NE on basolateral neutrophils compared to the mock-infected control (*N*=6 neutrophil donors, Paired Two-Way ANOVA) (**Figure 1C**). This was accompanied by an increase in levels of pro-inflammatory chemokine and cytokines including interferon γ-induced protein 10 (IP-10), interleukin 6 (IL-6) and IL-8 in the apical supernatant of RSV-infected AECs compared to mock-infected control cells (**Figure 1D**) (*N*=3 neutrophil donors, Paired Two-Way ANOVA).

CONCLUSIONS: We have shown that neutrophils present on the basolateral (blood) side of RSV infected AECs increase expression of NE, which is similar to the finding of increased NE in the blood of infants hospitalised with RSV infection. These findings demonstrate that our *in vitro* model can replicate key human disease outcomes and can therefore be used to identify critical mechanisms that mediate epithelial cell damage and promote inflammation in children with severe RSV disease. Future work will investigate the mechanism behind this and compare the

phenotype of neutrophils isolated from our model to those from the blood of RSV-infected infants, thereby allowing us to characterise a neutrophil sub-population that can be used as a biomarker of severe infection.

1. Shi T et al. (2017). Lancet 390, 946-958 2. McNamara PS et al. (2003). Arch Dis Child 88, 922-926 3. Emboriadou M et al. (2007). Ann Clin Lab Sci 37, 79-84 4. Abu-Harb M et al. (1999). Eur Respir J. 14, 139-143 5. Halfhide CP et al. (2011). J Infect Dis. 204, 451-458

PCB040

Dietary regulation of ruminal UT-B2 urea transporters in adult male fallow deer bucks: effects of season and wildlife feeding activity.

Gavin Stewart[1], Deirdre McLaughlin[1], Laura Griffin[1], Chongliang Zhong[1], Simone Ciuti[1]

[1]University College Dublin, Dublin, Ireland

The urea nitrogen salvaging process (**UNS**) supports the symbiotic relationship between ruminants and their gastrointestinal microbiome by both supplying nitrogen and buffering bacterially-derived short chain fatty acids (**SCFAs**). Our previous studies have shown the importance of the UT-B2 urea transporters in the rumen of wild fallow deer living in Phoenix Park, Dublin. In this current pilot study, we investigated the effects on these transporters of seasonal changes and feeding behaviour in adult male deer bucks. Restricted, authorized culling of the deer population was performed by Irish Government bodies (Office of Public Works; National Parks & Wildlife Service) under strict national laws. Ruminal tissue samples were obtained from culled animals and ethical approval obtained for their use from UCD Animal Research Ethics Committee's (AR-EC-E-18-28). Initial investigation of the rumen papillae revealed that animals culled in January had significantly longer papillae (8.4 +/- 1.0 mm, N=5) than those culled just after the rutting season in November (5.3 +/- 0.7 mm, N=12) (p=0.0270, Unpaired T-test) [NOTE: All values are mean +/- S.E.]. In contrast, western blotting analysis showed that there was no significant difference in the abundance of UT-B2 transporters between these two groups (22 +/- 3, N=4, versus 34 +/- 9, N=4) (p=0.2416, Unpaired T-test). Adult males that had displayed consistent begging behaviour to obtain food from human visitors to the park had a higher papillae density (54 +/- 5 per cm2, N=4) than non-begging adult males (40 +/- 2 per cm2, N=7) (p=0.0128, Unpaired T-test). Furthermore, these animals had a significantly higher UT-B2 transporter to total protein abundance ratio (0.56 +/- 0.10, N=3, versus 0.13 +/- 0.04, N=3) (p=0.0183, Unpaired T-test). This increase was also shown, qualitatively by immunolocalization studies, to be predominantly in the stratum basale layer of the begging animals' rumen papillae (N=3). The findings of this novel study therefore improve our understanding of basic rumen physiological processes, but also add insight into the profound unseen effects that humans feeding wildlife may have.

PCB041

Regular exercise ameliorates anxiety and memory dysfunction due to combined oral contraceptive treatment in female rats: role of allopregnanolone and the vagus nerve

Tulin Altinoluk[1], Zarife Nigar Ozdemir Kumral[1], Ece Bakiryol[1], Elif Pamukbasanoglu[1], İpek Ayata[1], Bersun Turan[1], Damla Anil Celebi[2], Dilek Akakin[2], Berrak Yegen[1], Alper Yildirim[1]

[1]Marmara University School of Medicine, Department of Physiology, Istanbul, Turkey, [2]Marmara University School of Medicine, Department of Histology and Embryology, Istanbul, Turkey

The combined oral contraceptives (COCs) are a mixture of synthetic oestrogen and progestogen. In contrary to beneficial action of COCs in protecting uterine endometrium, a growing number of studies indicate an increased risk of cardiovascular, psychophysiological, and cognitive side effects through a reduction in vagal tone. These side effects are mediated by the catalysation of progesterone into allopregnanolone, which is known to attenuate vagal neurotransmission within brain stem (1). It is widely accepted that physical exercise stimulates the vagus nerve by a non-pharmacological intervention (2). Our objective in this study was to investigate the underlying mechanism in the therapeutic action of exercise as a vagal stimulator in COC-induced side effects that include anxiety, memory dysfunction, and cardiovascular damage. Adult female Sprague-Dawley rats were randomly divided into 4 groups as control, exercise, non-exercised COC and exercised COC groups (n=8 each). The treadmill exercise protocol required 25 m/min, 1 h/day, 3 days/week, for a total of 1 0 weeks, which corresponds to 60% of the maximum aerobic power (3). The groups received vehicle (distilled water, p.o.) or COC containing levonorgestrel and ethinylestradiol (1.0 µg/kg/day and 5.0 µg/kg/day, respectively) for 10 weeks. At the end of the 10th week, passive avoidance test (PAT) was performed to evaluate 72 h memory retention. Spatial memory was measured using the Y-maze. The hole-board test was performed to evaluate anxiety level. Vagal tone was assessed by heart rate variability (HRV) under an irreversible urethane anaesthesia (1.2 g/kg), after which the contraction / relaxation responses of the aortic rings were obtained with carbachol following precontraction with phenylephrine. Serum and brain tissues were obtained to measure allopregnanolone level by ELISA. The data were analysed using one-way ANOVA followed by post hoc Tukey test. The non-parametric Kruskal-Wallis test was applied when appropriate. $p < 0.05$ was considered statistically significant. Serum allopregnanolone level was increased in the exercised COC group ($p<0.05$); however, brain tissue allopregnanolone levels were not different among experimental groups. The non-exercised COC group demonstrated a significant memory impairment in PAT as compared to the control group ($p<0.05$), which was accompanied by memory deficits in the Y-maze score ($p<0.05$). Despite that PAT score was increased in the exercised COC group, it was not statistically significant. Anxiety was increased in the COC group as compared to non-treated exercise group ($p<0.01$). The lowest HRV scores were measured in the non-exercised-COC group, without reaching statistical significance. The aorta strips of all treatment groups showed decreased contractile responses to phenylephrine when compared to control aortae ($p<0.001$), while relaxation responses were similar in all groups. Although COC treatment did not diminish cardiovascular functions, it increased anxiety and thereby inhibited exploratory behaviour. When added to COC treatment, exercise tended to increase allopregnanolone, which could have a role in increasing the vagal tone. Taken together, more research is needed to elucidate how exercise influences the metabolism of neurosteroids and brain stem autonomic circuits.

1. Littlejohn EL, Fedorchak S, Boychuk CR. Sex-steroid-dependent plasticity of brain-stem autonomic circuits. Am J Physiol Regul Integr Comp Physiol. 2020 Jul 1;319(1):R60-R68. 2. Daniela, Matei et al. “Effects of Exercise Training on the Autonomic Nervous System with a Focus on Anti-Inflammatory and Antioxidants Effects.” Antioxidants (Basel, Switzerland) vol. 11,2 350. 10 Feb. 2022. 3. Brooks GA, White TP. Determination of metabolic and heart rate responses of rats to treadmill exercise. J Appl Physiol. 1978;45(6):1009–15.

PCB042

The reproducibility of dendritic cell and T cell counts to a 30-minute high-intensity cycling protocol as a tool to highlight overtraining

Carla Baker[1], John Hough[1], Jessica Piasecki[1], John Hunt[1]

[1]Nottingham Trent University, Nottignham, United Kingdom

Introduction: Intensified training and sufficient recovery is required to improve athletic performance. Heavy training has been reported as immunosuppressive in athletes, possibly due to reduced immunosurveillance with increased exercise stress. A maladaptation of the hypothalamic pituitary adrenal (HPA) axis has been shown after periods of intensified training; specifically, a blunted cortisol response to an exercise stress test has been reported. Activation of the HPA axis elevates dendritic cell (DCs) numbers. DCs are key antigen presenting cells that present antigen to T cells to initiate the required immune response. The link between training stress, the HPA axis and DCs has yet to be examined. Before investigating whether DCs become dysfunctional with intensified training, a reproducible response of these cells to an exercise stress test must be confirmed. Reproducible cortisol responses to alternating blocks of 1-minute cycling at 55% maximum power output (W_{max}) and 4-minutes cycling at 80%W_{max} for 30-minutes (55/80) have been shown, and 20-minutes cycling at 80%VO_{2max} can elevate total DCs numbers. The 55/80 is based on percentage of work rate maximum, but large differences in homeostatic perturbations i.e. O_2 uptake kinetics and blood lactate responses are likely between participants in the 55/80. Therefore, submaximal anchors such as the ventilatory threshold (VT_1) should be used to prescribe intensity. **Aim:** Therefore, this study aims to assess the reproducibility of the DC and T cell responses to an adapted version of the 55/80 utilising VT_1 to prescribe intensity. **Methods:** 12 healthy males (age, 26.4 ± 5.8 years; VO_{2peak}, 48.58 ± 7.14 ml/kg/min) cycled for 1-minute at a work rate 20% below VT_1 and 4-minutes at 50% between VT_1 and VO_{2max} for 30-minutes (20/50 exercise test) with blood samples pre, post and 30-minutes post. This was repeated on two occasions, 2-7 days apart and at the same time of day. Using flow cytometry, total DCs were defined at Lineage– (CD3, CD19, CD20, CD14, CD56) HLA-DR+ and subsequently identified as plasmacytoid (CD11c- CD123+) (pDCs) or myeloid (CD11c+ CD123-) (mDCs). T-helper cells were identified as being CD3+CD4+ and T-cytotoxic cells were identified as being CD3+CD8+. Two-way repeated measures ANOVAs were used for all variables apart from pDCs which were analysed via a Wilcoxon signed-rank test for main effects of trial and post-hoc time effects, and a Friedmans test for main effect of time. **Results:** No significant effect of trial ($P > 0.05$) or interaction effects ($P > 0.05$) were found for any variable. A significant main effect of time for all variables were found with immune cell counts increasing from pre- to post-exercise and decreasing to baseline 30-minutes post-exercise ($P < 0.001$), apart from pDCs which remained elevated 30-minutes after exercise. Intraclass correlation coefficients showed excellent reliability for CD3+ T cells, CD8+ T cells and pDC responses between trials (ICC >0.75) and good reliability for Total DCs, mDCs and CD4+ T cells (ICC 0.6-0.74). **Conclusions:** These results suggest that the 20/50 exercise test induces reproducible DC and T-cell count changes, which, implemented before and after a period of intensified training, may highlight the negative states of overtraining.

PCB043

The Role of Oestrogen in Female Skeletal Muscle Ageing: A Systematic Review

Annabel Critchlow[1], Danielle Hiam[1], Ross Williams[1], David Scott[1], Séverine Lamon[1]

[1]School of Exercise and Nutrition Sciences, Institute for Physical Activity and Nutrition (IPAN), Deakin University, Geelong, Australia

Ageing is associated with a loss of skeletal muscle mass and function that negatively impacts the independence and quality of life of older individuals. Females demonstrate a distinct pattern of muscle ageing compared to males, potentially due to menopause where endogenous sex hormone production declines. This systematic review aims to investigate the current knowledge about the role of oestrogen in female skeletal muscle ageing. A systematic search of MEDLINE complete, Global Health, Embase, PubMed, SPORTDiscus, and CINHAL was completed from inception to 08/11/2022. The systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines and was registered in the international prospective register of systematic reviews (PROSPERO) (CRD42022374366). Studies were considered eligible if they compared a state of oestrogen deficiency (e.g. postmenopausal females) or supplementation (e.g. oestrogen replacement therapy) to normal oestrogen conditions (e.g. premenopausal females or no supplementation). Outcome variables of interest included measures of skeletal muscle mass, function, damage/repair, and energy metabolism. Quality assessment was completed with the relevant Johanna Briggs critical appraisal tool, and data were synthesised in a narrative manner. Thirty-two studies were included in the review. Nineteen studies (59%) had a low risk, 10 studies (31%) had a moderate risk, and three studies had a high risk (9%) of bias. Seventeen studies compared skeletal muscle outcomes in females across different menopausal stages. Overall, they showed that compared to premenopausal females, postmenopausal females display reduced muscle mass and strength, but the effect of menopause on markers of muscle damage and expression of the genes involved in metabolic signalling pathways remains unclear. Of 10 studies that investigated the effect of oestrogen supplementation, some suggest a beneficial effect of oestrogen replacement therapy on muscle size and strength, but evidence is largely conflicting and inconclusive, potentially due to large variations in the reporting and status of exposure and outcomes. The findings from this review points toward a potential negative effect of oestrogen deficiency in ageing skeletal muscle, but further mechanistic evidence is needed to clarify its role.

N/A

PCB044

Prevalence and translation of assessment and putative treatments for Long Covid-19 into clinical practice

Avijit Datta[1]

[1]*York and Scarborough Teaching Hospitals NHS Foundation Trust, York, United Kingdom*

INTRODUCTION

Long COVID-19 (LC) is defined as the persistent symptoms ≥12 weeks following acute COVID-19 infection. The Office for National Statistics, ONS, estimated that there are 2M self-reported UK patients with (LC) ie 3% population (REF1).

METHODS & RESULTS

We currently have 400 adult patients in our teaching hospital NHS trust (which serves a population of 800 000) being managed with LC ie 0.0005% and another 120 on the waiting list. Applied nationwide, this suggests that only a small proportion of self-reported LC cases have the benefit of Hospital-based treatment. Raised BMI, associated type 2 diabetes, anxiety-depression and asthma were common co-morbidities as was seen in the national study.

A recent analysis by Subramaniam et al (2022) of national GP records of 486,149 non-hospitalized patients with some of 62 possible recorded LC symptoms during the first two surges of the pandemic produced three symptom cluster classes. The three classes were heterogenous symptoms, respiratory and anxiety-depression. Mean age was 43.8 years (s.d. 16.9), 55.3% of participants were female. 64.7% were white, 12.2% were Asian origin, 4.0% were Black Afro-Caribbean; 16.2% had missing ethnicity data. 53.8% were overweight or obese (BMI data missing for 13.0%), and 22.5% were current smokers (smoking data missing for 4.3%).

Separately, three leading (but not mutually exclusive) hypotheses and some putative treatments for each have been proposed (Couzin Frankel, 2022) :

1. Microvascular blood clots (identified by single photon emission computed tomography SPECT-CT or Hyperpolarised hyperpolarized xenon 129 MRI (XeMRI) to identify alveolar capillary diffusion limitation - DOAC anticoagulants
2. Persistent virus-antiviral therapy
3. An aberrant immune system - antihistamines

The results from the available clinical trials of treatment are awaited. Stimulate ICP. (Symptoms, Trajectory, Inequalities and Management: Understanding Long-COVID to Address and Transform Existing Integrated Care Pathways) tests the effectiveness of repurposed drugs (participants are allocated to (1) usual care, (2) famotidine/loratadine antihistamines, (3) Colchicine anti-inflammatory or (4) Rivaroxaban DOAC anticoagulant groups for 3 months). to treat long COVID. The effects of 3 months of treatment is measured on peoples' symptoms, mental health and other outcomes in patients attending 6-10 UK LC clinics. Cluster randomisation is at level of primary care networks so that integrated care pathway interventions are delivered as "standard of care" in that area. (Forshaw et al 2023)

CONCLUSIONS

Given the paucity of patients being referred to hospital, the majority of patients do not have ready access to advanced diagnostic techniques, associated tailored putative treatments or the above clinical trials. Underpowered clinical trials have in the past failed to provide reliable results applicable to the general population.

New approaches using analysis of real-world data of vastly larger patient numbers combined with machine learning may deliver reliable results faster in the future. Such initiatives are in line with UK government ambitions to provide more services out of hospitals, a larger primary care workforce and greater integration with social care, so that care is more joined up to meet people's physical health, mental health and social care needs.

1. Estimates of the prevalence of self-reported long COVID and associated activity limitation, using UK Coronavirus (COVID-19) Infection Survey data. (ONS July 2022) 2. Subramaniam A et al (2022) Symptoms and risk factors for long COVID in non-hospitalized adults. Nature Medicine volume 28, pages1706–1714 3. Couzin-Frankel J (2022) Clues to Long Covid. Science 376, Issue 6599. 4. Forshaw D et al (2023) STIMULATE-ICP: A pragmatic, multi-centre, cluster randomised trial of an integrated care pathway with a nested, Phase III, open label, adaptive platform randomised drug trial in individuals with Long COVID: A structured protocol . PLoS ONE. 2023. Vol. 18(2). DOI: 10.1371/journal.pone.0272472

PCB045

Orai Ca2+ channels but not Ano1 or L-type Ca2+ channels contribute to adrenergic contractions of male mouse urethral smooth muscle

Neha Gupta[1], Caoimhin Griffin[2], Mark Hollywood[3], Keith Thornbury[4], Gerard Sergeant[5], Bernard Drumm[6]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland, [2]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland, [3]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland, [4]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland, [5]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland, [6]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland

Ca^{2+} dependent contractions of urethral smooth muscle (USM) prevent leakage of urine during bladder filling. In rabbit and pig, USM contraction relies on Ca^{2+} influx through L-type Ca^{2+} channels (Brading, 2006), and in rabbit this requires USM depolarization via Ca^{2+} activated-Cl^- channels (Ano1) (Fedigan *et al.*, 2017). Studies in mice demonstrated L-type channel inhibitors do not affect phenylephrine (PE) responses in male USM, but store-operated-Ca^{2+}-entry (SOCE) via Orai channels was critical (Drumm *et al.*, 2018). However, this study only examined L-type channel inhibitors on responses to supramaximal PE doses (10 μM). Thus, contributions of L-type or Ano1 channels by lower (more physiologically relevant) PE concentrations or nerve stimulation cannot be ruled out. We sought to examine potential roles for L-type and Ano1 channels in regulating male mouse USM across a range of PE concentrations and electrical field stimulation (EFS) frequencies. Intact USM rings from wildtype male C57 mice were mounted on an isometric force transducer inside a heated (37^{o}C) organ bath, perfused with oxygenated Krebs solution. Contractions and relaxations of USM were monitored using LabScribe. Tissues were stretched to an initial 2 mN of tension and equilibrated for 1 hour before experimentation. EFS was delivered via two platinum electrodes either side of USM rings, at 1, 2, 5, 10, 20 Hz for 30 sec. USM rings contracted in response to PE (30nM – 30 μM) in a concentration dependent manner, with an EC_{50} of 1.3 μM (n=60). EFS in the presence of L-NNA (nNOS synthase inhibitor), to prevent relaxations of USM due to nitric oxide release, evoked contractions whose amplitude was frequency dependent, with 20 Hz EFS evoking responses 80% larger than those at 2 Hz (n=113). The L-type channel activator FPL 64176 (300 nM) slightly (but significantly) increased the area under the curve (AUC) of PE-induced contractions (e.g. 1 μM PE AUC increased 24.8% in FPL, $P<0.05$, n=6), without affecting EC_{50}. Similarly, EFS response amplitude was increased by FPL, e.g., contractions at 10 Hz increased 10% (n=6, $P<0.05$). FPL effects were reversed by the L-type channel inhibitor nifedipine (1 μM, n=6, $P<0.05$), but nifedipine failed to significantly affect control PE or EFS responses (n=6, $P<0.05$) at any PE concentration or EFS frequency. In contrast, the Orai Ca^{2+} channel inhibitor GSK 7975A (10 μM), reduced EFS-induced contraction amplitude by 50% at all frequencies (n=13, $P<0.005$). Upon subsequent addition of nifedipine in the continued presence of GSK 7975A, there was a further 10 - 20% reduction in residual EFS-induced contractions (n=13, $p<0.05$). The Ano1 channel activator Eact (10 μM) and antagonist Ani9 (3 μM) failed to affect PE dose-response curves or EFS responses at any frequency (n=6, $P>0.05$). Furthermore, nifedipine sensitive components of EFS-induced responses (unmasked when Orai channels were inhibited) were unaffected by Ani9 (n=6, $P>0.05$). In conclusion, in male USM, L-type channels can be activated by appropriate agonists (FPL) but inhibition of these channels does

not affect PE or EFS responses under normal conditions. Ca^{2+} influx via SOCE is the dominant Ca^{2+} influx pathway required for male USM contraction.

Brading AF. (2006). Spontaneous activity of lower urinary tract smooth muscles: correlation between ion channels and tissue function. The Journal of physiology 570, 13-22. Drumm BT, Rembetski BE, Cobine CA, Baker SA, Sergeant GP, Hollywood MA, Thornbury KD & Sanders KM. (2018). Ca2+ signalling in mouse urethral smooth muscle in situ: role of Ca2+ stores and Ca2+ influx mechanisms. Journal of Physiology 596, 1433-1466. Fedigan S, Bradley E, Webb T, Large RJ, Hollywood MA, Thornbury KD, McHale NG & Sergeant GP. (2017). Effects of new-generation TMEM16A inhibitors on calcium-activated chloride currents in rabbit urethral interstitial cells of Cajal. Pflugers Archiv European Journal of Physiology 469, 1443-1455.

PCB046

The impact of indoor carbon dioxide on human cognition and the physiological response

Stefan Flagner[1,2,5], Steffen Kuenn[2], Thomas Meissner[3], Guy Plasqui[1]

[1]Department of Nutrition and Movement Sciences, Faculty of Health Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands, [2]Department for Macro, International & Labour Economics, School of Business and Economics, Maastricht University, Maastricht, Netherlands, [3]Department of Microeconomics and Public Economics, School of Business and Economics, Maastricht University, Maastricht, Netherlands, [4]Department of Nutrition and Movement Sciences, Faculty of Health Medicine and Life Sciences,, Maastricht, Netherlands, [5]Department of Finance, School of Business and Economics, Maastricht, Netherlands

Modern buildings often have automatized ventilation systems to reduce energy consumption, which leads to a higher air tightness of the building envelope and limit the control humans have over the ventilation. Therefore, there is an increasing research field on the impact of indoor air quality on human cognition and health. The indoor concentration of carbon dioxide is of particular interest because it can be used as a proxy for indoor air quality. However, past studies suggested that carbon dioxide itself can be a contributor to impaired cognition, but the findings are inconclusive due to several limitations [1]. Either a carbon dioxide exposure level higher than usually occurring indoors was used or the exposure time was short. Furthermore, studies differ in their applied cognition tests.

To cover these limitations, this interdisciplinary study used a cross-over design, in which 20 healthy office workers were exposed to two test days of eight hours to either 800 ppm carbon dioxide or 3,000 ppm carbon dioxide in a respiration chamber. Volatile Organic Compounds were filtered out from the air. Cognitive performance was measured using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Additionally, multiple price lists from economics literature were used to measure the risk and time preferences when faced with a financial decision-making problem [2]. A heuristics battery from psychology literature was used to measure sensitivity to bias behaviour in decision-making [3]. Lastly, subjects' satisfaction about the indoor air quality and their belief to which degree the air quality hinders their ability to answer the questionnaire was recorded on a 7-point Likert scale. Physiological parameters including oxygen consumption, heart rate, heart rate variability, respiration rate, blood carbon dioxide concentration, blood pressure, and skin temperature were measured continuously to investigate possible mechanisms. A linear model with group fixed effects on test subject and clustering of standard errors on the subject level was used. As robustness checks, the same linear model but with bootstrapped standard errors was estimated and a mixed linear model with a random intercept on the subject and a random slope on the carbon dioxide condition was applied. Multiple hypotheses testing has been applied to derive corrected p-values [4].

The statistical analysis indicated no significant effect of carbon dioxide on any test results of either the CANTAB test battery, the economic preferences, or the heuristics battery after applying multiple hypotheses testing. Furthermore, no significant effect on the physiological parameters could be found after correcting for multiple hypotheses testing. The same insignificance was derived using the other regression models in the robustness checks. Also, there was no significant difference in satisfaction levels with the air quality. However, subjects rated that in the high carbon dioxide condition the air quality hindered them less to answer the questionnaire ($p < 0.05$, after multiple hypotheses testing).

We could not replicate the negative effects of pure carbon dioxide exposure found in past studies. It seems that carbon dioxide is not causing the impaired cognition. The absence of a physiological response cannot confirm any adaptive behaviour which could explain why cognition is unaffected.

[1] Du, B., Tandoc, M. C., Mack, M. L., & Siegel, J. A. (2020). Indoor CO2 concentrations and cognitive function: A critical review. Indoor Air, 30(6), 1067–1082. [2] Falk, A., Becker, A., Dohmen, T. J., Huffman, D., & Sunde, U. (2016). The preference survey module: A validated instrument for measuring risk, time, and social preferences. [3] Toplak, M. E., West, R. F., & Stanovich, K. E. (2011). The Cognitive Reflection Test as a predictor of performance on heuristics-and-biases tasks. Mem Cogn, 39, 1275–1289. [4] Hommel, G. (1988). A stagewise rejective multiple test procedure based on a modified Bonferroni test. Biometrika, 75(2), 383–386. https://doi.org/10.1093/biomet/75.2.383

PCB047

The effects of β-GPA on resistance training adaptations in rat skeletal muscle

Naoki Fukao[1], Junya Takegaki[2], Ryo Takagi[3], Koki Okumura[4], Satoshi Fujita[5]

[1]Graduate School of Sport and Science, Ritsumeikan University, Kusatsu, Japan, [2]Research Organization of Science and Technology, Ritsumeikan University, Kusatsu, Japan, [3]Research Organization of Science and Technology, Ritsumeikan University, Kusatsu, Japan, [4]Graduate School of Sport and Science, Ritsumeikan University, Kusatsu, Japan, [5]Faculty of Sport and Health Science, Ritsumeikan University, Kusatsu, Japan

Resistance training induces not only muscle hypertrophy but also mitochondrial adaptation (Kitaoka et al. 2016). However, it is also known that resistance training-induced mitochondrial adaptation is limited and smaller than endurance training-induced mitochondrial adaptation (Wilkinson et al. 2008).
β-guanidinopropionic acid (β-GPA) inhibits creatine transport into the cell and decreases creatine in skeletal muscle. Previous studies have reported that β-GPA activates mitochondrial biogenesis and increases mitochondrial content markers in skeletal muscle (Williams et al. 2009).
However, the effects of β-GPA on resistance training are not clear. Therefore, the aim of this study was to investigate the effects of β-GPA on resistance training adaptation in rat skeletal muscle.

This experiment was approved by the Ethics Committee for Animal Experiments at Ritsumeikan University (BKC2022-009). 8-week-old male SD rats were randomized to (1) placebo group or (2) β-GPA group (n = 5/group). β-GPA (1000 mg/kg) was administered once daily in the β-GPA group by oral ingestion by a sonde. Resistance exercise was performed according to a previous study (Takegaki et al. 2019). Briefly, the ankle joint was positioned at 90 degrees, and a 3-second maximal isometric contraction was performed 10 times with a 7-second interval (1 set), for a total of 5 sets. Resistance exercise was performed only on the right gastrocnemius muscle and the left gastrocnemius muscle was treated as a resting sample. During exercise, rats were anesthetized with 2% concentration of isoflurane. Resistance exercise was performed for a total of 12 sessions, and 48 hours after the last exercise session, rats were anesthetized and gastrocnemius muscles were removed. Western blotting was used to evaluate the expression levels of proteins involved in mitochondrial biogenesis. Two-way ANOVA was used for statistical analysis, and multiple comparisons were made only when an interaction was observed. Unpaired t-test was only used for the analysis of the % change in muscle mass.

A significant interaction was found for protein expression levels of PGC-1α, a key regulator of mitochondrial biogenesis ($p < 0.05$). Multiple comparisons showed that PGC-1α protein expression was significantly higher in β-GPA + exercised leg (+82.5% vs placebo + rested leg) than in the placebo + exercised leg (+34.3% vs placebo + rested leg) ($p < 0.05$). A significant interaction was also observed in total OXPHOS (Complex I-V) protein expression, markers of mitochondrial content ($p < 0.05$). Multiple comparisons showed that total OXPHOS protein expression in β-GPA + exercised leg (+61.8% vs placebo + rested leg) was significantly higher than in exercised leg (+33.0% vs placebo + rested leg) ($p < 0.05$). Main effects of training and β-GPA were observed in muscle wet weight ($p < 0.05$). The % change in muscle mass with resistance training was significantly lower in β-GPA group (+3.92%) than in placebo group (+9.92%) ($p < 0.05$).

The study suggests that β-GPA, a creatine inhibitor, enhances resistance training-induced mitochondrial biogenesis. Furthermore, β-GPA can attenuate, but not completely abolish, resistance training-induced gains in muscle mass.

Takegaki et al. (2019). Biochem Biophys Res Commun, 520(1), 73–78. Wilkinson et al. (2008). J Physiol, 586(15), 3701–3717. Williams et al. (2009). Am J Physiol Endocrinol Metab, 296(6), E1400–E1408. Kitaoka et al. (2016). J Applied Physiology, 121(3), 806–810.

PCB048

The effect of electrical pulse stimulation, an in vitro model of exercise, on metabolic characteristics of differentiated primary human muscle cells: comparison of two different protocols

Klára Gabrišová[1], Tímea Kurdiová[1], Natália Pálešová[2], Jozef Ukropec[1], Barbara Ukropcová[1,3]

[1]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia, [3]Department of Pathological Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction: Regular physical activity is capable of improving and maintaining human health. However, the molecular mechanisms of exercise-induced adaptations are still not fully understood. Electrical pulse stimulation (EPS) is used to induce visually detectable contractions of differentiated muscle cells, myotubes, to mimic exercise *in vitro*.

Aim: Our aim was to identify the EPS protocol that can induce significant physiological response in cultured human primary myotubes, by comparing the effectiveness of two EPS protocols, with 1. continuous, and 2. intermittent stimulation.

Materials and methods: Differentiated primary human skeletal muscle cells derived from healthy, lean men (n=3, 31±2.45 yrs, 23,72±0.82 kg/m^2) were exposed to two EPS (Ionoptix, USA) protocols: (i) commonly used 24h continuous stimulation (frequency 1Hz, pulse duration 2ms), and (ii) 24h intermittent stimulation, where higher frequency stimulation (5Hz, pulse duration 2ms) is followed by subthreshold stimulation (0.2Hz, pulse duration 4 ms). This cycle was repeated 9 times in 24h period. Oxidation of radioactively labeled ^{14}C-glucose and ^{14}C-palmitate, incorporation of ^{14}C-glucose into glycogen, changes in content of mitochondrial respiratory chain proteins (western blot) and in fiber-type specific gene markers (qPCR) were determined. Control cells were exposed to electrodes without EPS. Data are presented as average±SEM and differences were analyzed with paired T-test (GraphPad Prism 9.4.1).

Results: Both types of EPS led to visually detectable contractions of myotubes and facilitated the incorporation of glucose into glycogen (continuous stimulation: 19.72±5.09 pmol/3h/µg, p=0.0063; intermittent stimulation: 17.97±4.88 pmol/3h/µg, p=0.0051 vs. control: 15.14±4.79 pmol/3h/µg, n=3). However, oxidative glucose utilization tended to increase only after the intermittent stimulation (intermittent stimulation: 9.41±1.76 pmol/3h/µg, vs. control: 7.58±1.77 pmol/3h/µg, p=0.0835, n=3). Intermittent stimulation also led to significantly increased total glucose disposal (intermittent stimulation: 27.38±6.49 pmol/3h/µg, vs. control: 22.72±6.30 pmol/3h/µg, p=0.0031, n=3), while after continuous stimulation we observed only a trend (continuous stimulation: 26.48±6.93 pmol/3h/µg, vs. control: 22.72±6.30 pmol/3h/µg, p=0.0744, n=3). Importantly, we observed a 20% increase in total fatty acid oxidation in cells exposed to intermittent stimulation (intermittent stimulation: 983.5±149.582 pmol/3h/mg, vs. control: 799.6±98.8 pmol/3h/mg, p=0.0669, n=4), while continuous stimulation did not induce significant change due to high response variability (continuous stimulation: 1067.4±249.0 pmol/3h/mg, , vs. control: 799.6±98.8 pmol/3h/mg, p=0.33, n=4). There were no changes in the protein content of respiratory chain complexes in response to two protocols (n=4, p>0.1). However, there was an increase in mRNA for MYH2 (marker of fast IIa fibers) specifically after continuous stimulation (continuous stimulation: 45.33±5.16 AU, vs. control: 27.342±4.34 AU, p=0.0356, n=4).

Conclusion: Electrical pulse stimulation is *in vitro* model used for studying exercise-related adaptative mechanisms in muscle cells. We identified intermittent EPS as more effective in inducing relevant physiological changes in metabolism, as exemplified by an improvement in glucose and palmitate oxidation.

Ethical approval: The study was approved by the ethics committee of the University Hospital Bratislava, Comenius University Bratislava and the Ethics Committee of the Bratislava Region Office and conforms to the ethical guidelines of the Declaration of Helsinki. All participants provided witnessed written informed consent prior to entering the study.

PCB049

Whole-body cardiopulmonary fitness and local skeletal muscle function in people living with Long COVID compared with healthy controls

Alexandra Jamieson[1], Ronan Astin[2], Lamia Al Saikhan[7], Robert Bell[3], Mark Hamer[4], Lee Hamill Howes[1], Toby Hillman[5], Michele Orini[1], Helen Purcell[5], Marie Scully[6], Melissa Heightman[5], Thomas Treibel[1], Hugh Montgomery[2], Nishi Chaturvedi[1], Alun Hughes[1], Siana Jones[1]

[1]MRC Unit for Lifelong Health and Ageing, UCL, London, United Kingdom, [2]Centre for Human Health and Ageing, UCL, London, United Kingdom, [3]Hatter Cardiovascular Institute, UCL, London, United Kingdom, [4]Division of Surgery and Interventional Science, UCL, London, United Kingdom, [5]Department of Respiratory Medicine, UCLH, London, United Kingdom, [6]Department of Haematology, London, United Kingdom, [7]Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Introduction
The mechanisms underlying symptoms of persistent fatigue and severe exercise intolerance which can follow SARS-CoV-2 infection, termed 'Long COVID' (LC), are not fully understood. The objective of this study was to compare whole-body cardiopulmonary fitness ($\dot{V}O_2$) and local skeletal muscle measures of oxygen consumption ($mus\dot{V}O_2$), oxidative capacity (τ) and microvascular reactivity (post-occlusive reactive hyperaemia (PORH)) between individuals with LC and healthy controls (HC).

Methods
Participants with LC were recruited from the University College London Hospital (UCLH) Long COVID Clinic and HC from students and staff at University College London. Cardiopulmonary fitness was measured using analysis of expired gases during a sub-maximal (85% of predicted maximum heart rate) ramp cardiopulmonary exercise test (CPET) performed on a semi-recumbent cycle ergometer. Near Infrared Spectroscopy (NIRS) was applied to the gastrocnemius in combination with arterial occlusions and a short bout of muscle contractions for assessment of local skeletal muscle oxygen consumption ($mus\dot{V}O_2$), oxidative capacity (recovery of $mus\dot{V}O_2$, τ) and microvascular time to 95% PORH. Descriptive statistics are presented as n(%) and mean±standard deviation. Outcome measures were compared between LC and HC using potential outcome means (POMs) calculated by an augmented inverse probability weighted estimator with a linear outcome model and logit treatment model. Estimates were adjusted for potential confounders (age, sex and ethnicity) and are summarised as POM(95% confidence intervals). The level of significance was set at $p<0.05$.

Results
Analysis includes 32 adults (10(31%) men, 44±12 years old) with LC and 19 HC (6(32%) men, 40±13 years old). In patients with LC, cardiopulmonary fitness was lower ($\dot{V}O_2$ at Anaerobic Threshold (AT): 12.8(11.7,13.9) versus 16.3(15.0,17.5) ml/Kg/min, $p<0.001$; AT: 47.7(44.1,51.2) versus 56.6(53.1,60.0) % of predicted $\dot{V}O_2$max, $p<0.001$; $\dot{V}O_2$ Work Rate: 8.5(8.1,9.0) versus 9.4(9.0,9.8) ml/min/W, $p=0.008$), oxidative capacity was poorer (τ: 38.7(31.9,45.6) versus 24.6(19.1,30.1) seconds, $p=0.001$) and resting $mus\dot{V}O_2$ was lower (0.11(0.08,0.15) versus 0.15(0.12,0.18) μM/s, $p=0.09$) compared to healthy controls. There were no observed

differences for time to 95% PORH between the groups (28.0(23.7,32.3) versus 27.3(22.3,32.4) seconds, p=0.86).

Conclusion

Results from this study suggest that, compared to healthy controls, individuals living with Long COVID have lower whole-body cardiopulmonary fitness and lower local skeletal muscle measures of oxygen utilisation and oxidative capacity but similar skeletal muscle microvascular function.

PCB050

Sex differences in the effect of physical activity throughout adolescence on VO2max in early adulthood

Siana Jones[1], Michele Orini[1], Chloe Park[1], Nishi Chaturvedi[1], Alun Hughes[1]

[1]MRC unit for Lifelong Health and Ageing, University College London, London, United Kingdom

Motivation

Low cardiopulmonary fitness (CPF) predicts future morbidity in adults. Participation in physical activity (PA), particularly moderate to vigorous physical activity (MVPA), can improve CPF but the impact of MVPA throughout adolescence on CPF in early adult life remains unclear and potential differences in this association between men and women have not previously been quantified. We investigate sex-differences in the impact of MVPA throughout adolescence on early adult CRF.

Methods

Participants enrolled in a UK birth cohort (*The Avon Longitudinal Study of Parents and Children*, ALSPAC) undertook measures of physical activity at 11, 13, 15 and 24 years (y) old using a hip-worn accelerometer. Time in MVPA (average minutes/day) was derived from accelerometer data at each time. Cumulative MVPA participation was calculated as a life-time average across all measurement time points. CPF (VO_2max) was estimated from a Tecumseh step test at age 24. Ethical clearance for all procedures was granted by the ALSPAC Law and Ethics Committee and the Local Research Ethics Committee and all participants provided written and informed consent.

Structural equation modelling was used to compare associations between MVPA measured at each time and VO_2max at age 24 within the same model and cumulative MVPA participation and VO_2max. Direct and indirect effects of MVPA at each time point on VO_2max are presented. The full information maximum likelihood method was used to account for missingness under the assumption of missing at random. Maternal socioeconomic group was included in models as a predictor of missing observations. Skewed data were log transformed. Analysis was sex-stratified.

Results

Participants who undertook the step test at 24 and had undertaken at least two PA measurements during adolescence were included in this analysis (n=1347 (476 men)). Cumulative MVPA was positively associated with VO_2max in both men and women, but the association was slightly stronger for men (Table 1). In men, after adjustment for MVPA at each measurement time (direct effects), only MVPA at 24 remained strongly associated with VO_2max at 24 (Table 1). In women, we observed strong direct effects of MVPA at age 13 and age 24 on VO_2max at 24 (Table 1).

Conclusion

Cumulative MVPA participation throughout adolescence is an important determinant of CRF in early adulthood. These data also suggest that MVPA in early adolescence is an important determinant of early adult CPF in women independent from MVPA participation in early adulthood.

PCB051

Evaluation of the Vapor Phase Toxicity of the Thyme Essential Oil and Thymol in Fibroblasts and Lung Tumor Cells.

Jacqueline Gonçalves Santos[1], Marilene Biavatti[2], Carla Cristine Kanunfre[2]

[1]Federal University of Paraná (UFPR), Curitiba/PR, Brazil, [2]State University of Ponta Grossa (UEPG), Ponta Grossa/PR, Brazil

Essential oils (EO) are source of compounds with several pharmacological activities, which due to their volatile components can be directly delivered to the lung tissue by inhalation, an interesting characteristic for the lung cancer treatment. The species *Thymus vulgaris* L. (thyme) has been used for a long time in traditional medicine due to its therapeutic potential. In addition, the antitumor activity of thyme EO has already been demonstrated *in vitro* and *in vivo* assays. The main component of thyme EO, thymol and ρ-cymene, also present the cytotoxic activity against cancer tumor cell lines, but the effects of the vapor phase remains unknown. The aim of this study was to evaluate the cytotoxicity of the vapor phase of the *Thymus vulgaris* L. (thyme) EO and thymol, on the lung cancer cells A549 and H292, as well on the MRC-5 line, a model of lung fibroblast non-tumor. To perform the treatments with the vapor phase, was used the methodological strategy described by Seal *et al*. (2012) with some modifications. Cells were seeded ($1x10^4$ cells/well) in the 8 central wells of 24-well plates. In the 16 wells free from cells, was added 1 mL of solubilized thyme EO (62.5-1000 µg/mL) or thymol (31.25-500 µg/mL) diluted in different concentrations. The cells were treated 48 h. The cytotoxicity was assessed by MTT reduction and Sulforhodamine B staining assays and expressed as concentration capable of generating vapor to generate 50% reduction of cell viability (ICV_{50}). The chemical analysis of thyme EO by CG/MS confirmed thymol (41.84%) as the major compound. Both OE and thymol were cytotoxic to the three cell lines in a dose dependent manner, however thymol showed greater toxicity. The A549 cells were more responsive than H292. The ICV_{50} calculated to thyme OE for A549 was 305 µg/mL and to thymol was 150 µg/mL, for the H292 it was 648 µg and 138 µg/mL, respectively. The vapor phase of the thymol was less toxic for the non-tumor cells (MRC-5), with the selectivity index (SI) equal to or greater than 2. To evaluate the effect of thymol in association with radiation, A549 cells were treated for 6 hours with thymol (125 µg/mL) before exposure to 9 Gray (Gy) of radiation. After 48 hours, cell viability assays were performed. The vapor phase of thymol reduced the viability of tumor cells in synergy with the radiation. The AO/EB staining (fluorescent microscopy) revealed that the association of treatments (thymol + IR) resulted in cell death by necrosis. In the group control (cells irradiated) was observed also necrotic cells, however a high number of apoptotic cells were found. Corroborating with these results, the caspase assay (CellEvent™ Caspase-3/7 Green Detection Reagent) showed a high activation in only control group. The results indicate that the cytotoxicity of the vapor phase of the thyme OE is due mainly to its major compound, thymol, which was more cytotoxic and more selective than the thyme OE, and it could be a great adjunct when used in association with radiation.

Seal, S et al. (2012). PLoS ONE, 7(10), 1–11.

PCB052

The impact of a mixed exercise countermeasure on muscle proteomic dynamics and muscle atrophy in active healthy older adults undergoing a 14-day head-down tilt bedrest

Jean-Christophe Lagacé[1], Philippe St-Martin[1], Mathil Ruel[1], Jamie S. McPhee[2], Karen Lambert[3], Eléonor Riesco[1], Isabelle J. Dionne[1]

[1]Faculty of Physical Activity Sciences & Research Centre on Aging, Université de Sherbrooke, Sherbrooke, Canada, [2]Department of Sport and Exercise Sciences & Institute of Sport, Manchester Metropolitan University, Manchester, United Kingdom, [3]PhyMedExp, Université de Montpellier, INSERM, CNRS, Montpellier, France

Introduction Prolonged muscle disuse, as is often observed in long-term hospitalization, can have deleterious effects on muscle health. The aging population, which is more prone to lengthy hospital stays, has shown an increased risk of suffering from the impact of bedrest and presents greater rate of muscle atrophy and muscle weakness. An exercise countermeasure therefore presents itself as a prime solution to mitigate the effect of bedrest. Yet, little to no studies have investigated the impact of an exercise countermeasure on muscle atrophy, strength loss and muscle proteome dynamics in an aging population subjected to prolonged muscle disuse.

Methods We therefore sought to verify how a mixed exercise program affects muscle health and performances in active older adults aged 55 to 65 (n=20, 9 men and 11 women) subjected to 14 days of continuous head-down tilt bedrest. Half of the participants engaged in a daily mixed exercise program for an hour in addition to receiving passive mobilizations, while the other half received only passive mobilizations. Total muscle mass was estimated with DXA, and muscle volume and fat infiltration were measured with MRI. Knee extensor strength was measured isometrically with Biodex system at a 90° angle. To better understand the mechanisms at play, muscle biopsies were taken on the outer part of the quadriceps muscle on day 1, 3, 8 and 14 of bedrest and muscle proteomics analyses were done by tandem mass spectrometry. Finally, inflammatory markers (TNF-α, IL-6, IL-8), myostatin, and heat-shock protein 27 and 72 were measured in plasma before and after bedrest. Follow-up measurements were repeated after 4 weeks and 4 months. The effect of time and group, and their interaction was verified with mixed-effect models.

Preliminary results 14 days of continuous head-down bedrest induced changes in knee extensor strength in both group ($p<0.001$) without a group-by-time interaction ($p=0.37$). Total lean mass estimated with DXA decreased after bedrest ($p=0.004$) and returned to normal after 4 weeks without a group effect ($p=0.93$). MRI however showed a group-by-time interaction for changes in quadriceps muscle volume (Change from baseline to end of bedrest: Control: -6.2% CI [-8.3, -4.0%]; Exercise: -0.8% CI [-2.5, 0.9%], $p<0.01$), with exercise mitigating the impact of bedrest. So far, the changes in protein abundance between day 1 and 14 of bedrest are related to biological processes of innate immunity and inflammation. These changes in protein abundance were however not reflected in systemic inflammation as there was no change from baseline in IL-6, IL-8 or TNF-α throughout the duration of the study ($p\geq0.2$ for all).

Conclusion Altogether, our preliminary analyses show that engaging in an hour of mixed exercise everyday mitigated the effect of 14 days of head-down bedrest on quadriceps muscle volume. It did not, however, counter the loss of strength. Additional analyses will be done in

muscle biopsies and in plasma to better understand the mechanisms at play and full data will be presented at the Physiological Society Conference.

Ethical statement Participants gave their informed and written consent before participating in this study.

PCB053

Influence of anxiety, breathing vigilance and respiratory muscle strength on respiratory load perception

William Scott[1], Christos Aristidou[1], Michael I Polkey[2], Timothy O Jenkins[1,2], Vicky MacBean[1]

[1]Brunel University London, London, United Kingdom, [2]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom

Introduction: Respiratory effort perception is a complex phenomenon, essential to maintenance of homeostasis, and is influenced by a number of factors. Anxiety is associated with greater breathlessness in clinical populations. Anxiety and vigilance towards respiratory sensations have previously been shown to be inter-related but have not yet been explored in a controlled laboratory setting. Perceived breathing effort is often viewed in the context of load-capacity balance, but the influence of the absolute magnitude of pressure generation is under-explored.

Methods: Maximum inspiratory pressure (PImax) was measured with a differential pressure transducer during a sustained maximal inspiratory effort against an occlusion and calculated as the greatest one-second mean pressure. Breathing vigilance and anxiety were assessed prior to loading using the Breathing Vigilance Questionnaire (BVQ) and State-Trait Anxiety Inventory (STAI).

Inspiratory threshold loading was applied at 30%, 50% and 80% of each participant's PImax using an inspiratory muscle training device (POWERbreathe Plus IMT). A "sham" load at 4cmH_2O was delivered using an inverted Philips Threshold PEP device.

Each load was applied for twelve breaths, with five minutes of tidal breathing between loads. Order was randomised. Participants rated breathing difficulty using a 100mm visual analogue scale (VAS-D) immediately after each load. Friedman's ANOVA with Dunn's post hoc test using Bonferroni correction for multiple comparisons was used to examine differences in VAS-D at each load. Linear mixed effects modelling (LMM) was used to quantify the relationship between load and VAS-D, and the influence of BVQ score, STAI score and PImax.

Results: Thirty healthy adults (eighteen female) were studied (median (IQR) age 32.0 (24.3 – 44.5) years, mean (SD) PImax 119 (48)cmH_2O. Mean (SD) BVQ score was 10 (4), STAI-state was 24 (7), STAI-trait was 32 (6). Only three and seven participants respectively scored above the accepted threshold of 37 for "no or low anxiety".

Median (IQR) VAS-D varied with IMT dose (Baseline: 4 (0 – 10)mm, sham 11 (3 – 18)mm, 30% 29 (12 – 54)mm, 50% 41 (28 – 66)mm, 80% 73 (47 – 94mm), $p<0.001$). Individual values are shown in Table 1. On post hoc testing, all VAS-D values differed significantly from one another (p values 0.048 to <0.001), with the exception of baseline *versus* sham and 30% *versus* 50% ($p=1.00$ and $p=0.604$ respectively).

LMM showed a significant ($p<0.001$) relationship between VAS-D and load: slope (95% confidence interval) 0.72 (0.61 – 0.83)mm/%PImax. Neither BVQ nor STAI score influenced this relationship significantly. PImax significantly influenced the load-perception relationship: slope

(95% CI) of load *versus* VAS-D in the combined model 0.37 (0.09 – 0.65)mm/%PImax, p=0.01), additional influence of baseline PImax 0.003 (0.001 – 0.005)mm/%PImax/cmH_2O, p=0.009.

Conclusions: Perceived difficulty of breathing increases with applied threshold load. In a population with low state and trait anxiety and low levels of breathing vigilance, this relationship is not modulated by anxiety or breathing vigilance scores. Underlying respiratory muscle strength does however exert a significant relationship on load perception, suggesting that absolute magnitude of imposed load in addition to the fraction of the individual's capacity determines response to sensory feedback from the respiratory system.

PCB054

Lower Limbs Motor Index (LLIMI-DXA): a standardisation parameter of muscle mass in professional football players

Antonio Molina López[1], Heliodoro Moya Amaya[1], Antonio Jesús Berral Aguilar[1], Daniel Rojano Ortega[1], Pedro Estevan Navarro[1], José Naranjo Orellana[1], Francisco José Berral de la Rosa[1]

[1]Universidad Pablo de Olavide, Sevilla, Spain

Introduction

Currently, the somatotype of elite football players has become towards a greater mesomorphic and ectomorphic component[1]. In the UEFA Expert Group Statement[2] research, the range of fat mass has been established by Dual Photon X-ray Absorptiometry (DXA) between 8 and 13%, but no reference values for lean mass have been calculated. At present, there is a lack of useful reference parameters for setting lean mass targets in lower limbs of professional football players[3,4].

For this reason, the aim is to obtain lower limb lean mass reference ranges for this elite population.

Methods

The total sample consisted of 31 elite football players (Table 1), from the first team of Udinese Calcio (Italy).

Table 1. Characteristics of the players evaluated. n=31.

Variable	Mean ± SD
Age (years)	26,11 ± 4,73
Weight (kg)	82,31 ± 8,39
Height (cm)	184,76 ± 7,65

Full body DXA analysis was performed with a GE Healthcare Lunar iDXA device during the competitive stage of the 21/22 and 22/23 seasons, gathering a total of 298 measurements. Injured players were excluded from the total sample.

Lower limb lean mass was assessed using the index: lower limb lean mass/height^2 which we have named LLIMI-DXA.

This work was approved by the Ethics Committee of the Pablo de Olavide University.

Results

In our group we obtained mean reference values of the LLIMI-DXA index of 8.13 ± 0.86 kg/m2. Approximately 70% of the players are in the range (x±SD) of 7.27-8.99 kg/m2. Approximately 96% of our players are in the range (x±2SD) of 6.41-9.85 kg/m2. The evolution of this index during the 21/22 and 22/23 seasons is shown in table 2.

Table 2. DXA measurements season 21/22 and 22/23. n=31.

	LLIMI-DXA Evolution			
Period	**July-August**	**September to December**	**January - February**	**March to May**
Number of measures	62	128	91	17
LLIMI-DXA **Mean ± SD**	8,07 ± 0,87	8,12 ± 0,84	8,15 ± 0,89	8,41 ± 0,80

LLIMI-DXA: Lean mass/stature index^2 by dual photon X-ray absorptiometry on lower limbs. Total number of DXA measurements: 298

Conclusions

The LLIMI-DXA index has proved very useful in our elite football players for monitoring the evolution of the lean mass of the lower limbs during the season, so we consider this index to be very useful for analysis by the technical and health staff of a professional football club. Given that this is a novel index not evaluated in other teams, our data from the interval in which 70% of the players are found can serve as a reference for adequate values of this index, while the second interval can serve as a reference for a more exhaustive monitoring of muscle mass.

However, it would be advisable for each club to obtain the average values of the LLIMI-DXA index in order to be able to compare and analyse their athletes.

1. Moya-Amaya H, Molina-López A, Berral-Aguilar AJ, Rojano-Ortega D, Berral-de la Rosa FJ (2022). Somatotype Migration in Professional Soccer Players Over the Last Decades. Int J Morphol;40(2):327-33. https://doi.org/10.4067/S0717-95022022000200327. 2. Ofenheimer A, Breyer-Kohansal R, Hartl S, Burghuber OC, Krach F, Schrott A, Wouters EFM, Franssen FME, Breyer MK (2020). Reference values of body composition parameters and visceral adipose tissue (VAT) by DXA in adults aged 18-81 years-results from the LEAD cohort. Eur J Clin Nutr. Aug;74(8):1181-1191. doi: 10.1038/s41430-020-0596-5. 3. Collins J, Maughan RJ, Gleeson M, Bilsborough J, Jeukendrup A, Morton JP, et al. (2021). UEFA expert group statement on nutrition in elite football. Current evidence to inform practical recommendations and guide future research. Br J Sports Med;55(8):416. https://doi.org/10.1136/bjsports-2019-101961. 4. Sebastiá-Rico, J.; Soriano, J.M.; González-Gálvez, N.; Martínez-Sanz, JM (2023). Body Composition of Male Professional Soccer Players Using Different Measurement Methods: A Systematic Review and Meta-Analysis. Nutrients, 15, 1160. https://doi.org/10.3390/nu15051160

PCB056

The protective mechanisms of Osteoprotegerin on C2C12 myogenic differentiation.

Aaron Owens[1,2], Lívia Dos Santos[1], Mathew Piasecki[2], John Hunt[1], Jessica Piasecki[1]

[1]Nottingham Trent University, Nottingham, United Kingdom, [2]University of Nottingham, Nottingham, United Kingdom

Skeletal muscle mass progressively decreases with advancing age, increasing the risk of developing Sarcopenia, characterised by low appendicular muscle mass (Dufresne., 2016; Ageing and health., 2022). This currently poses a significant cost to global healthcare systems. Low skeletal muscle mass is more likely to cause a loss of independence and, ultimately, a reduced longevity. Osteoprotegerin (OPG), a decoy receptor for RANK/RANKL is known to prevent bone resorption within the bone remodelling cycle, yet it has been suggested to exhibit protective effects on dystrophic skeletal muscle in animal models (Dufresne et al., 2015).

To further understand the potential of OPG as a therapeutic target to combat age related muscle loss, a better mechanistic insight is required. Therefore, we aimed to identify if OPG exhibits protective effects in C2C12 myoblasts in the presence of TNFα, used to represent the inflammatory response associated with ageing, having previously shown deleterious properties in C2C12 cells. To achieve this aim we had 3 objectives 1; Ascertain concentrations of both TNFα and OPG that will exhibit observable effects on C2C12 myogenic differentiation, 2; develop a protocol to grow and treat differentiating C2C12 myoblasts with OPG and TNFα and, 3; treat C2C12 myotubes with TNFα and OPG, simultaneously, in order to examine any effect OPG may exhibit on differentiation parameters.

C2C12 myoblasts were seeded at a density of 5000 cells/cm^2 in growth media (GM) and grown until confluent. A titration of OPG and TNFα was conducted, concluding with 30ng/ml^{-1} TNFα and 20ng/ml^{-1} OPG as the most effective treatment doses for differentiation. Cells were then seeded in 24 well plates and grown to confluence in GM (n=4) after which GM was replaced with Differentiation Media (DM). Experimental treatments were then added at 0 and 24 hours of incubation to observe OPGs effects on the formation of myotubes with TNFα present. Mean myotube diameter and mean number of myonuclei per myotube were measured as differentiation measurement parameters. Mixed effects analysis of variance and One-way ANOVA tests were used and significance accepted at $p<0.05$.

Between controls (DM) and groups treated with TNFα and OPG simultaneously, significant differences in mean myotube diameter and mean number of myonuclei/myotube were observed ($p<0.05$). In all conditions containing both TNFα and OPG, mean myotube diameter was non-significantly increased when compared to conditions containing only OPG or TNFα ($p>0.05$). Significant increases in mean number of myonuclei per myotube between groups treated with TNFα or OPG at 0h, and groups treated with TNFα or OPG at 0h followed by OPG or TNFα at 24h ($p<0.05$) were also observed (Figure 1). The data herein provides a sound, viable method for investigating the effects TNFα and OPG exhibit on differentiating C2C12 myotubes. In addition, OPG demonstrates protective effects on C2C12 differentiation in a model replicative of the ageing skeletal muscle. It is clear OPG has the potential to interact with bone and muscle, however, further insight is required to understand its mechanistic actions to confirm OPG as a therapeutic target to help overcome the musculoskeletal declines throughout the ageing process.

Ageing and health (2022). Available at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health (Accessed: 1 March 2023), Dufresne, S. et al. (2016) ‘Physiological role of receptor activator nuclear factor-kB (RANK) in denervation-induced muscle atrophy and dysfunction’, Receptors & Clinical Investigation. Available at: https://doi.org/10.14800/rci.1323., Dufresne, S.S. et al. (2015) ‘Osteoprotegerin protects against muscular dystrophy’, American Journal of Pathology, 185(4). Available at: https://doi.org/10.1016/j.ajpath.2015.01.006.

PCB057

End Stage Liver Disease is Associated with Increased Quadriceps Intermuscular Adipose Tissue Compared to Age and Sex Matched Controls

Jonathan Quinlan[1,2], Colette Bell[1], Armitpal Dhaliwal[1,2], Felicity Williams[1,2], Sophie Allen[1,2], Surabhi Choudhary[3], Alex Rowlands[4,5], Leigh Breen[1,2,6], Gareth Lavery[7], Janet Lord[1,2,6], Ahmed Elsharkawy[3], Matthew Armstrong[3], Carolyn Greig[1,2,6]

[1]University of Birmingham, Birmingham, United Kingdom, [2]NIHR Birmingham Biomedical Research Centre, Birmingham, United Kingdom, [3]University Hospitals Birmingham, Birmingham, United Kingdom, [4]NIHR Leicester Biomedical Research Centre, Leicester, United Kingdom, [5]Diabetes Research Centre, University of Leicester, Leicester, United Kingdom, [6]MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, University of Birmingham, Birmingham, United Kingdom, [7]Nottingham Trent University, Nottingham, United Kingdom

Background

The investigation of muscle quality is important to understand underlying muscle health and pathology in disease states, such as End Stage Liver Disease (ESLD). Myosteatosis, i.e., fat infiltration into skeletal muscle, is a key indicator of muscle quality and may negatively affect muscle function. Previous studies have shown that myosteatosis occurs in patients with non-alcoholic fatty liver disease[1]. However, most studies involving patients with ESLD have typically assessed myosteatosis in L3/L4 muscle groups[2,3]. Thus, an in-depth investigation of myosteatosis in the lower limbs of patients with ESLD, and in particular the quadriceps, remains to be completed.

Aims and objectives

The primary aim was to investigate whether quadriceps intermuscular adipose tissue (IMAT) differs between patients with ESLD and healthy age/sex-matched controls (HC), and whether IMAT differs based on anatomical location and/or quadriceps muscle head. A secondary aim was to explore the impact of IMAT on muscle function.

Methods

33 patients with ESLD (55.0±10.5 years) and 17 HC (49.6±15.4 years) participated in this observational study. Quadriceps IMAT was estimated at 20,40,50,60 and 80% of muscle length (distal = 0%) via Magnetic Resonance Imaging (MRI) Dixon technique. Vastus lateralis, vastus medialis, vastus intermedius, and rectus femoris IMAT was also calculated at 50% of muscle length. Bioelectrical impedance analysis and maximal knee extensor isokinetic assessments were also completed to assess body composition and muscle strength respectively. Finally, wrist worn accelerometers were worn for up to 14 days to assess habitual physical activity. The study was approved by the Health Research Authority - West Midlands Solihull (REC reference: 18/WM/0167) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. ClinicalTrials.gov identifier: NCT04734496.

Results

Two-way ANOVA showed a main effect of anatomical location ($P<0.0001$) and condition ($p<0.0001$), in addition to a significant interaction (i.e., location x condition) ($P<0.01$) for quadriceps IMAT. Šidák's post hoc comparison showed quadriceps IMAT was greater in ESLD at all anatomical locations compared to HC. Similarly, when comparing individual quadricep muscles, two-way ANOVA showed a main effect for muscle ($P<0.0001$) and condition ($P<0.0001$) with a significant interaction (i.e., muscle x condition) ($P<0.01$). Šidák's multiple comparisons revealed significant differences between quadriceps muscles in ESLD but not HC. Pearson r correlation showed significant positive correlations between quadriceps IMAT (at 50% muscle length) and BMI ($r=0.62$, $P<0.0001$, $n=50$), body fat percentage ($r=0.65$, $P<0.0001$, $n=50$) and age ($r=0.36$, $P<0.01$, $n=50$). In addition, negative correlations existed between quadriceps IMAT and both maximal knee extensor strength ($r=-0.50$, $P<0.001$, $n=50$) and habitual physical activity ($r=-0.51$, $P<0.001$, $n=42$).

Conclusions

Quadriceps IMAT is greater in patients with ESLD compared to HC, irrespective of the anatomical location or muscle analysed. Correlations suggest that quadriceps IMAT is positively associated with overall body fat percentage and BMI, and negatively associated with physical activity. Importantly, quadriceps IMAT may negatively impact muscle function and strength.

References 1: Linge et al, 2021. JHep 3:1, 100197 2: Montano-Loza et al, 2016. JCSM 7:2, 126-35 3: Bhanji et al, 2018. Hepatol. Int. 12;4, 377-86

PCB059

Modelling skeletal muscle ageing and repair in vitro

Janelle Tarum[1], Hans Degens[2], Mark Turner[3], Claire Stewart[4], Craig Sale[5], Livia Santos[1]

[1]Department of Sport Science, School of Science and Technology, Nottingham, United Kingdom, [2]Department of Life Sciences, Research Centre for Musculoskeletal Science & Sports Medicine, Manchester Metropolitan University, Manchester, United Kingdom, [3]Department of Biosciences, School of Science and Technology, University of Nottingham, Nottingham, United Kingdom, [4]Research Institute for Sport & Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom, [5]Institute of Sport, Manchester Metropolitan University, Manchester, United Kingdom

AIM: One of the hallmarks of ageing muscle is the decreased ability to regenerate. This has been attributed to dysfunctional satellite cells that can result in reduced myogenic capacity. Although reduced myogenic capacity can impair muscle regeneration, other factors including restricted muscle repair programming are also at play (1). Muscle regeneration mirrors foetal development and thus, we hypothesise that the transcriptome (the full range of messenger RNA molecules expressed by an organism) involved in muscle development is affected by ageing, resulting in a diminished regeneration potential. The aim of this study was to develop a high-throughput *in vitro* model enabling cellular and molecular investigations of muscle regeneration across the life course.

Methods: Myotubes, differentiated from human myoblasts from an older donor (male, aged 68 years; from Promocell) and a younger donor (male, aged 20 years; from Lonza), were injured after exposure to 12% barium chloride. Myotube repair was assessed by morphological analysis of myotube fusion and width, cell cycle and the transcriptome. For morphological analysis, the myotubes were stained with phalloidin and DAPI (4´,6-diamidino-2-phenylindole) to label the cytoskeleton and nuclei of muscle cells. This enabled us to estimate the fusion index and myotube width. The cell cycle was investigated using the EdU (5-ethynyl-2´-deoxyuridine) assay, which detects cells entering the S-phase (proliferative). In both morphological and proliferation assays, images were acquired with the Leica fluorescence microscope and analysed using ImageJ. Data were expressed as mean ± SEM. The transcriptome was assessed by RNA-seq. Timepoints for each analysis were pre-injury (control), post-injury, end of proliferation and end of differentiation (4 independent experiments). Statistical analyses of morphological and proliferation assays were performed using a two-sided unpaired t-test and RNA-seq using the DESeq2 R package (1.20.0).

Results: After repair, the fusion index (p=0.04) and myotube diameter (p=0.0008) were smaller in older myotubes compared to pre-injury (control). Younger myotubes exhibited a fusion index and width similar to their pre-injury state. With regards to the cell cycle, the number of EdU+ cells increased during the proliferation phase in myotubes derived from both young (p=0.00003) and aged (p=0.0008) myoblasts. Transcriptome analysis of older myotubes showed significant enrichment of the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway, PI3K-Akt signalling, and of GO (Gene Ontology) biological processes associated with muscle development and the extracellular matrix. Many of the genes involved in muscle cell development were downregulated (Figure 1). In young myotubes, the most overrepresented KEGG pathways were cytokine receptor interaction and protein digestion and the most enriched GO biological process were extracellular matrix-related processes.

Conclusion: This model provides a high-throughput platform enabling cellular and molecular investigations of muscle regeneration across the life course. As expected, older myotubes showed impaired regeneration as evidenced by reduced myofusion index and width after repair. We postulate that this is due to the down-regulation of genes involved in muscle development and function.

(1) Barberi L, Scicchitano BM, De Rossi M, Bigot A, Duguez S, Wielgosik A, et al. Age-dependent alteration in muscle regeneration: the critical role of tissue niche. Biogerontology 2013;14:273-292.

PCB060

Spectral decomposition of different pulse wave signals – a pilot study

Henrique Silva[1,2,3], Carlota Rezendes[4]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal, [2]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal, [3]Biophysics and Biomedical Engineering Institute (IBEB), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisbon, Portugal, [4]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal

Pulse wave analysis (PWA) is commonly employed for the calculation of a multitude of physiological parameters related to cardiac pumping mechanics, arterial stiffness and peripheral vascular resistance. These parameters have been increasingly used in the last decades for the assessment of cardiovascular risk, which highlights their usefulness. In recent years new analytic strategies have focused on the spectral decomposition of pulse wave signals for the assessment of the dynamics of cardiac autonomic regulation, the best example being pulse rate variability (PRV) analysis. Spectral decomposition of pulse wave signals also provides insight into the mechanisms (central and local) regulating tissue perfusion through the assessment of the relative contribution of the different frequency intervals of the signals over time. Although there are striking differences between pulse wave signals from different anatomical regions, few studies have attempted to compare them on the basis of their frequency spectra. This study aimed to compare the frequency spectra of pulse wave signals obtained from the neck and fingertip regions and their respective underlying mechanisms. Ten young healthy subjects (23.4 ± 4.9 y.o.; 6 females, 4 males) participated in this study after giving informed consent. Pulse wave signals were obtained with photoplethysmography (PPG) sensors placed over a random common carotid artery and over the pulp of the second finger of the ipsilateral upper limb. PPG signals were recorded for 10 minutes while subjects were sitting upright and performing a simple postural modification – 5 min with both arms at heart level (phase I) and 5 min with one random arm placed 40 cm below heart level (phase II). The wavelet transform was used to decompose the raw PPG signals into their different frequency regions (high, low and very low frequency). The amplitude ratio of each frequency region was assessed over time and compared between phases of the protocol, as well as between signals. Nonparametric statistics were employed and a $p<0.05$ was adopted. Significant differences in the amplitude ratio of the frequency intervals were identified between signals, highlighting their different physiological origin. Significant differences were also detected between the different phases, with the finger PPG signals showing more pronounced changes during the postural change when compared to the carotid signals. Although preliminary, our results show that the wavelet transform is a useful tool to provide a spectral decomposition of pulse wave signals from different anatomical regions. In addition, spectral analysis provides useful insights into the physiological origins of these signals.

PCB061

Suprasystolic limb occlusion and its impact on contralateral limb perfusion - an insight into flowmotion

Henrique Silva[1,2,3], Nicole Lavrador[4]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal, [2]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal, [3]Biophysics and Biomedical Engineering Institute (IBEB), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisbon, Portugal, [4]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal

Suprasystolic limb occlusion (SLO) is a common challenge for assessing endothelial activity and cardiovascular risk. It is commonly carried out by inflating a pneumatic pressure cuff above systolic pressure for a certain period of time, during which perfusion in distal territories decreases significantly. Upon cuff release perfusion increases producing a well-known reactive hyperemia. Although it remains underexplored, the contralateral limb also responds to SLO. Previous results from our group suggest that this contralateral response is a sympathetically-driven perfusion decrease. This study aims to further expand the knowledge on the physiological response to SLO by exploring the physiological mechanisms underlying the contralateral vascular response. Ten healthy male subjects (mean 20.2 ± 2.3 y.o.) participated in this study after giving informed written consent. After acclimatization, subjects performed a standard SLO protocol on a random upper limb while sitting upright, as follows: 10 min resting with both arms at heart level (phase I), 5 min random arm occlusion (200 mmHg, phase II) and 10 min recovery in the initial position (phase III). Photoplethysmography (PPG) signals were acquired from the second finger of the occluded (test) and non-occluded (control) arms and then decomposed into their main frequency components (cardiac, respiratory, myogenic, sympathetic, endothelial) with the wavelet transform (WT). The electrodermal activity (EDA) was also acquired from the third and fourth fingers of both hands. Nonparametric statistics were used for comparing the activity of each frequency between phases and arms ($p<0.05$). As previously reported, occlusion caused a significant decrease in cardiac, respiratory, myogenic and sympathetic activities together with a significant increase in NO-dependent and NO-independent endothelial activities in the test arm. The contralateral arm responded to occlusion with a significant decrease in perfusion, however no significant changes in the signal components. Nevertheless, the cardiac activity decreased, whereas the myogenic, sympathetic and endothelial NO-dependent increased. In contrast, the respiratory and endothelial NO-independent activities remained unchanged. Therefore, the perfusion decrease of the contralateral limb should be explained by the decrease of the cardiac and by the increase of the sympathetic activities. EDA increased significantly in both limbs during occlusion. These results show an overall agreement between EDA and the PPG sympathetic activity, reinforcing the usefulness of WT for assessing the mechanisms underlying perfusion regulation. They also highlight that only the non-occluded arm can be used for measuring the sympathetic nervous activity during SLO with decomposed PPG signals.

PCB062

Exploring the fractal behaviour of photoplethysmography perfusion signals

Henrique Silva[1,2,3], Nicole Lavrador[2]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal, [2]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal, [3]Biophysics and Biomedical Engineering Institute (IBEB), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016, Lisbon, Portugal, [4]Department of Pharmacy, Pharmacology and Health Technologies, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal

The regulation of blood flow to any organ results from a complex regulation from several mechanisms, both central and local. Several mathematical tools have contributed to the better understanding the interaction between these mechanisms by exploring more detailed features of perfusion signals. Fractality is one of such features and can be defined as the self-similarity of a signal at different scales. Photoplethysmography (PPG) is a non-invasive and low-cost technology that allows the recording of skin/muscle perfusion over time. Previous publications have shown that PPG signals result from the contribution of the different physiological activities that affect perfusion (cardiac, respiration, myogenic, sympathetic, endothelial), each with its specific frequency interval. This study aimed at characterizing the fractal behaviour of PPG signals obtained during a suprasystolic limb occlusion (SLO) protocol. Ten healthy male subjects (mean 20.2 ± 2.3 y.o.) participated in this study after giving informed written consent. After acclimatization, subjects performed a standard SLO protocol on a random upper limb while sitting upright, as follows: 10 min resting with both arms at heart level (phase I), 5 min random arm occlusion (200 mmHg, phase II) and 10 min recovery in the initial position (phase III). PPG signals recorded from the index finger of both occluded and non-occluded limbs. These signals were first decomposed into their respective frequencies with the wavelet transform. Then, both the raw signal and the components were processed with a detrended fluctuation analysis (DFA) algorithm and the alpha exponent was calculated for each phase. The Wilcoxon signed rank test was used for comparing the alpha exponents between phases and the Mann-Whitney test for independent samples for limb comparisons ($p<0.05$). The magnitude of alpha exponents increased with decreasing frequency of the PPG components. Furthermore, occlusion significantly changed the alpha exponents of the PPG signal and several of its components from the occluded limb. These results show that PPG perfusion signals exhibit fractal behaviour and that the DFA-derived alpha exponent could serve as new descriptor of perfusion regulation phenomena.

PCB063

Lifelong exposure to high-altitude hypoxia in humans is associated with improved redox homeostasis and structural-functional adaptations of the neurovascular unit

Benjamin Stacey[1], Ryan Hoiland[2,3], Hannah Caldwell[4], Connor Howe[4], Tyler Vermeulen[4], Michael Tymko[4,5,6], Gustavo Vizcardo-Galindo[7], Daniella Bermudez[7], Rómulo Figueroa-Mujíica[7], Christopher Gasho[8], Edouard Tuaillon[9], Christophe Hirtz[10], Sylvain Lehmann[10], Nicola Marchi[11], Hayato Tsukomoto[12], Francisco Villafuerte[7], Philip Ainslie[4], Damian Bailey[1]

[1]Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Pontypridd, United Kingdom, [2]Department of Anaesthesiology, Pharmacology and Therapeutics, Vancouver General Hospital, West 12th Avenue, University of British Columbia, Vancouver, Canada, [3]Department of Cellular and Physiological Sciences, Faculty of Medicine, University of British Columbia, Vancouver, Canada, [4]Centre for Heart, Lung and Vascular Health, University of British Columbia-Okanagan Campus, School of Health and Exercise Sciences, Kelwona, Canada, [5]Faculty of Kinesiology, Sport, & Recreation, University of Alberta, Edmonton, Canada, [6]Department of Medicine, Faculty of Medicine, University of British Columbia, Vancouver, Canada, [7]Laboratorio de Fisiología Comparada, Departamento de Ciencias Biológicas y Fisiológicas, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru, [8]Division of Pulmonary and Critical Care, Loma Linda University School of Medicine,, Loma Linda, CA, United States, [9]Department of Infectious Diseases, University of Montpellier, INSERM, Montpellier, France, [10]University of Montpellier, CHU of Montpellier, INSERM, Montpellier, France, [11]Laboratory of Cerebrovascular and Glia Research, Department of Neuroscience, Institute of Functional Genomics, University of Montpellier, Montpellier, France, [12]Faculty of Sport and Health Science, Ritsumeikan University,, Kusatsu, Shiga, Japan

Background: The neurovascular unit (NVU) is a functionally integrated cellular network responsible for maintaining structural integrity of the blood-brain barrier (BBB) and regulation of cerebral blood flow (CBF) via neurovascular coupling (NVC). Upon exposure to high-altitude (HA) in lowlanders born and bred at sea-level (SL), BBB integrity may become compromised due to autoregulatory breakthrough subsequent to local elevations in oxidative-nitrosative stress (OXNOS) reflected by a free radical-mediated reduction in vascular nitric oxide (NO) bioavailability (Bailey *et al*., 2009a; 2009b). Furthermore, MRI evidence of extracellular (vasogenic) edematous brain swelling (Kallenberg *et al*., 2007) combined with hemosiderin deposits (Kallenberg *et al*., 2008) implying erythrocyte extravasation, have been collectively interpreted to reflect BBB disruption (Bailey *et al*., 2009a), predisposing to impaired cerebral bioenergetic function and cognitive decline (Bailey *et al*., 2019). The aim of the present study was to determine how the hypoxia of HA across the temporal continuum of chronic through to lifelong exposure impacts the NVU phenotype and to what extent this is subject to altered redox homeostasis.

Methods: Nine male lowlanders were examined at SL (~344m) and after 14-days acclimatisation to 4,300 m (chronic HA) in Cerro de Pasco (CdP), Péru, alongside nine sex, age and body mass index-matched healthy highlanders native to CdP (lifelong-HA). Venous blood was assayed for serum proteins (S100B, neuron specific enolase [NSE], glial fibrillary acidic protein [GFAP], neurofilament light-chain [NFL], ubiquitin carboxy-terminal hydrolase-L1 [UCHL-1] and Total-tau [T-Tau]), reflecting NVU integrity via automated high-sensitivity clinical grade ELISA and single molecule array (Simoa) technology. Free radicals and NO were determined

using electron paramagnetic resonance spectroscopy and ozone-based chemiluminescence, respectively. Regional cerebral blood flow (CBF) was examined in conjunction with cerebral substrate delivery, dynamic cerebral autoregulation (dCA, transfer function analysis of spontaneous oscillations of middle/posterior cerebral artery blood velocity [MCA_V/PCA_V] and mean arterial blood pressure [MAP]), cerebrovascular reactivity to carbon dioxide (CVR_{CO2}, +9 mmHg end-tidal partial pressure of carbon dioxide) and NVC (PCA_V responses to visual stimulation) using Transcranial doppler (MCA_V/PCA_V) and Duplex ultrasound (internal carotid/vertebral artery blood flow: ICA_Q/VA_Q). Global cerebral blood flow (gCBF) was calculated as (ICA_Q + VA_Q) × 2, and substrate (oxygen/glucose) delivery as: gCBF × arterial oxygen content/glucose. Psychomotor tests and the Montreal Cognitive Assessment (MoCA) were employed to examine cognitive function.

Results: Compared to lowlanders at SL, highlanders exhibited elevated basal plasma and red blood cell NO bioavailability (P = 0.003 and P = 0.026, respectively), improved anterior and posterior dCA (↓MCA and PCA LF Gain, P = 0.029 and P = 0.017, respectively), elevated anterior CVR_{CO2} (↑MCA and ICA CVR_{CO2}, P = 0.036 and P = 0.042, respectively), preserved cerebral substrate delivery and NVC (all P = >0.050). In highlanders, S100B, NFL and T-tau were consistently lower (P = 0.018, P = 0.037 and P = <0.001, respectively) and cognition comparable all (P = >0.050) to lowlanders following chronic-HA.

Conclusions: These findings highlight novel integrated adaptations towards regulation of the NVU in highlanders that may represent a neuroprotective phenotype underpinning successful adaptation to the lifelong stress of HA hypoxia.

Bailey DM, Bartsch P, Knauth M & Baumgartner RW. (2009a). Emerging concepts in acute mountain sickness and high-altitude cerebral edema: from the molecular to the morphological. Cellular and Molecular Life Sciences 66, 3583-3594. Bailey DM, Taudorf S, Berg RM, Lundby C, McEneny J, Young IS, Evans KA, James PE, Shore A, Hullin DA, McCord JM, Pedersen BK & Moller K. (2009b). Increased cerebral output of free radicals during hypoxia: implications for acute mountain sickness? Am J Physiol Regul Integr Comp Physiol 297, R1283-1292. Bailey DM, Brugniaux JV, Filipponi T, Marley CJ, Stacey B, Soria R, Rimoldi SF, Cerny D, Rexhaj E, Pratali L, Salmon CS, Murillo Jauregui C, Villena M, Smirl JD, Ogoh S, Pietri S, Scherrer U & Sartori C. (2019). Exaggerated systemic oxidative-inflammatory-nitrosative stress in chronic mountain sickness is associated with cognitive decline and depression. J Physiol 597, 611-629. Kallenberg K, Bailey DM, Christ S, Mohr A, Roukens R, Menold E, Steiner T, Bartsch P & Knauth M. (2007). Magnetic resonance imaging evidence of cytotoxic cerebral edema in acute mountain sickness. J Cereb Blood Flow Metab 27, 1064-1071. Kallenberg K, Dehnert C, Dorfler A, Schellinger PD, Bailey DM, Knauth M & Bartsch PD. (2008). Microhemorrhages in nonfatal high-altitude cerebral edema. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 28, 1635-1642.

PCB064

The effect of hypoxia on physiological and behavioural outcomes during simulated driving in healthy subjects

Jaspreet Kaur[1], Michael Thynne[2], Feisal Subhan[1]

[1]School of Biomedical Sciences, Faculty of Health, University of Plymouth, PL4 8AA, Plymouth, United Kingdom, [2]Chest Clinic, University Hospitals Plymouth NHS Trust, PL6 8DH, Plymouth, United Kingdom

Hypoxia is a common condition mainly caused by neurological, cardiac and respiratory disease. Hypoxia is known to affect cognition and driving is dependent on satisfactory cognitive ability. Currently those who are prescribed supplemental oxygen have not been given any guidance from regulatory bodies on whether to use supplemental oxygen while driving. There is very limited research on the possible effects that hypoxia may have on persons whilst driving. This study investigates whether hypoxia influences driving behaviours in healthy subjects using simulated driving. Breathing frequency, oxygen saturation (SpO_2), heart rate variability (HRV) and subjective comments were also collated.

52 healthy subjects participated in this study with written informed consent. The study was approved by the Science & Engineering Ethical Committee, University of Plymouth and procedures were in accordance with the Declaration of Helsinki. Inclusion criteria were ≥18 years old and no history of cardiorespiratory or chronic disease. All subjects had their anthropometric and resting blood pressure, heart rate and SpO_2 data collected. Then they were attached to ECG electrodes, a chest plethysmograph, and an oximeter before they started the simulated driving on an Xbox 360 game console using Forza Horizon 4 software. Each subject had four driving sessions; a 10-minute practice and three randomised interventions: 20-minute normoxic room air (FIO_2 21%), 20-minute normoxic medical air (FIO_2 21%) and 20-minute hypoxic air (equal to 15% FIO_2). Driving behaviours (DB) were assessed by the sum of positive and negative scores for each session. HRV and breathing frequency were collected by using LabChart software. Short term HRV was assessed using time domain (heart rate - HR, standard deviation of the RR interval - SDRR), frequency domain (low and high frequency - LF and HF) and Poincaré analysis (SD1 and SD2). The results were statistically analysed in SPSS by repeated measures ANOVA. $p < 0.05$ was considered as significant.

HR ($p<0.0001$), SDRR ($p=0.03$), SD1 ($p<0.0001$), breathing rate ($p=0.01$) and SpO2 ($p<0.0001$) were all significantly different over the three gas interventions (n=52). LH, HF, DB all showed no significant difference. Pairwise comparisons showed that during hypoxia HR increased, while SDRR, SD1, breathing rate and SpO2 were lower, when compared to both normoxic interventions.

The main finding of our study was that hypoxia did not significantly affect simulated driving behaviours in our subjects. Therefore, we believe that the level of hypoxia (FiO2 15%) used in

the present study, may not have a great impact during driving. These findings add important significance for legislators and policy makers when making decisions with regard to the road safety of hypoxic patients who drive. Interestingly, HRV was negatively affected by hypoxia whilst driving and provides a starting point for conducting further research on the impact hypoxia may have on driving performance for patients with cardiovascular disease.

PCB066

Identification of a miRNA Signature as a Diagnostic and Prognostic Marker in Clear Cell Renal Cell Carcinoma

Muhammad Ammar Zahid[1], Abdelali Agouni[1]

[1]Department of Pharmaceutical Sciences, College of Pharmacy, QU Health, Qatar University, Doha, Qatar

Clear cell renal cell carcinoma (ccRCC) is the most common type of renal cell carcinoma and is associated with high morbidity and poor prognosis. Micro-RNAS (miRNAs) have emerged as promising biomarkers for cancer diagnosis and prognosis due to their involvement in cancer progression and development (Ghafouri-Fard et al., 2020). The integration of big omics data from GEO and TCGA, along with data mining and machine learning, has revolutionized the identification of reliable diagnostic and prognostic signatures for various types of cancer. The present study aims to identify a diagnostic and prognostic signature for ccRCC using miRNA data from microarray and NGS experiments in GEO and TCGA.

Differentially expressed miRNAs (DEmiRs) in ccRCC samples compared to normal renal tissue were identified using GEO2R packages in R, with adjusted $P<0.05$ and log2FC> 1.5 as cutoff criteria. The overlapping DEmiRs were identified, and the target genes of these DEmiRs with strong experimental validation were obtained using miRTargetLink 2.0 (Kern et al., 2021), and the pathway enrichment analysis was performed using ClusterProfiler package in R with KEGG annotation database (Wu et al., 2021). Kaplan-Maier (KM) survival analysis was performed to correlate the survival of patients with higher or lower expression of the identified miRNAs (Lánczky & Győrffy, 2021). A support vector machine model was trained and cross-validated to classify tumor samples from matched solid normal tissue samples.

Six datasets, namely GSE11016, GSE12105, GSE47582, GSE73342, GSE151423, and a dataset from TCGA were chosen for the analysis. Results revealed that 14 DEmiRs were consistently differentially expressed in RCC tissues in the microarray datasets, and 26 DEmiRs in the NGS datasets. We identified 9 mRNAs that exhibited a consistent expression trend across all datasets included in the study. We identified 637 genes as targets of the miRNAs under investigation. Pathway enrichment analysis demonstrated that these target genes were significantly enriched in several crucial pathways, including but not limited to AGE-RAGE signaling, MAPK signaling, cellular senescence, toll-like receptor signaling, TNF signaling, PD-L1 expression, and PD-1 checkpoint pathways. Survival analysis revealed that among the 9 signature miRNAs, higher expression of 4 and lower expression of 5 miRNAs were significantly associated with poor survival. Based on these findings, we hypothesize that these identified key miRNAs have the potential to serve as prognostic biomarkers for patients with ccRCC. Using the nine miRNAs identified earlier as features, we trained a support vector machine model on the TCGA dataset. The results of the 10-fold cross-validation demonstrated a high accuracy of 99.23+-0.89% and an AUC of 0.99+-0.007. These findings suggest that the model can accurately and reliably classify tumor samples from normal solid tissue samples.

In summary, this study has identified a nine-miRNA signature that is associated with poor survival outcomes in patients with ccRCC. Moreover, our machine learning model, based on this signature, is capable of distinguishing between tumors and normal tissue samples. Further

validation of this model in a clinical cohort would aid in translating our findings into clinical practice, potentially leading to earlier detection and improved follow-up care for ccRCC patients.

Ghafouri-Fard, S., Shirvani-Farsani, Z., Branicki, W., & Taheri, M. (2020). MicroRNA Signature in Renal Cell Carcinoma. Frontiers in Oncology, 10. Kern, F., Aparicio-Puerta, E., Li, Y., Fehlmann, T., Kehl, T., Wagner, V., Ray, K., Ludwig, N., Lenhof, H.-P., Meese, E., & Keller, A. (2021). miRTargetLink 2.0—Interactive miRNA target gene and target pathway networks. Nucleic Acids Research, 49(W1), W409–W416. Lánczky, A., & Győrffy, B. (2021). Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. Journal of Medical Internet Research, 23(7), e27633. Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X., & Yu, G. (2021). clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. The Innovation, 2(3).

PCB067

Effect of obesity on cognitive functions in school children

Nouralsalhin Alaagib[1], Alshima Ahmed[2]

[1]Assistant professor, Faculty of Medicine, Sulaiman Alrajhi University, Qassim, Saudi Arabia,
[2]Lecturer, Faculty of Medicine, Omdurman Islamic University., Omdurman, Sudan

Background: The prevalence of overweight and obesity in school children between 10-18 years old in Khartoum state in Sudan in 2010, was 10.8% and 9.7% respectively [1]. Obesity is a major risk factor of many health problems. Recently, researchers suggested that increased adiposity is associated with poor cognitive performance, independently of associated medical conditions [2]. This study aims to investigate the effect of obesity on cognitive function in primary school children.

Methods: This is a descriptive, cross sectional study that included 290 primary school children (150 boys and 140 girls) between 9 and 14 years old. Participants were chosen for the study using stratified multistage random sample technique from two of the biggest primary public schools in Omdurman city. Brief medical history was taken and general examination was done to exclude any abnormalities. Blood pressure (BP), weight and height were measured and body mass index (BMI) was calculated. Students were classified according to WHO BMI chart percentiles 2007 into: underweight (< 3rd), normal weight (≥3rd-< 85th), over weight (≥ 85th- < 97th), and obese (≥ 97th). Cognitive function was assessed using Mini-Mental State Examination Test (MMSE) [3].

Results: Results of the study showed that 15.51% (n= 45) of students were obese and 17.93% (n= 52) were overweight. The mean BMI was 18.95 ± 4.68 kg/m². BMI showed insignificant difference between the three socioeconomic status (P=0.538). Mild cognitive impairment was detected in 9.3% (n=27) of students, 0.7% (n=2) has moderate cognitive impairment. Only 2 out of the 45 (4.4%) obese students had mild cognitive impairment, and one (2.2%) had moderate cognitive impairment. The association between cognitive impairment and BMI was insignificant (P=0.098). Mother's education showed a significant positive association with language and praxis (P=0.027). Whereas father's educational level had correlated positively with orientation (P =0.001) and with MMSE test results (P =0.037).

Conclusion: Obesity seems to have insignificant effect on cognitive function in obese school children. Parent's educational background has a major effect on their children's cognition.

[1] Nagwa M et al. (2011). European journal of clinical nutrition 65(3):409-11. [2] Smith E et al. (2011). Obesity reviews 12(9):740-55. [3] Folstein MF et al. (1975). Journal of psychiatric research 12(3):189-98.

PCB068

Plasmodium berghei infection associated with adverse birth outcomes in pregnant Swiss albino mice

David audu[1], Olufunmilayo Ajoke Idowu[1], Adewumi Babatunde Idowu[1]

[1]Federal University of Agriculture, Abeokuta, Abeokuta, Nigeria

Introduction: Malaria in pregnancy has been seen to cause poor pregnancy and foetal outcomes.

Aims/Objective: In this study, mice infected with *Plasmodium berghei* (*P. berghei*) during the second and third stages of pregnancy were examined for their pregnancy's outcome and changes in their blood's biochemical composition after delivery. Additionally, the physical and behavioural reactions of the mice's pups were also investigated.

Methods: Thirty pregnant female Swiss Albino mice were randomly divided into three groups; two received intraperitoneal injections of 10^6 *P. berghei*-infected red blood cells on gestational days (GD12 and 17), while the third group was left uninfected (control). The study was conducted with the approval of the department of pure and applied Zoology, Federal University of Agriculture Abeokuta, Nigeria. This study also followed the national institute of health guide for using and caring for laboratory research animals (NIH publication 8023, revised 1978). Data were reported as means and standard errors, and analysis of variance was used to determine a significant difference from the control group at $p<0.05$.

Results: Pregnancy termination occurred in 20% of mice infected during GD12, whereas mortality before parturition occurred in 40% and 30% of mice infected during GD12 and GD17, respectively. Non-infected group's total protein and glucose concentrations were significantly higher ($p<0.05$), while cholesterol and triglyceride concentrations were significantly lower ($p<0.05$) when compared to the infected groups. The Mean birth weights (1.82 ± 0.37 g) of pups were higher ($p < 0.05$) in control mice compared to pups from infected groups. Offspring born to infected mothers exhibited poor physical and behavioural responses.

Conclusion: Mice infection by *P. berghei* during pregnancy resulted in adverse birth outcomes, altered measured biochemical parameters, poor physical and behavioural responses in their offspring and was more severe during the second stage of pregnancy.

PCB069

High-density lipoprotein protein composition differs between white Europeans and South Asians but is not related to its anti-inflammatory function

Jack Beazer[2], Stefan Ljunggren[3], Helen Karlsson[3], James McLaren[1], Jason Gill[1], Dilys Freeman[1]

[1]School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, United Kingdom, [2]School of Cardiovascular and Metabolic Health, College of Medical Veterinary and Life Science, University of Glasgow, Glasgow, United Kingdom, [3]Department of Health, Medical and Caring Sciences, Linköping University, Linköping, Sweden

Aims

Insulin resistance is known to alter high-density lipoprotein (HDL) composition and function (1). South Asians in the United Kingdom develop type 2 diabetes mellitus younger and at a leaner BMI than white Europeans(2). Our aim was to compare HDL composition and function with weight gain in young healthy white European (EU) and South Asian (SA) men.

Methods

White Europeans (n = 21) and South Asians (n = 14) were recruited with ethical approval from the University of Glasgow and NHS Greater Glasgow and Clyde according to the Declaration of Helsinki. These participants comprised the Glasgow Visceral & Ectopic Fat with Weight Gain in South Asians study (ClinicalTrials.gov Identifier: NCT02399423). Biochemical and anthropometric measures were taken at baseline and after ~7% weight gain over 6 weeks. Body fat distribution was measured by MRI. HDL was isolated from fasted plasma samples using sodium bromide sequential density ultracentrifugation. HDL apolipoprotein AI (ApoAI) was measured by ELISA. HDL cholesterol content was measured by commercial assay. HDL protein was measured by Bradford assay. HDL proteomics was performed by nano-liquid chromatography tandem mass spectrometry and protein levels expressed as a label free quantitation (LFQ) intensity. HDL anti-inflammatory function was assessed by measuring percentage inhibition of TNFα stimulated vascular cell adhesion molecule-1 expression in human microvascular endothelial cells. HDL was dosed onto cells at a concentration of 300 μg/mL apolipoprotein AI. Data was analysed using mixed effects models followed by post-hoc Tukey test. Statistical significance was considered at $p < 0.05$ and for interaction effects $p < 0.15$.

Results

HDL ApoAI, cholesterol and protein content were unchanged by ethnicity or weight gain. Of 50 proteins identified on HDL, 12 were higher in South Asians irrespective of weight gain, including apolipoprotein A-IV (EU, LFQ 2.22×10^7 [1.49×10^7, 3.53×10^7] SA, 3.63×10^7,[2.60×10^7, 4.94×10^7], p = 0.011, Median [Q1, Q3]). Five proteins had interaction effects where South Asians and white Europeans responded differently to weight gain, including apolipoprotein C-III, apolipoprotein D, apolipoprotein F and cholesteryl ester transfer protein (CETP) (Table 1). The change in apolipoprotein C-III LFQ intensity post weight gain negatively correlated with the change in liver fat fraction after weight gain (Spearman r -0.42, p = 0.023, Figure 1). HDL anti-

inflammatory function did not differ between white Europeans and South Asians or post weight gain.

Conclusion

South Asians have a markedly different HDL protein composition to white Europeans which responds differently to weight gain. However, this did not affect HDL anti-inflammatory function. HDL protein composition may therefore act as a biomarker of systemic pathophysiology, in this case impaired lipid metabolism with weight gain in South Asians. In this light, these findings suggest CETP inhibition and reduced triglyceride transfer to HDL, and increased lipase activity in South Asians. This may favour triglyceride transfer down the lipolytic pathway and into adipose tissue. The association between the change in HDL apolipoprotein C-III and liver fat fraction after weight gain offers a potential mechanism of ectopic lipid overspill in South Asians (3). This may contribute to ethnic differences in type 2 diabetes mellitus aetiology.

1. Beazer JD, Patanapirunhakit P, Gill JMR, Graham D, Karlsson H, Ljunggren S, et al. High-density lipoprotein's vascular protective functions in metabolic and cardiovascular disease – could extracellular vesicles be at play? Clin Sci. 2020;134(22):2977–86. 2. Mukhopadhyay B, Forouhi NG, Fisher BM, Kesson CM, Sattar N. A comparison of glycaemic and metabolic control over time among South Asian and European patients with Type 2 diabetes: Results from follow-up in a routine diabetes clinic. Diabet Med. 2006;23(1):94–8. 3. Iliodromiti S, McLaren J, Ghouri N, Miller MR, Dahlqvist Leinhard O, Linge J, et al. Liver, visceral and subcutaneous fat in men and women of South Asian and white European descent: a systematic review and meta-analysis of new and published data. Diabetologia. 2023;66(1):44–56.

PCB070

Activation of pancreatic stellate cells evokes signalling and metabolic changes promoting cellular resilience

Agnieszka Kusiak[1], Katarzyna Fryt[1], Kinga Stopa[2], Jacek Litewka[1], Ole Petersen[3], Monika Jakubowska[2], Pawel Ferdek[1]

[1]Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland, [2]Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland, [3]Cardiff School of Biosciences, Cardiff University, Cardiff, United Kingdom

Activated pancreatic stellate cells (PSCs) are involved in the excessive deposition of extracellular matrix (ECM) proteins resulting in the development of fibrosis in alcoholic pancreatitis or fibrotic stroma in pancreatic tumours [1]. In response to tissue injury, quiescent fibroblast-like PSCs undergo activation, that is assume a myofibroblast-like phenotype characterised by upregulation of α-SMA expression, increased contractile capacity and production of ECM components [2]. This phenotype transition significantly affects the physiology of PSCs, including changes in cell signalling and metabolism, which may have profound implications in diseases of the pancreas.

To investigate this, we used quiescent and TGF-β-activated (for 48 h or 7 days) human PSCs, in which we compared pathophysiological Ca^{2+} signals, mitochondrial potential and cell death in response to ethanol (EtOH) and palmitoleic acid (POA) – the major inducers of alcoholic pancreatitis. We also measured mitochondrial parameters using the Seahorse Cell Mito Stress Test and compared the expression of Ca^{2+} channels and pumps between the quiescent and activated phenotypes.

Our data show that, compared to quiescent PSCs, activated cells differ significantly, in terms of Ca^{2+} signalling and metabolic activity. Activated PSCs are much less prone to EtOH/POA-induced cytosolic Ca^{2+} overload and cell death, predominantly due to downregulation of the TRPA1 channel (a decrease to 17.4% and 23.2% in PSCs 48 h and 7 days post-activation, respectively) [3]. In quiescent PSCs, inhibition or silencing of TRPA1 expression reduces cytosolic Ca^{2+} responses to 50 mM EtOH / 50 μM POA (from 4342.5±486.9 a.u. to 1508.0±205.4 a.u., $p=0.0051$) and protects these cells from cell death (a decrease of cell death from 70.5% to 19.8%, $p=0.0006$), mimicking the activated phenotype. In addition, activated PSCs had their basal respiration, ATP production and spare respiratory capacity increased (by approx. 1.6x, 1.4x and 12-16x respectively), compared to quiescent cells. EtOH/POA disrupted the mitochondrial potential in quiescent PSCs (an average decrease of 231.9±15.8 a.u. below baseline levels), but this effect was inhibited in cells 7 days post-activation (30.1±8.0 au, $p<0.0001$). Activated PSCs were also less sensitive to menadione-induced oxidative stress compared to quiescent cells.

Our results reveal significant changes in Ca^{2+} signalling machinery and the condition of mitochondria between quiescent and activated PSCs. These changes are directly responsible for the increased resilience of activated PSCs to noxious signals, which likely allows them to divide and deposit collagen and other components of the ECM even under harsh pathophysiological conditions such as ongoing inflammation. Better understanding of the

activation-induced alterations in the cellular physiology of PSCs provides new insights into the mechanisms of pancreatic disorders, particularly those associated with fibrosis.

1. Ferdek PE & Jakubowska MA. (2017). Pflugers Arch 469, 1039-1050. 2. Ferdek PE et al. (2022). J Physiol 600, 2579-2612. 3. Kusiak AA et al. (2022). Cell Death Dis 13, 744.

PCB071

Neurogenic activation of lipolysis in white adipose tissue ex vivo

Kayleigh Goddard[1], Samuel J. Fountain[1]

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

Motivation White adipose tissue (WAT) is a major energy store, endocrine organ and is critical to metabolic homeostasis. Adipocytes release stored energy through lipolysis; the hydrolysis of stored triglycerides to liberate free fatty acids and glycerol. WAT is known to be innervated by sympathetic and sensory nerves [1], though the direct contribution of nerves in controlling lipolysis is currently unclear. It is well established that lipolysis is stimulated by catecholamines through activation of β-adrenergic receptors expressed by adipocytes [2]. Sympathetic nerves innervating WAT are a potential source of norepinephrine, but evidence of their involvement in lipolytic control is limited. Here we have developed a model to study the effects of nerve stimulation on lipolysis in WAT *ex vivo*.

Methods Adult male C57BL/6J mice (8-10 weeks) were sacrificed by CO_2 asphyxiation. Inguinal fat pads were identified and *Immunocytochemistry*: whole-mount immunocytochemistry was performed on whole fat pads, following previously described methods [3] Paraformaldehyde-fixed tissue was imaged by confocal microscopy. Nerves were stained with a chicken polyclonal antibody against β3-tubulin (1:500; Abcam), vasculature was stained with isolectin B4 (1:500; Invitrogen) and BODIPY used to visualise adipocytes. *Ex vivo lipolysis assay*: Sections (11 – 25mg) of inguinal fat pad were added to Dulbecco's Modified Eagle's medium containing 5.5 mM glucose and 2% (w/v) fatty acid-free bovine serum albumin. Tissue was challenged with pharmacological agents for 3 hours at 37°C, 5% CO_2 and 95% relative humidity. Media samples were removed to assay free glycerol as an indirect measurement of lipolysis. Glycerol was quantified by a colorimetric absorbance assay.

Results β3-tubulin immunoreactivity revealed nervous innervation throughout the inguinal fat pad (*N*=5), with nerve bundles commonly observed tracking the length of the tissue. Finer innervation was mostly restricted to innervating large/medium-sized blood vessels. Parenchymal nervous innervation of white adipose was also observed (*N*=3). In lipolysis assays, it was observed that glycerol was constitutively released over 3 hours (*N*=5). Statistical analysis was conducted by ANOVA with post-hoc Tukey tests.

Norepinephrine (2μM) stimulated glycerol production above levels of constitutive glycerol production ($P<0.01$; *N*=5). We next employed veratridine to stimulate nerves, a natural product that inhibits voltage-gated Na^+ channel inactivation, increasing nerve excitability. In these experiments, veratridine (100μM) stimulated glycerol production to the same level of norepinephrine ($P>0.05$; *N*=5). The effect of veratridine was abolished by tetrodotoxin (1μM; 30 mins preincubation) ($P<0.01$; N=5), with no significant difference observed from constitutive glycerol production ($P>0.05$; N=5).

Conclusions Our data reveal that white adipocytes and blood vessels of the mouse inguinal fat pad are innervated by nerves. Norepinephrine or pharmacologically increasing nervous excitability both stimulate lipolysis *ex vivo* in inguinal fat. Glycerol was also produced constitutively, suggesting basal lipolysis occurs in inguinal fat. Application of TTX was able to abolish veratridine-induced lipolysis to basal levels.

1. Blaszkiewicz et al., (2019). The Importance of Peripheral Nerves in Adipose Tissue for the Regulation of Energy Balance. Biology. 8, 10. 2. Frühbeck et al., (2014). Regulation of adipocyte lipolysis. Nutr. Res. Rev. 27, 63-93. 3. Willows et al., (2021). Visualization and analysis of whole depot adipose tissue neural innervation. Cell Press. 24, 10.

PCB072

Age-related differences in skeletal muscle fibre-specific mTOR-mediated signalling proteins via immunofluorescent microscopy

Marie Korzepa[1], Ryan Marshall[2], Benoit Smeuninx[3], Jonathan Quinlan[1], Paul Morgan[4], Leigh Breen[1]

[1]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom, [2]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom, [3]Cellular & Molecular Metabolism Laboratory, Monash Institute of Pharmacological Sciences, Monash University, Melbourne, Australia, [4]Department of Sport and Exercise Sciences, Institute of Sport, Manchester Metropolitan University, Manchester, United Kingdom

Background: Ageing results in a gradual loss of skeletal muscle mass and type II fibre atrophy, through dysregulated muscle protein synthesis (MPS). Traditional immunoblotting techniques have revealed dysregulated mTOR-mediated signalling as a mechanistic driver of impaired MPS in older adults. However, immunoblotting cannot determine fibre-type specific protein abundance, localisation, or translocation of these molecular regulators within cells. Immunofluorescence microscopy (IF) has the capacity to address the shortcomings associated with immunoblotting and uncover mechanisms of age-related MPS impairment and muscle decline. This study aimed to use IF to measure age-related and fibre-specific differences in the abundance and localisation of mTOR-mediated proteins.

Methods: Resting muscle biopsies from eight young males (YM; 24 ± 4 years, BMI; 24 ± 4 kg·m2) and seven older males (OM; 67 ± 5 years, BMI; 25 ± 2 kg·m2) were embedded in OCT compound, frozen in liquid nitrogen-cooled isopentane for IF subsequent analysis. Embedded muscle samples were cut using a microtome blade, and cryosections (7mm) were collected on room-temperature uncoated glass slides. Sections were fixed and incubated in MHC-I, Rheb, TSC2 or WGA, mTOR and Sestrin-2 primary antibodies and incubated in contrasting Alexa Fluor secondary antibodies. Images were captured at 10x for fibre type distribution and 20x for analysis of fibre-type immunofluorescent abundance. Full ethical approval was granted (18/EM/0004) and procedures were conducted in accordance with the Declaration of Helsinki.

Results: OM displayed a significantly (P=0.0151) lower proportion of type I fibres than YM (YM, 57.6 ± 0.6% vs OM, 47.6 ± 2.8%). Mean CSA was not different between groups for type I (YM, 4745.8 ± 98.1 mm2 vs OM, 6133.0 ± 408.6 mm2) or type II fibres (YM, 6556.7 ± 196.0 mm2 vs OM, 5752.3 ± 246.8 mm2). Notably, a 2-fold higher abundance of TSC2 in OM compared with YM (YM, 12.71 ± 0.28 A.U. vs OM, 25.45 ± 0.64 A.U.; P<0.001) was observed. OM displayed a ~31% (YM, 68.86 ± 3.36 A.U. vs OM, 47.22 ± 2.77 A.U., P=0.020) and a ~63% (YM, 39.21 ± 2.15 A.U. vs OM, 14.49 ± 0.57 A.U., P<0.001) lower abundance in Sestrin-2 and mTOR, respectively, compared with YM. Rheb abundance was ~43% higher in OM compared to YM (YM, 18.66 ± 0.92 A.U. vs OM, 26.71 ± 0.58 A.U., P<0.001). There were no differences in protein target abundance between fibre types for either group. Notably, peripheral and membrane bound fluorescence was higher in OM.

Discussion: Using IF, it is possible to characterise fibre-specific molecular regulators of skeletal muscle. Whilst the selection of regulatory proteins we investigated were similarly abundant in type I and II fibres, differences in mean fluorescence relative to fibre area of mTOR, TSC2,

Sestrin-2 and Rheb may be implicated in age-related MPS impairment and muscle decline. IF could be used to identify dysregulated signalling events that underpin age-related anabolic resistance (e.g., impaired MPS response to amino acids and/or contraction).

Conclusion: IF characterisation of the fibre-type-specific abundance of key regulators of mTORC1 revealed age-related differences that may be linked to dysregulated proteostasis.

N/A

PCB074

Higher stromal vascular fraction TGFB1 transcription and adipocyte overexpression of COL4A1 may explain reduced capacity for adipocyte expansion in pre-eclampsia

Zoë J. Lees[1], Róisín Ramsay[1], Xiwen Chen[1], Zuzanna Boguslawska[1], Ian P. Salt[2], Dilys J. Freeman[1]

[1]School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, United Kingdom, [2]School of Molecular Biosciences, University of Glasgow, Glasgow, United Kingdom

Pre-eclampsia (PE) is a disorder of human pregnancy, which is defined as new onset hypertension with proteinuria. In recent years, a role for adipose tissue (AT) dysfunction has emerged in PE pathology. Women with PE display exaggerated insulin resistance in subcutaneous AT (SAT) at third trimester compared to normotensive (NT) controls, without evidence of hypertrophy[1]. This may indicate a reduced capacity in PE to store gestationally-acquired fat within AT. We hypothesised that increased AT fibrosis in PE, defined as excessive accumulation of extracellular matrix components including collagens, could limit healthy AT expansion in PE.

Non-labouring pregnant women in the third trimester with PE, as defined by ISSHP guidelines, and age- and BMI-matched NT controls undergoing elective C-section at the Glasgow Royal Infirmary were consented for SAT and visceral AT (VAT) biopsy collections. Ethical approval was granted from the Glasgow Royal Infirmary Local Research Ethics Committee (06/S0704/14). Adipocyte diameter was assessed by manual microscopic sizing of a minimum of 100 adipocytes. Fibrosis was quantified in paraffin embedded AT sections using picrosirius red staining (PE n=11, NT n=6). Acquired images of stained whole tissue sections were converted to 8-bit images, background area subtracted and total red staining of collagen fibres (%) measured in ImageJ. Taqman qRT-PCR was used to measure mRNA expression relative to the endogenous control *PPIA* in whole AT (n=6 per group) and isolated adipocytes (n=9 per group). Data were analysed using repeated measures mixed-effects models of PE status (NT or PE) and AT depot (SAT or VAT) and their interaction; mean ± SD reported.

There was no difference in mean adipocyte diameter between PE (SAT: 113.6 ± 9.6μm, VAT: 90.6 ± 10.6μm) and NT (SAT: 111.7 ± 7.9μm, VAT: 88.2 ± 8.3μm) ($P_{\text{PE status}}$=0.53, $P_{\text{AT depot}}$<0.001, $P_{\text{interaction}}$=0.90). There was no difference in tissue collagen content between NT and PE ($P_{\text{PE status}}$=0.56, $P_{\text{AT depot}}$=0.55, $P_{\text{interaction}}$=0.39). Whole AT *COL6A3* ($P_{\text{PE status}}$=0.41, $P_{\text{AT depot}}$=0.005, $P_{\text{interaction}}$=0.51), *COL1A1* ($P_{\text{PE status}}$=0.76, $P_{\text{AT depot}}$=0.16, $P_{\text{interaction}}$=0.21) and *COL4A1* ($P_{\text{PE status}}$=0.17, $P_{\text{AT depot}}$<0.001, $P_{\text{interaction}}$=0.20) mRNA expression was not different between PE and NT. Interestingly, whole AT mRNA expression of *TGFB1* was higher in PE compared to NT ($P_{\text{PE status}}$=0.012, $P_{\text{AT depot}}$=0.039, $P_{\text{interaction}}$=0.73). Isolated adipocyte *COL4A1* mRNA expression was higher in PE compared to NT ($P_{\text{PE status}}$=0.044, $P_{\text{AT depot}}$<0.001, $P_{\text{interaction}}$=0.64). Adipocyte *TGFB1* expression did not differ between PE and NT ($P_{\text{PE status}}$=0.91, $P_{\text{AT depot}}$=0.001, $P_{\text{interaction}}$=0.48).

In conclusion, while there was no effect of PE on AT fibrosis, higher adipocyte expression of *COL4A1*, a key basement membrane component was observed. This has also been seen in adipocytes from obese individuals[2] suggesting similar localised restriction of adipocyte hypertrophy in PE which may contribute to impaired AT expansion. Higher whole AT, but not adipocyte, *TGFB1* expression (a regulator of *COL4A1* transcription) indicates that TGFβ is

produced by stromal vascular cells. Understanding the relationship between TGFβ and adipocyte collagen type IV may shed light on defective adipocyte differentiation in PE pregnancies.

1) Huda SS et al. (2014). Hypertension 63, 1094-1101 2) Reggio S et al. (2016). JCEM 101, 2578-2587

PCB075

The effect of ex vivo human serum on insulin signalling mechanisms in C2C12 skeletal muscle cells: a randomised parallel trial of diets differing in carbohydrate and fat content.

Deaglan McCullough[2], Tanja Harrison[4], Katie Lane[3], Claire Stewart[3], Ian Davies[3]

[1]Leeds Beckett University, Leeds, United Kingdom, [2]Carnegie School of Sport, Leeds Beckett University, Leeds, United Kingdom, [3]Research Institute of Sport and Exercise Science, Liverpool John Moores University, Liverpool, United Kingdom, [4]Department of Clinical Sciences and Nutrition, Chester University, Chester, United Kingdom

Type 2 diabetes affects 462 million adults globally (1) and skeletal muscle has shown to be a key regulator of glucose homeostasis (2). Both high-carbohydrate (HC) and low-carbohydrate (LC) diets reduce markers of insulin resistance (3) however, the underlying mechanisms are yet to be elucidated. *In vitro* models provide insight to niche responses, however, in isolation, cannot elucidate the impact of the change in metabolic factors on insulin signalling. Conditioned serum, in combination with conventional cell culture methods, may allow for such cross talk to be investigated (4). Therefore, the purpose of this study was to investigate the effects of diet on insulin resistance markers in adults and how their serum impacts skeletal muscle insulin signalling. Procedures received ethical approval from LJMU Research Ethics Committee (16/ELS/029). Participants were randomly assigned to a HC (n=8, ≥50% of energy from carbohydrates) or LC (n=8, consume <50 g/day of carbohydrates) diet. At 0, 4 and 8 weeks, serum samples were analysed for insulin resistance markers (5). Skeletal muscle C2C12 cells (n=3) underwent fusion in differentiation medium (DM; 2% horse serum). Myotubes were next incubated with 2% pooled human serum from 0, 4 and 8 weeks of intervention. After 30 min, the impact of serum on cellular energy status was assessed by immunoblotting for p/t-AMPK^{thr172}. Following incubation of serum for 3 hours, cells were stimulated with 100nM of insulin for 20 min and immunoblotted for p/t-Akt^{ser473} while glucose uptake was assessed via measuring 2NBDG uptake. Data are presented as mean ± SEM and underwent a 3 x 3 mixed ANOVA. Both groups reduced ($P < 0.01$) markers of insulin resistance (5). Serum from LC and HC significantly ($P < 0.05$) increased p/t-AMPK^{thr172} from 0 to 30 mins. p/t-AMPK^{thr172} significantly ($P = 0.04$) decreased with serum from 0, (LC; 6.51 ± 2.16, HC 4.54 ± 0.83), 4 (LC; 2.16 ± 0.43, HC; 2.93 ± 0.64) to 8 (LC; 1.33 ± 0.2, HC; 1.96 ± 0.44) weeks. Insulin stimulation significantly ($P < 0.001$) increased p/t-Akt^{ser473} with serum from 0 (LC; 3.25 ± 0.62, HC; 3.33 ± 1.29), 4 (LC; 1.95 ± 0.23, HC; 2.59 ± 0.42) and 8 (LC; 2.13 ± 0.31, HC; 2.6 ± 0.60) weeks however, no change in glucose uptake was observed. The fold change of p/t-AMPK^{thr172} was positively associated ($r = 0.62$, $P < 0.01$) with insulin stimulated p/t-Akt^{ser473}. As AMPK can regulate insulin sensitivity, p/t-Akt^{ser473} at each week were relativised to their p/t-AMPK^{thr172}. The fold change in insulin-induced p/t-Akt^{ser473} now showed a tendency ($P = 0.067$) of increasing from 0 (LC; 0.5 ± 0.01, HC; 0.73 ± 0.28) to 4 (LC; 0.9 ± 0.11, HC; 0.89 ± 0.14) and 8 weeks (LC; 1.6 ± 0.24, HC; 1.33 ± 0.3) with both diets. To conclude, human derived sera can impact the *in vitro* skeletal muscle response to insulin stimulation. Both a LC and HC diet can improve markers of insulin resistance and improve the cellular environment. Further research is required to determine the potential of *ex vivo* serum at elucidating cellular adaptations.

1 - Abdul, M., et al. (2020). J Epidemiol Global H, 10, pp. 107-111. 2 - DeFronzo, R. A. and Tripathy, D. (2009). Diabetes Care, Vol 32 Suppl 2. 3 - Apekey, T. A., et al. (2022). Nutrients, 14

(20). 4 - Allen, S. L., et al. (2023). Am J Physiol Cell Physiol 324: C420–C427. 5 - McCullough, D., et al. (2022). Nutrients, 14, (18).

PCB076

Adipose depot dependency of chloride channels expression in murine white fat adipocytes

Yan Meng[1], Olena Fedorenko[2], Nneoma Akaniro-Ejim[3], Maria Toledo-Rodriguez[1], Paul Smith[1]

[1]University of Nottingham, Nottingham, United Kingdom, [2]Unversity of Cambridge, Cambridge, United Kingdom, [3]University of Oxford, Oxford, United Kingdom

Plasma membrane ion channels are important for cell homeostasis with their combined properties often defining the function of that cell; however, for white fat adipocytes (WFA) their expression and associated roles remain unclear. Given the importance of chloride channels in WFA membrane potential (Pulbutr et al., 2007; Bentley et al., 2014), we aimed to identify these at the molecular level. We explored this issue in adipocytes isolated from different adipose depots of adult rats, mice and 3T3-L1 cells: a common adipocyte cell line model. RNAseq revealed the expression of various chloride channel isoforms in the epididymal WFA of adult male rats. Among these, putative plasma membrane chloride channels were the volume-regulated chloride channels Lrrc8a/b/c/d and Ttyh2, the calcium-activated chloride channels, Ano1 and Ttyh3 and the glutamate aspartate transporter, Eaat1. Relative expression was confirmed by RT-qPCR. Data are given as mean ratios (95% CI, number of determinations) relative to rSWFA. Statistical significance, with $p<0.01$ to account for repeated measures, was determined by ANOVA with Dunnet's multiple comparison test relative to rSWFA. Protein expression was determined by western blot in undifferentiated and differentiated 3T3-L1 cells with statistical significance determined by unpaired T-test. Confocal immunofluorescence was used to identify the cellular location of chloride channels on WFA. To investigate the role of chloride channels in adipogenesis, the effect of their inhibitors on 3T3-L1 cells was determined by measuring Nile Red accumulation analysed using One-way ANOVA.

RT-qPCR showed no difference in depot expression for Lrrc8a, Lrrc8c, Lrrc8d, Ttyh2 and ANO1, whereas, in rMWFA and rPWFA, Lrrc8b was expressed by 4.9 (2.5 to 13, n=5) and 3.3 (1.6 to 9, n=5) fold greater, respectively. Western blot demonstrated that Lrrc8a and Ttyh3 were expressed in differentiated 3T3-L1 cells by 4.1 (3.0 to 5.1, n=16) and 5.4 (-6.4 to 17.2, n=3) compared to undifferentiated 3T3-L1 cells, respectively. Data also showed that Eaat1 was exclusively expressed in differentiated 3T3-L1 cells while Ano1 was exclusively expressed in undifferentiated 3T3-L1 cells. Immunofluorescence showed that Eaat1, Ano1, Ttyh2 and Ttyh3 are primarily located to the plasma membrane, while Lrrc8a was all around the cells. During 3T3-L1 differentiation, 25μM DCPIB, a selective blocker of the volume-regulated anion channels Lrrc8a and Ttyh2, and 5μM quercetin, which inhibits Ca^{2+}-activated Cl^- channels Ttyh3 and Ano1, significantly reduced adipogenesis by 34% (16% to 53%, n=5) and 19% (6% to 31%, n=5) respectively. However, 50μM DIDS, which also inhibits Ttyh3, Ano1, and 10μM UCPH101, which inhibits Eaat1, were without effect.

This study provides evidence for the existence of chloride channels with various expression patterns among different WFA depots and in 3T3-L1 cells. Lrrc8a, Ttyh2, Ttyh3 and Eaat1 were all expressed in the plasma membrane of murine WFA, Lrrc8a was also found intracellularly. Ano1 was expressed only in rat WFA. The observation that DCPIB and Quercetin significantly inhibited adipogenesis suggests that chloride channels play a role in adipocyte differentiation.

Bentley et al (2014). Am J Physiol - Endocrinol Metab 307, E161–E175. Pulbutr P et al (2007). Proc Life Sci PC192.

PCB077

Effects of the administration of a combined oral contraceptive composed by ethinylestradiol and drospirenone on adiposity and liver histopathology in female mice in reproductive age

Rosane Aparecida Ribeiro[1], Janaina de Oliveira Chaves[2], Gésily de Souza Aguiar[2], Isabelle Vollero Manosso[1], Helene Nara Henriques Blanc[2]

[1]Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil, [2]Universidade Federal do Rio de Janeiro, Centro Multidisciplinar UFRJ-Macaé, Macaé, RJ, Brazil

Combined oral contraceptives (COC) is the type to birth control used for several women, but, little is known about its organic effects when it is combined with an obesogenic diet. Here, we aimed to investigate the effects of administration of a COC composed of 17α-ethinylestradiol (EE2) and drospirenone (DRSP) upon obesity, glucose tolerance and non-alcoholic fat liver disease (NAFLD) in female mice of reproductive age. Eighty-days old *Swiss* female mice were fed on a standard diet (SD) or a high-fat diet (HFD) and daily received, via gavage, 0.2 mL of distilled water [CTL-SD (n=13) and CTL-HFD (n=16) groups, respectively], containing or not, 0.6 µg EE2 plus 60 µg DRSP [COC-SD (n=13) and COC-HFD (n=16) groups, respectively], for 65 days. At the end of the treatment, all female mice groups were submitted to an intraperitoneal (IP) glucose tolerance test (GTT) and two days after were weighted and euthanized by decapitation. Total blood was collected to measure plasma triglycerides. Total adipose tissue stores and the liver were excised for visceral adiposity evaluation and hepatic fat extraction and histology, respectively. Results were analyzed using two-way ANOVA followed by Bonferroni-Sidak's test ($p < 0.05$). Procedures performed in mice were in accordance with brazilian's ethical standards and were approved by the animal use committee (certificate number: MAC039). Consumption of HFD increased total BW gain in CTL-HFD females (11.9 ± 1.2 g), when compared with CTL-SD (0.5 ± 1.0 g). In accordance, CTL-HFD exhibited increased final BW (49.1 ± 1.4 g) and total visceral adiposity (147.2 ± 9.6 mg/g BW) than those observed for CTL-SD (35.3 ± 1 g and 57.5 mg/g BW, respectively). COC administration attenuated obesity induced by HFD, since COC-HFD females displayed high visceral adiposity (88.1 ± 12.5 mg/g BW), but similar BW gain (4.4 ± 1.5 g) and final BW (36.4 ± 1.8 g) than COC-SD (47.0 ± 5.0 mg/g BW, 4.4 ± 1.5 and 35.8 ± 0.4 g, respectively). But all these parameters in COC-HFD were lower than those observed for CTL-HFD. Also, CTL-HFD displayed high total glycemia during the GTT (40031 ± 2763 mg/dL.min-1) in comparison with CTL-SD (28577 ± 2000 mg/dL.min-1). COC treatment prevented glucose intolerance induced by HFD, since COC-HFD females displayed lower total glycemia during this test (31113 ± 1439 mg/dL.min-1) when compared to CTL-HFD. HFD augmented plasma (85.6 ± 5.7 mg/dL) and hepatic triglycerides levels (24.7 ± 2.1 µg/mg), and increased NAFLD score (3.2 ± 0.1) in CTL-HFD liver, mainly in part due to increase the hepatic steatosis score (3.1 ± 0.1), but not, inflammation score (0.1 ± 0.1). COC-HFD females displayed lower hepatic TG content (16.2 ± 2.0 µg/mg) of CTL-HFD, while their liver parenchyma displayed NAFLD associated features, such as hepatocytes with microvesicular steatosis (score = 1.6 ± 0.4), higher inflammatory foci (0.5 ± 0.2) and hyperemia. Therefore, COC administration to female mice of reproductive age, attenuated some HFD inducing obesity impairments, such as adiposity, glucose intolerance, but not prevented signs of liver damage that characterizes NAFLD development.

not applicable

PCB078

Effects of a resistance training programme in people living with HIV in Zimbabwe

Victor Mbayo[1], Takshita Sookan Kassie[2]

[1]Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, Durban, South Africa, [2]Discipline of Biokinetics, Exercise and Leisure Sciences, School of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

Purpose: Combination antiretroviral therapy (cART) increases life expectancy in people living with HIV (PLWH). However, receiving cART coupled with physical inactivity increases the risk of developing non-communicable diseases. Resistance training (RT) has also been found to increase muscle strength and mass, mitigate muscle wasting, improve psychological status, glucose tolerance, insulin sensitivity and lipid profile in PLWH. However, this has not been fully investigated in sub-Saharan Africa. Therefore, the purpose of this study was to investigate the effect of RT on body composition, laboratory analysis and strength values in PLWH receiving cART in Zimbabwe.

Methods: One-hundred and twenty-eight PLWH receiving cART, aged 18–45 years were purposely recruited to saturation. The study obtained ethical approvals from the University of Kwa-Zulu Natal's Biomedical Research Ethics Committee (BREC) BF293/15 and the Medical Research Council of Zimbabwe (MRCZ)
MRCZ/B/948. All participants signed an informed consent following initial BL assessments. Two districts in Zimbabwe were used. Participants in Budiriro were randomly assigned for convenience to an experimental (EXP) group (n = 64) performing RT 3 days/week and participants in Mabvuku to a control (CON) group (n = 64) for 12 weeks of no exercise. Body mass index, waist-to-hip ratio, percentage body fat, lean body mass (body composition), laboratory analysis profiles and one-repetition maximum (1RM) strength were measured at baseline (BL) and after 12 weeks (W12) in both groups. Significance was set at $p < 0.05$.

Results: Lean body mass increased in the EXP group (n=64) from 52.42 ± 8.360 kg to 53.07 ± 8.225 kg (mean difference -0.65 kg), suggesting that the 12-week RE intervention programme significantly increased ($p < .001$) lean body mass in the EXP group. In the CON group, lean body mass reduced from 50.87 ± 6.340 kg to 47.43 ± 7.829 kg (mean difference 3.44 kg) during the same period. Fasting blood glucose decreased significantly ($p < .001$) in the EXP group from 4.440 ±0.445 mmol/l to 4.240 ± 0.488 mmol/l (mean difference 0,2 mmol/l), compared to the CON group at BL 3.68 ±0.711 mmol/l to W12 3.98 ±0.818 mmol/l (mean difference -0.3 mmol/l). Fasting total blood cholesterol decreased significantly ($p<.0005$) in the EXP group from 4.440 ±0.526 mmol/l to 4.240 ± 0.488 mmol/l (mean difference 0.2 mmol/l), compared to the CON group, which increased from 4.556 ± 0.445 mmol/l to 4.672 ± 0.497 mmol/l (mean difference -0.116 mmol/l). In the EXP group, 66% of participants improved resting blood pressure, a significant change from BL to W12 ($p<.0005$). In the EXP group, 1RM muscular strength increased significantly ($p < .001$) for bench press (mean difference -3.9 kg), squat (mean difference -26 kg), biceps curl (mean difference -7.84 kg) and leg curl (mean difference -11.44 kg) from BL to W12 compared to the CON group.

Conclusions: These findings highlight the benefits of RT for PLWH receiving cART. This demonstrates the need for additional public health initiatives involving RT in this population in sub-Saharan Africa.

Keywords HIV-infected · Resistance training · Body composition · Chronic disease · Strength

1. American College of Sports Medicine (2014) ACSM's guidelines for exercise testing and perscription: eighth edition. In: W. R. Thompson, N. F. Gordon, L. S. Pescatello (eds.), 8th edition. Wolters Kulwer - Lippincott Williams and Wilkins, USA. 2. Sookan T, Motala A, Ormsbee M, Antonio J, Magula N, Lalloo U, McKune A (2019) Improvement in muscular strength in Hivinfected individuals receiving antiretroviral therapy. J Fun Morphol Kinesiol 4(66):1–13. https://doi.org/10.3390/jfmk4 03006 6 3. Lima LRA, Back I, Nunes EA, Silva DAS, Petroski EL (2019) Aerobic fitness and physical activity are inversely associated with body fat, dyslipidemia and inflammatory mediators in children and adolescents living with HIV. J Sports Sci 37(1):5058. https ://doi.org/10.1080/02640 414.2018.14817 24 4. Guariglia DA, Pedro RE, Deminice R, Rosa FT, Peres SB, De Moraes SMF (2018) Effect of combined training on body composition and metabolic variables in people living with HIV: a randomized clinical trial. Cytokine 111:505–510 5. Kamitani E, Sipe GTA, Higa DH, Mullins MM, Soares J (2017) Evaluating the effectiveness of physical exercise interventions in persons living with HIV: overview of systematic reviews. AIDS Educ Prev 29(4):347–363. https ://doi.org/10.1521/aeap.2017.29.4.347

PCB079

Altered cardiac mitochondrial respiratory complex activities in adult mice born to obese dams

Benjamin Thackray[1], Denise Fernandez-Twinn[2], Kwun Kiu Wong[2], Susan Ozanne[2], Andrew Murray[1]

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

Introduction: Exposure to gestational obesity increases cardiometabolic disease risk in both human longitudinal cohorts and animal models. Male mice born to obese dams display cardiac hypertrophy and declining systolic and diastolic function. Despite alterations in cardiac mitochondrial respiratory function and ultrastructure in the offspring of obese dams, the role of mitochondrial metabolic changes in the programming of cardiac dysfunction by maternal obesity remains poorly defined.

Objective: To evaluate myocardial mitochondrial respiratory capacity in a well-established murine model of maternal obesity, we subjected banked cardiac tissue from 8wk-old offspring of both sexes to a protocol optimised for respirometry in frozen samples to profile electron transport chain (ETC) capacities.

Methods: Animal work was conducted in accordance with the UK Home Office Animal (Scientific Procedures) Act 1986 and following local ethical approval. Cardiac tissue was previously collected from 8wk-old C57BL/6J offspring mice of both sexes born to dams fed either a control (CC group) or obesogenic diet (OC group) for 10 weeks prior to mating and throughout gestation. Frozen cardiac tissue was homogenised in MiR06 respiration medium and transferred to Oxygraph-O2K chambers. A substrate inhibitor titration was initiated to determine uncoupled capacities of ETC complex I (CI; NADH), complexes I+II (maximal ETC capacity; NADH + succinate), complex II (CII; succinate + rotenone), and complex IV (CIV; TMPD + ascorbate). Respiratory oxygen fluxes (JO_2) were normalised to chamber homogenate dry weight and maximal ETC capacity, and analysed by two-way ANOVA for sex and maternal diet (n = 7).

Results: Uncoupled respiratory complex activities normalised to tissue weight did not significantly differ between the 2 offspring groups, suggesting no overt changes in mass-specific mitochondrial respiratory capacity, although the CI-linked JO_2/maximal ETC capacity ratio was higher in the OC group (control: 0.37 vs. obese: 0.41, P = 0.041). Mass-specific CI-linked ETC activity (male: 171.97 pmol O_2/[s×mg dw] vs. female: 117.29 pmol O_2/[s×mg dw], P = 0.077) and CI/maximal ETC capacity ratio (male: 0.42 vs. female: 0.37, P = 0.0065) were higher in male offspring, with females exhibiting a trend towards lower CII activity normalised to maximal ETC (male: 0.69 vs. female: 0.73, P = 0.054).

Conclusion and further work: Despite no differences in mass-specific uncoupled respiratory complex activities, suggesting an overall preservation of cardiac mitochondrial respiratory capacity, CI activity as a proportion of maximal ETC capacity was increased in maternal obesity exposed offspring, potentially indicating an early remodelling of relative respiratory complex

activities. Further work is necessary to profile the stoichiometry of respiratory complex expression, and to define the wider mitochondrial metabolic phenotype in these samples.

PCB080

PUTATIVE GENETIC IMPACT ON DEPRESSIONAND SUICIDAL IDEATION AMONGST A POPULATION OF YOUNG FEMALES WITH PREMENSTRUAL DYSPHORIC DISORDER

FREDDY AGOREYO, Ese Onuyoh-Adaitire, Blessing Agoreyo, B Onuyoh-Adaitire

undefined

Premenstrual Dysphoric Disorder (PMDD) is a pathological spectrum of emotional and somatic symptoms observed during the luteal phase of menstrual cycle interfering with the physical and social life of the individual. WHO in 2016 revealed that Nigeria had the highest suicidal number in Africa with over 17,000 lives lost to Suicide.The aim of this study was to evaluate the putative genetic impact on depression and suicidal ideation amongst a population of young females with premenstrual dysphoric disorder. The study was carried out across Benin metropolis. A total of 200 apparently healthy young female adults were recruited in this study with age range between 18 and 30 years. Subjects were grouped into 3 groups: Control subjects (without symptoms of PMS), subjects with symptoms of PMS only and those with PMS and Suicidal tendencies. To assess the subjects' subjective perception of health, each subject was asked to fill out the self-reporting luteal phase depression and distress measurements. Five (5.0) mls of whole blood was

collected and dispensed into 2.5ml DNA shield container. Analyses were carried out in the University of Benin, University of Benin Teaching Hospital (UBTH) and Federal University of Technology, Akure (FUTA), Nigeria. All data were presented as mean ± standard deviation. Statistical analyses were done using graph pad prism 8.1. The data was evaluated using two-way

analysis of variance (ANOVA) utilizing the F test. Data was expressed as the mean value ± SD for the control and test groups. Differences within the groups were then assessed using least significant difference (LSD) and p-values less than 0.05 ($p<0.05$) was considered statistically

significant. The Beck's Depression Inventory showed a significant rise in the test participants when compared with the control participants ($p<0.05$). Cytochrome P450 -17 gene expression was significantly up regulated in the test participants compared to control participants during the luteal phase of the menstrual cycle ($p<0.05$). The Extra sex comb/Enhancer of Zestes genes were

significantly down regulated in the test participants when compared with the control participants ($p<0.05$).

PCB081

Regulation of synaptic AMPA receptor function by TARP combinations

Cecile Bats[1], Thomas P. McGee[2], Mark Farrant[3], Stuart G. Cull-Candy[4]

[1]University College London, London, United Kingdom, [2]University College London, London, United Kingdom, [3]University College London, London, United Kingdom, [4]University College London, London, United Kingdom

AMPA receptors (AMPARs) are responsible for fast excitatory synaptic signalling in the brain. A majority of AMPARs are heterotetramers composed of four pore-forming subunits (GluA1-4), co-assembled with transmembrane AMPAR regulatory proteins (TARPs). Six TARP family members have been described which differ in their effects on receptor trafficking and function (Jackson & Nicoll, 2011). While many central neurons contain more than one type of TARP, it is unknown whether different TARPs can co-associate within with the same receptor and further modify individual AMPAR function.

Structurally, TARPs possess four transmembrane domains and intracellular C- and N- termini. While the TARP's backbone mediates binding to AMPARs, its C-terminal tail (CT) binds to postsynaptic density protein (PSD-95) through a PDZ binding motif (Bats, 2007). Functionally, TARP family members fall into two categories, type I (gamma-2, -3, -4 and -8) and type II (gamma-5 and -7), according to their ability to rescue excitatory postsynaptic currents (EPSCs) in stargazer cerebellar granule cells (CGCs). Stargazer mice are a spontaneous mutant that lack gamma-2 (stargazin) expression in homozygous individuals. CGCs of these mice lack AMPAR-mediated synaptic currents, suggesting these neurons rely on gamma-2 for AMPAR surface expression and synaptic clustering. Transfecting any type I TARP in stargazer CGCs can compensate for loss of endogenous gamma-2. While only type I TARPs promote AMPARs surface delivery and synaptic expression, TARPs from both subtypes enhance AMPAR channel function. They do this by increasing the AMPAR deactivation- and desensitization time, increasing net cation influx. Of the TARPs, gamma-4 produces the slowest decaying AMPAR-mediated currents.

We made patch clamp recordings from CGCs cultured from postnatal stargazer mice. Transfection of a chimera containing the CT of gamma-2 fused to the backbone of gamma-4 gave rise to miniature EPSCs that decayed remarkably slowly when compared with wild type gamma-2 and gamma-4 (τ_w 22.7 ± 1.7 ms versus 3.2 + 0.2 ms and 4.8 ± 0.6 ms; all n = 5; p = 0.007 and p < 0.001, two-sided permutation t-test, Ho, 2019). Such combined action of the gamma-4 backbone and gamma-2 CT within the chimera suggests that gamma-2 and gamma-4 may use different mechanisms to control AMPARs kinetics. We therefore asked whether gamma-2 and gamma-4 could co-associate within individual AMPARs to confer distinct kinetic properties. To address this, we expressed a chimera containing the CT of gamma-7 fused to the backbone of gamma-4 (gamma-4_7CT) in cultured CGCs. As this chimera lacks the ability to promote the trafficking of AMPARs to synapse, any increase in mEPSCs decay time is expected to reflect the synaptic insertion of receptors containing both gamma-2 and gamma-4_7CT. Indeed, mEPSCs in these neurons displayed significantly slower decay times than those seen in GFP-transfected controls (τ_w 4.2 ± 0.3 ms versus 2.2 ± 0. 3ms, n = 5 and 7; p = 0.0026). Together, these results suggest that, in CGCs that contain endogenous gamma-2 and transfected gamma-4_7CT, a population of synaptic AMPARs contained both TARPs, and that the receptors can show some features derived from each.

Jackson AC & Nicoll RA (2011). Neuron 70(2), 178-99. Bats C et al. (2007). Neuron 53(5), 719–734. Ho J et al. (2019). Nature Methods 16(7), 565-566.

PCB082

Cognition is selectively impaired in males with spinal pain: A retrospective analysis of data from the Longitudinal Study of Ageing Danish Twins

David Byfield[2], Benjamin Stacey[1], Alister DuRose[3], Damian Bailey[1]

[1]Neurovascular Research Laboratory, University of South Wales UK, Pontypridd, United Kingdom, [2]Neurovascular Research Laboratory, University of South Wales, Pontypridd, United Kingdom, [3]AECC University College, Bournemouth, United Kingdom

Background: Cognitive decline and dementia represent a major health and social care challenge in the twenty-first century as a leading cause of morbidity and mortality affecting over 55 million people globally. Back pain (BP) and neck pain (NP), often referred to collectively as spinal pain, contribute to additional health challenges in the elderly and is acknowledged as the primary cause of years lived with disability often co-existing with cognitive decline and neurodegeneration. Despite their co-existence, our current understanding of the mechanisms that potentially link BP and NP to accelerated cognitive decline remains uncertain.

Hypothesis: We hypothesise that elderly adults reporting spinal pain would exhibit lower cognition scores, and that this would be more pronounced in females, given their established vulnerability to later-life neurodegeneration.

Methods: To investigate the potential relationships between BP/NP and cognitive decline, we conducted a retrospective cross-sectional analysis of the Longitudinal Study of Aging Danish Twins Database as part of the Danish Twins Registry adjusting for age, sex, educational and socioeconomic status. Ethical approval was granted by the Faculty of Life Sciences and Education Ethics Committee at the University of South Wales (#19DB0501LR).

Results: A total of 4,731 adults (2,788 females/1,943 males) aged 78±6 (SD) years were included in the analysis. We observed a one-month prevalence of 25% with BP, 21% with NP and 11% for combined BP/NP. While there were no differences in cognition scores for male and females reporting combined BP/NP, compared to those without combined BP/NP (34.38 points; 95% CI=31.88, 36.88 vs 35.72 points; 95% CI=35.19, 36.26; *P*=0.180; and 35.72 points; 95% CI=35.19, 36.26 vs 35.85 points; 95% CI=35.39, 36.31; *P*=0.327, for male and females respectively), an adjusted analysis revealed that males with combined BP/NP presented with lower cognitive scores compared to males without combined BP/ NP (79.48 points; 95% CI=70.31, 88.66; *P*=0.043 vs 81.26 points; 95% CI=73.80, 88.72, respectively).

Conclusions: In the current study, males reporting combined BP and NP exhibited lower composite cognitive scores compared to males without combined BP and NP when adjusting for age, sex, educational and socioeconomic status. The fact that male twins reporting both BP and NP presented with lower cognitive scores may be due to males carrying more cardio-cerebrovascular risk factors (i.e., greater vascular disease burden) than females. Accordingly, an elevation in cardiometabolic risk factors and the pattern of combined BP and NP symptomatology may have the potential to increase systemic oxidative-inflammatory-nitrosative stress (OXINOS). Given this was only observed in males, these findings suggest a 'sex-specific susceptibility' to cognitive decline and supports the notion that combined BP and NP may be considered as an additional cardio-cerebrovascular risk factor for later life neurodegeneration warranting further investigation.

PCB084

Use of SH-SY5Y cell line to develop an in vitro model to study autism spectrum disorders

Süeda Tunçak[2,3], Vanessa Vulovic[3], Nikhil Menezes[3], Isadora Tassinari[3,4], Amanda Thomaz[3], Victorio Bambini-Junior[3]

[1]Bursa Uludag University, Bursa, Turkey, [2]Department of Physiology, Faculty of Medicine, Bursa Uludağ University, Bursa, Turkey, [3]Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Lancaster, United Kingdom, [4]Graduation Program in Physiology, Federal University of Rio Grande do Sul (UFRGS), Porto Alerge, Brazil

Valproic acid (VPA) is an environmental risk factor for Autism Spectrum Disorders (ASD), especially during the first trimester of pregnancy, that is widely used in experimental designs for its ability to model ASD both morphologically and behaviourally on animals but was not studied on cell lines. Cell lines provide cheaper, ethically less problematic and reusable models (Xicoy et al., 2017). The aim of this study was to use SH-SY5Y, a human neuroblastoma cell line, to develop an *in vitro* model for studying ASD and observing VPA's effects directly on cells during different stages of differentiation.

SH-SY5Y cells were seeded to a 96-well plate with 2500 cells per well. 24hrs after seeding, culture media was changed to differentiation media and this was accepted as the first day of differentiation. The differentiation protocol was 7 days in total (Kovalevich and Langford, 2013). Cells were treated with VPA for 24 hours on different days of differentiation and each treatment day had three different concentrations (1mM, 5mM and 10mM of VPA). Experimental groups were the following: Control group (vehicle); VPA on the first day of differentiation; VPA on the third day of differentiation; VPA on the fifth day of differentiation. The MTT assay was performed as triplicates to determine cell viability. Statistical analysis was performed with One Way ANOVA and on Sigma Plot.

MTT assay showed that VPA affects cell proliferation/viability when compared to the control group on the first day of differentiation for each dose group ($p<0,001$; $p<0,001$; $p<0,05$). Cells exposed to VPA at the third day of differentiation also had reduced cell viability when compared to control cells except for the lower concentration of VPA ($p<0,001$; $p<0,001$). VPA treatment on the fifth day of differentiation significantly affected cell viability, when treated with concentrations of 5mM and 10mM ($p<0,001$; $p<0,001$). However, the lowest concentration of VPA had the opposite effect, increasing cell counting when compared to control cells ($p<0,001$). Effect of VPA exposure on different days of differentiation was compared within the same dose groups. In 5mM and 10mM doses, VPA exposures on third day of differentiation resulted in significantly lower cell count compared to first and fifth day of differentiation ($p<0,05$; $p<0,05$).

Consistent with literature, our results showed that 1mM of VPA does not have negative effects on cell viability whereas 10mM showed detrimental effects (Jang et al., 2021). Our results also showed that third day of differentiation is the most vulnerable time for VPA exposure in terms of cell viability. We suggest that 5mM VPA should be used for further analysis for cellular and morphological parameters that are disrupted in *in vivo* models of ASD.

Xicoy H et al. (2017). Molecular neurodegeneration 12, 1-11. Kovalevich J & Langford D (2013). Neuronal cell culture: methods and protocols 9-21. Jang EH et al. (2021). Cells 10(10), 2522.

PCB085

Characterising Lipopolysaccharide induced Tumour Necrosis Factor Alpha release by BV-2 cells in Normoxia and Hypoxia (1% O2)

John Bennett[2], Stuart Jenkins[3], Mark Dallas[1], Ruoli Chen[2]

[1]School of Pharmacy, University of Reading, Reading, United Kingdom, [2]School of Pharmacy and Bioengineering, Keele University, Newcastle Under Lymn, United Kingdom, [3]School of Medicine, Keele University, Newcastle Under Lyme, United Kingdom

Microglial cells are the key immunogenic cells in the CNS, and significantly contribute to overall brain function by participating in phagocytosis during development, homeostasis, and diseased states. It has been demonstrated that oxygen levels have significant impacts on microglial morphology and behaviour. Hypoxia is reported to either induce, or attenuate externally induced, inflammatory signalling. This study aimed to characterise the LPS induced inflammatory response, including its potential dose dependency, of BV-2 cells, and discover how this response is altered when stimulation occurs during hypoxia. BV-2 cells between passage 3-11 were exposed for 24h to LPS, at 1, 10, 100, 1000, or, 10,000 ng/mL, in normoxia (21% O_2), or hypoxia (1% O_2). Media samples were collected and assessed for inflammatory response, via TNF-α ELISA assay. Cell viability, via LDH assay, and metabolic activity, via MTT assay, were also determined. Lastly expression of inflammation and hypoxia associated genes was analysed via qPCR, using only 100ng/ml LPS as a dosage.

MTT assays demonstrated decreased metabolic activity in hypoxia at all LPS doses, with 10,000, 10 and 1ng/mL being significant at $p<.05$, n=4 (Figure 1). This decrease is likely the reduced oxygen limiting aerobic respiration, though cell death from hypoxia, suggested from our LDH results, may also be responsible. LDH concentration was significantly increased by hypoxia compared to normoxic cells when LPS was present, but not by hypoxia alone. All LPS doses 10ng/mL or higher showed significantly increased LDH concentration compared to hypoxic control (min sig .05), n=4 (Figure 2). Predictably, all normoxic LPS dosages, and all doses above 1ng/mL in hypoxia, showed significantly higher TNF- α than control, n=4 (Figure 3). Not all doses differed significantly, however increasing TNF- α release trended with increasing LPS concentration. The exception was normoxic 10,000ng/mL LPS where a biphasic high (1000-3000pg/mL,) or low response (<50pg/mL, excluded herein) appeared between technical replicates. We suspect this results from the cytotoxic effect of LPS at high dosages limiting TNF- α production. qPCR analysis showed LPS 100ng/mL induced increases in TNF-α (x41.58), GAPDH (x42.86), and PDK (x24.32) expression at 21% O_2, which were reduced when combined with Hypoxia (x5.62, x4.42, x1.30 respective) n=4 (Figure 4). TNF-α (x30.58) and IL-6 (x25.64) were also notably increased in LPS dosed hypoxia samples compared to hypoxic controls. This indicates that while LPS promotes a proinflammatory response, 24h of hypoxia suppressed this induction.

In conclusion, this study demonstrates LPS stimulates TNF-α gene expression in both normoxia and hypoxia and TNF-α release shows evidence of dose dependency, with a potency threshold as low as 1ng/mL regardless of hypoxic status. This work further shows the combination of LPS and Hypoxic stimulation may induce cell death where LPS stimulation alone does not. Lastly, we demonstrated that 24h hypoxia (1% O_2) reduced BV-2 cell metabolism, including reduced expression of metabolically associated genes and reducing the inflammatory response of BV-2

cells induced by LPS at some concentrations. With these results, this study furthers our understanding of neuroinflammation under hypoxic conditions.

PCB086

The efficacy of induced pluripotent stem cells in animal models of stroke: a systematic review

Isabel Birt[1], Abigail Dos Santos[1], Catriona Jane Cunningham[1]

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

Background: Stroke is a leading cause of death and disability which affects around 13 million people annually. Despite this, treatment options remain limited. The development of induced pluripotent stem cell (iPSC) technology by Yamanaka and colleagues offers a new opportunity for restoring function to stroke survivors. While iPSCs are showing potential in animal models, to the best of our knowledge no systematic review has yet been conducted.

Methods: We performed a keywords literature search in PubMed and Embase to identify relevant studies. Our inclusion criteria included controlled studies, any adult animal model of stroke and transplantation of iPSCs or iPSC-derived cells. Study quality and risk of bias was assessed using a 10-point CAMARADES checklist.

Results: Following screening, a total of 27 studies were included. The majority used human iPSCs (n=21) and induced ischaemic stroke using a middle cerebral artery occlusion (MCAO) model (n=19). The median score of the CAMARADES checklist was 5/10 (IQR: 4-7). While all studies were peer-reviewed and the vast majority complied with welfare regulations (88.9%), reporting of blinding to induction of stroke and assessment of outcome was low (44.4% and 48.2% respectively). A total of 21 studies reported that iPSCs lead to significant improvements in outcomes.

Conclusions: The results suggest that iPSCs show great potential for the treatment of stroke leading to improvements in neurological function and lesion volume. However, improvements in study design and reporting in future research are required.

PCB087

Blockade of hyperpolarization-activated channels with ivabradine attenuates mechanical, but not heat, hypersensitivity in two rat models of diabetic neuropathy

Laiche Djouhri[1]

[1]*Qatar University, Doha, Qatar*

Introduction: peripheral neuropathic pain (PNP), is associated with many types of injury/diseases, including diabetes mellitus (DM) that affects hundreds of millions of people worldwide. Hyperglycaemia in patients with longstanding diabetes can cause damage to peripheral nerves. This damage, known as diabetic neuropathy (DN), is the most common complication of both type 1 and type 2 diabetes mellitus, and is the most common cause of PNP. Indeed, up to 50% of people with diabetes have some degree of DN, and around 25% develop diabetic PNP (DPNP). DPNP usually experience a range of unpleasant symptoms including mechanical hypersensitivity. Despite its clinical importance, the pathophysiology of DPNP is still illusive. However, hyperpolarization-activated cyclic nucleotide–gated (HCN) ion channels, which have been implicated in the pathogenesis of other types of PNP are likely to be involved.

Aims of the study: To examine, in rat models of DPNP, whether blocking HCN channels with ivabradine, a peripherally restricted drug that is devoid of CNS side effects, and the only clinically available HCN channel blocker, would reverse/attenuate behavioural signs of DPNP.

Methods: Male Sprague Dawley rats (250-300 g, n=64) were used, and the experimental protocols were approved by University of Qatar Ethical review committee. Two models of DPNP were used: the streptozotocin (STZ) model of type 1 DM that involved a single injection of STZ (60 mg/kg, i.p.), and the high fat diet-fed, STZ (HFD/STZ) model of type 2 DM that involved a single injection of a low dose of STZ (35mg/kg, i.p. n=32 rats) after 2 weeks of feeding the rats on HFD (Skovso, 2014). Three groups of rats were used in each model: (1) vehicle (control) group (n=10); (2) Ivabradine group (10 mg/kg, i.p, n=12) and (3) Gabapentin (positive) group (n=10). Behavioural testing for mechanical and heat hypersensitivity was performed using a dynamic plantar aesthesiometer touch stimulator, and Hargreaves analgesiometer, respectively (Djouhri et al. 2019). Data were presented as the mean ± SEM, and One-way ANOVA with post hoc tests was used.

Results: Both STZ and HFD/STZ rats exhibited behavioural signs of mechanical and heat hypersensitivity as indicated by significant decreases ($P<0.001$) in the mean paw withdrawal threshold (PWT) and mean paw withdrawal latency (PWL) respectively at 35 days post treatment. A single injection of ivabradine caused a significant ($P<0.05$) increase in the mean PWT from 20.6 ± 2.6 g to 37.0 ± 2.1 g in STZ rats, and from 26.6 ± 2.9 g to 35.0 ± 2.2 g in HFD/STZ rats, at 2h, but not at 24h, post treatment. Ivabrdaine was as effective as the positive control gabapentin in attenuating mechanical hypersensitivity, but had not effect on heat hypersensitivity (no significant change in the mean PWL).

Conclusions: The findings suggest that HCN channels are involved in the mechanisms of mechanical, but not heat hypersensitivity associated with DPNP, and that their blockade with ivabradine may prove to be effective in treating DPNP in humans.

1. Skovsø, S (2014). J Diabetes Invest, 5, 349-358 2. Djouhri, L et al., (2019). J. drug. Targeting, 27, 118–1126

PCB088

Minor effects of 12 week high-fat diet and/or prebiotic Xylo-oligosaccharides on cognition and brain metabolites in rats.

Sanna Lensu[1,2], Jukka Hintikka[2], Elina Mäkinen[2], Marko Lehtonen[3], Satu Pekkala[2]

[1]University of Jyväskylä, Department of Psychology, Jyväskylä, Finland, [2]University of Jyväskylä, Faculty of Sport and Health Sciences, Jyväskylä, Finland, [3]University of Eastern Finland, Faculty of Health Sciences, School of Pharmacy, Kuopio, Finland

Intro: Prevalence of obesity is expanding world-wide. Obesity is a severe risk factor for fatty liver and brain diseases, and problems in mental health. The role of gut microbiota (GM) in health maintenance is widely accepted; thus, the modification of GM owns huge potential for treating many diseases. *Feacaelibacterium prausnitzii* is one of the gut microbes associating with low hepatic fat (Munukka et al. 2014), and suggested to act as a possible psychobiont, a tool to modify and support mental health (Borkent et al. 2022). Because *F. prausnitzii* is extremely anaerobic, the gut level of it should be targeted via dietary means. Aims/objectives: We previously showed that a prebiotic XOS enhanced growth of *F. Prausnitzii* and ameliorated hepatic steatosis in rats (Lensu et al. 2020). Here we show the effects of high-fat diet and/or XOS supplementation on behavior and cognition, before and during the dietary intervention in adult male Wistar rats. Method: The ethical permission for the study was achieved from the National Animal Experiment Board of Southern Finland. To enhance the level of *F. Prausnitzii*, the diet of the rats (n=10 per group) was supplemented or not with prebiotic dose of XOS (0.12%, Shandong Longlive Biotechnology, CAS 87099-0), and the rats were having a simultaneous high-fat diet (60% energy from fat) to induce obesity or control diet (10% energy from fat, 'low-fat'), the details of the experiment can be found in Lensu et al. 2020. Behavior and cognition were studied with openfield, context-object recognition, and sucrose preference tasks. Untargeted metabolites from half of the brain tissue were measured by liquid chromatography – high resolution mass-spectrometry (LC-MS/MS) and curated using MS-DIAL and R. Data were tested with Generalized linear and mixed models (effect of high-fat diet and/or XOS, measurement time: pre-post) and between groups comparisons were done using non-parametric Kruskall-Wallis test. Results and conclusions: The activity of rats in the openfield-arena diminished during the 12-week intervention, independent of the group ($F [1,34] = 44.2$, $p < 0.001$), and low-fat diet enhanced anxiety-related behavior ($F [1,35.8] = 5.4$, $p < 0.05$). Low-fat diet attenuated the performance in the context-object recognition task in comparison to high-fat diet ($F [1,36] = 14.45$, $p<0.001$), explained by increased preference of the familiar object in the low-fat group during the post-measurement. In the end of the intervention, rats having high-fat diet preferred sucrose more than those having low-fat diet ($F([1,36] = 6.59$, $p = 0.015$). For the brain metabolites, the high-fat diet group showed separation from the other groups in principal component analysis and t-stochastic neighbor embedding. Using the pathway analysis in Metaboanalyst, eight metabolic pathways were found to be significantly different (q-value < 0.05 and pathway impact > 0.1) between high- and low-fat groups, including purine, pyridimine, histidine and tryptophan metabolism. In conclusion, our 12-week intervention caused only minor effects on behavior and brain metabolites, and they were mainly affected by high-fat diet. Thus, our research suggests that XOS is not highly psychoactive prebiotic although it beneficially affects the GM and host's physiology.

Borkent et al. (2022). Psychol. Med., 52(7): 1222-42. doi:10.1017/S0033291722000897. Lensu S et al. (2020). Nutrients, 12:3225. doi:10.3390/nu12113225. Munukka et al. (2014). J. Hepatol. 61: 132-8. doi:10.1016/j.jhep.2014.02.020.

PCB089

Effect of GSG1L on the modulation of calcium-permeable AMPA receptor single-channel conductance by intracellular spermine

Thomas McGee[1], Cecile Bats[1], Mark Farrant[1], Stuart Cull-Candy[1]

[1]University College London, London, United Kingdom

Calcium-permeable AMPA-type glutamate receptors (CP-AMPARs) play essential roles in synaptic transmission and plasticity in the CNS. Type 1 transmembrane AMPAR regulatory proteins (TARPs) are CP-AMPAR auxiliary subunits that slow gating, increase single-channel conductance and relieve channel block by intracellular polyamines (Soto et al., 2007). Conversely, the atypical auxiliary subunit GSG1L (germ cell-specific gene 1-like protein) (Shanks et al., 2012; Schwenk., et al 2012) decreases single-channel conductance and increases polyamine block, suppressing current flow and excitatory synaptic transmission (McGee et al., 2015; Gu et al., 2016). The aim of this study was to investigate the role of polyamines in the opposing effects of these different auxiliary subunits by examining the effect of spermine on CP-AMPARs expressed with and without GSG1L and the type 1 TARP γ2. AMPARs were transiently transfected into Human Embryonic Kidney (HEK 293) cells and currents were recorded from outside-out patches in response to rapid application of glutamate (10 mM) achieved through piezoelectric translation of a theta-barrel application tool. Macroscopic current-voltage relationships were examined from −120 to +100 mV to characterise spermine-dependent rectification. To estimate the weighted mean single-channel conductance we used nonstationary fluctuation analysis (NSFA). We also analysed directly resolved channel openings in the tail of macroscopic currents at −120 mV. In the absence of auxiliary subunits, elevating intracellular spermine from 100 μM to 1 mM unexpectedly, decreased single-channel conductance of GluA2(Q), as measured with NSFA, by ~50% (from 20.6 ± 1.6 to 9.1 ± 2.2 pS; $p < 0.0001$; Welch *t* test; $n = 12$ and 5, respectively). As TARP γ2 greatly reduces the block of CP-AMPARs by intracellular spermine, we predicted that in its presence the polyamine-dependent reduction of GluA2(Q) conductance would also be reduced. This was indeed the case. With co-expression of γ2 the effect spermine was eliminated (30.9 ± 2.3 and 27.4 ± 3.7 pS with 100 μM and 1 mM spermine, respectively; $p = 0.45$; unpaired Welch two-sample *t* test; $n = 10$ and 4). In contrast, GSG1L increased the effect of spermine on channel conductance. In the presence of just 100 μM intracellular spermine, the single-channel conductance was 12.7 ± 0.6 pS ($n = 18$; $p = 0.00031$ compared to GluA2(Q) alone and $p = 0.011$ compared to the spermine-free condition; $n = 14$). This reduction in NSFA-estimated single-channel conductance of GluA2(Q)/GSG1L by 100 μM spermine was mirrored by its effect on the mean amplitude of directly resolved single-channel events (reduced from 21.3 ± 1.4 to 15.7 ± 0.3 pS; $p = 0.016$; unpaired Welch two-sample *t* test; $n = 5$ and 5). Finally, we observed that GSG1L and spermine reduced channel conductance only when aspartate was present at the AMPARs Q/R +4 site in the channel's ion selectivity filter. Together, our results demonstrate that polyamines and GSG1L cooperate to attenuate CP-AMPAR conductance. Crucially, this unexpected property of intracellular polyamines is apparent at physiologically relevant negative membrane potentials.

Gu X et al. (2016) Nat Commun 7:10873. McGee TP et al. (2015) J Neurosci 35:16171-16179. Soto D et al. (2007) Nat Neurosci 10:1260-1267. Schwenk J et al. (2012) Neuron 74:621-633. Shanks NF et al. (2012) Cell Rep 1:590-598.

PCB090

The role of BK in glioblastoma multiforme membrane potential

Mathuscha Ratnasingham[1], Michaela Griffin[2], Stuart Smith[2,3], Jonathan Rowlinson[2], Paul Smith[4]

[1]School of Life Science, University of Nottingham, Nottingham, United Kingdom, [2]Children's Brain Tumour Research Centre, Biodiscovery Institute, University of Nottingham, Nottingham, United Kingdom, [3]Department of Neurosurgery, Queens Medical Centre, Nottingham, United Kingdom, [4]School of Life Science, University of Nottingham, Nottingham, United Kingdom

Introduction

Glioblastoma multiforme (GBM), is an aggressive brain tumour that accounts for nearly half of all glial brain tumours. Large conductance voltage and Ca^{2+}-activated potassium channels, BK, are overexpressed in GBM and are thought to play a role in their invasion and migration. Although these processes are modulated by changes in resting membrane potential, Vm, little is known about the origin of Vm and the role of BK. We have used cell-attached and whole-cell patch clamp in the glioblastoma cell line, SF188 to investigate the role of BK in GBM Vm.

Method

Single-channel BK currents were measured with cell-attached patch-clamp (CA). Pipettes contained 140 mM K^+. Currents were measured with holding potentials from 0 mV to -90mV. Vm of intact cells were estimated from the reversal potential of CA single-channel BK current-voltage plots (i-V) . Vm was then also measured with current clamp immediately after forming the whole-cell (WC) configuration. WC current-voltage-relationships (I-V) were characterised with voltage step protocol from a holding potential of -60mV. Data were normally distributed and are expressed as means ± S.D with n the number of cells. Statistical significance is defined as $p<0.05$ with tests stated.

Results

At a pipette-potential of 0 mV, KB was spontaneously active in 23 out of 49 CA patches. CA I-V analyses indicated voltage-dependent activation of BK with a median slope-conductance of 202 pS. Vm estimated from the BK I-V reversal potential, -34.9±10.9 mV (n=21) was similar (p=0.9221, Paired t-test) to that subsequently measured under WC current clamp: -29.7±13 mV (n=7) ([Ca^{2+}] = 45 nM). With a high [Ca^{2+}] pipette solution (1.5 mM) Vm became significantly hyperpolarized in WC current clamp (-46.4±16.3 mV, n=14; p=0.001, Unpaired t-test). With the high pipette [Ca^{2+}] the WC input resistance, Rm, was 221±194 MW (n = 8; p=0.0443, Unpaired t-test); a value significantly smaller (p=0.0443, Unpaired t-test) to that measured with low pipette [Ca^{2+}]: 394±194 MW (n=8). In 100% of CA patches, BK activity was abolished following perfusion of either 1 µM paxilline (n=3) or 200 µM quinine (n=3). With a low pipette [Ca^{2+}], WC Vm was unaffected by 1 mM TEA (n=15) or 1 µM paxilline (n=7) , but was significantly depolarised by 21±3.3 mV with 200 µM quinine (n=3) relative to perfusion control (n=4; p=0.0091 ANOVA Dunnet's multiple comparison test).

Conclusion

GBM SF188 cells exhibit spontaneous K^+ channel activity in CA patches, with biophysical and pharmacological properties typical for BK. At low intracellular $[Ca^{2+}]$ BK does not appear to be responsible for the resting Vm, however the hyperpolarization of Vm associated with a decrease in Rm that is seen on elevation of $[Ca^{2+}]_i$ are indicative of BK activation The reversal potential of BK in CA patches appears to be an accurate non-invasive measure of the resting membrane potential of SF188 cells. Further studies are required to determine what underlies the BK activation observed in CA patches on SF188 and to find under what physiological conditions does BK become activated to contribute to Vm in this cell line.

PCB091

Inra-Arterial Injection of Bradykinin In Femoral Artery Elicits Cardiorespiratory Reflexes Involving Perivascular Afferents In Rat Models

Sanjeev Kumar Singh[1]

[1]*Institute of Medical Sciences, Banaras Hindu University, Varanasi, India*

Introduction: The physiology of baroreceptors and chemoreceptors present in the heart is well documented in regulation of cardiorespiratory functions. Since large blood vessels of the heart and peripheral blood vessels are of same origin, therefore, the involvement of the peripheral blood vessels in regulation of cardiorespiratory system can be anticipated. **Aims:** In this study, role of peripheral blood vessels in regulation of cardiorespiratory system was examined using bradykinin (BK) as a nociceptive tool. **Methods:** Ethical Approval was taken from the Institutional Ethical Committee, Banaras Hindu University, Varanasi, India prior to the beginning of the experiments. Role of perivascular sensory nerves in mediating cardiorespiratory responses produced after intra-arterial injection of BK (1 μM, a pure nociceptive agent) was examined in urethane anesthetized male rats. Respiratory frequency, blood pressure, and heart rate were recorded for 30 min after the retrograde injection of BK/diclofenac/saline. Additionally, paw edema was estimated and water content was expressed as percentage of wet weight. **Results:** The results are presented as mean ± SEM values. Injection of BK produced immediate tachypnoeic (86 ± 2.7 to 125 ± 5.6 per min), hypotensive (82 ± 4.1 to 49 ± 3.6 mm Hg) and bradycardiac (321 ± 4.2 to 266 ± 5.2 beats/min) responses of a shorter latency i.e. 5-8 s. Injection of equi-volume of saline did not produce any responses and served as time matched control. Paw edema was observed in the ipsilateral hind limb and contralateral hind limb as control. Ipsilateral femoral and sciatic nerve sectioning attenuated BK-induced tachypnoeic (87 ± 2.3 to 98 ± 3.2 per min), hypotensive (89 ± 3.5 to 75 ± 5.8 mm Hg) and bradycardiac (316 ± 6.2 to 303 ± 7.3 beats/min) responses significantly which indicate the origin of responses from the local vascular bed. Pretreatment with diclofenac sodium significantly attenuated the BK-induced tachypnoeic, (88 ± 3.1 to 94 ± 3.3 per min) hypotensive hypotensive (85 ± 1.8 to 78 ± 1.8 mm Hg) and bradycardiac (320 ± 5.1 to 308 ± 5.9 beats/min) responses and also blocked the paw edema. Post-Hoc correction using Dunnett's t-test (two sided) and Student's t-test for paired observations were used and a p value < 0.05 was considered as significant. **Conclusions:** Administration of BK in the segment of an artery produced reflex cardiorespiratory changes by stimulating the nociceptors surrounding the blood vessels involving prostaglandins. This is a novel study exhibiting the role of peripheral blood vessels in regulation of cardiorespiratory system.

1. Bagchi S, Deshpande SB: Indian red scorpion venom-induced augmentation of cardiac reflexes is mediated through the mechanisms involving kinins in urethane anesthetized rats. Toxicon. 36, 309-320 (1998) 2. Davis AJ, Perkin MN: The involvement of bradykinin B1 and B2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in rat. Br. J. Pharmacol. 113, 63-68 (1994) 3. Deshpande SB, Bagchi S, Rai OP, Arya NC: Pulmonary edema produced by scorpion venom augments phenylediguanide-induced reflex response in anesthetized rats. J. Physiol. 521, 537-544 (1999) 4. Donnerer J, Lembeck F: Analysis of the effects of intravenously injected capsaicin in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 320, 54-57 (1982) 5. Donnerer J, Lembeck F: Capsaicin-induced reflex fall in rat blood pressure is mediated by afferent substance P-containing neurons via a reflex centre in the brainstem. Naunyn-Schmiedeberg's Arch. Pharmacol. 324, 293-295 (1983) 6. Julieus D, Basabaum AI: Molecular

mechanism of nociception. Nature. 413, 203-210 (2001) 7. McQueen DS, Bond SM, Moores C, Chessell L, Humphrey PPA, Dowd E: Activation of P2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anesthetized rats. J. Physiol. 359, 1-18 (1998) 8. Singh SK, Deshpande SB: Injection of Mesobuthus tamulus venom in distal segment of femoral artery evokes hyperventilatory and hypertensive responses in anesthetised rats. Neurosci. Lett. 438, 64-66 (2008a) 9. Singh SK, Deshpande SB: Intra-arterial injection of Mesobuthus tamulus venom elicits cardiorespiratory reflexes involving perivascular afferents. Toxicon. 46, 820-826 (2005) 10. Singh SK, Deshpande SB: Vasosensory responses elicited by Indian red scorpion venom last longer than capsaicin-induced responses. Ind. J. Exp. Biol. 46, 755-759 (2008b)

PCB092

Testing the Albus model of cerebellar learning in human subjects

Neil Todd[1], Sendhil Govender[2], Peter Keller[3], James Colebatch[1]

[1]School of Clinical Medicine, University of New South Wales, Sydney, Australia, [2]Neuroscience Research Australia, UNSW, Sydney, Australia, [3]Center for Music in the Brain, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

The cerebellum is accepted to have a crucial role in classical conditioning. Following the classical work of Eccles and co-workers in elucidating its detailed neurophysiology (Eccles et al., 1967), several theorists developed computational models of cerebellar learning (Albus, 1971). In Albus' original conception, the so-called 'inactivation response' of a Purkinje cell (PC), a pause in PC spontaneous activity associated with a climbing-fibre response (CFR), could be interpreted as the internal neural representation of the overt unconditional response (UR). He further suggested that mossy/parallel fibre (MF/PF) activity produced by the conditional stimulus (CS) could be considered its internal neural representation. The effect of learning, by changing PF-PC synaptic weights with conjunctive CF/PF inputs, and the acquisition of a conditioned response would, he hypothesized, be accompanied by a conditioned pause of Purkinje neurones. Direct recordings from animal models have since provided evidence to support the Albus hypothesis. There is also strong evidence from the effects of lesions in humans that the cerebellum is required for the acquisition of classically conditioned eye blink responses. However, to date the Albus model has not been directly tested in intact human subjects.

It had been widely thought that the cerebellum is particularly difficult to record from non-invasively. However, recent work using EEG/MEG techniques supports the view that non-invasive electrophysiology of the cerebellum is indeed viable. In our own work we have reported cerebellar evoked potentials produced by vestibular and axial stimuli from scalp electrodes from placed over the posterior fossa the properties of which are consistent with a CFR (Todd et al., 2017). In addition, we also observe an 'inactivation response' manifest as changes in the high frequency electrocerebellogram (ECeG: Todd et al, 2018) which has a higher frequency content than cerebral EEG. These observations suggest that it may be possible to directly test the Albus hypothesis in humans and this was the aim of the study reported here.

Electrophysiological activity was recorded in 14 healthy subjects (compliant with the Declaration of Helsinki) before, during and after a classical eye-blink conditioning procedure with a 500 ms auditory tone as CS and a maxillary nerve US (Todd et al 2023). Electrodes recorded EMG/EOG at peri-ocular sites, EEG over frontal eye-fields and the ECeG over the posterior fossa. ECeG high frequency power was computed using the continuous wavelet transform and each epoch segmented in time for statistical analysis before and after conditioning.

Of the 14 subjects half strongly conditioned while the other half were resistant. However, inhibition of cerebellar activity in the form of a significant reduction in high frequency ECeG power was observed in all subjects, prior to the CR, as shown in Figure 1. Pairwise comparisons of baseline, with conditioned and unconditioned pausing are given: *, $p < .05$, **, $< .01$, ***, $< .005$, ns = not significant.

We conclude that while conditioned cerebellar pausing may be necessary, it is not sufficient alone to produce overt behavioural conditioning, implying the existence of another central

mechanism. The outcomes of this experiment indicate the value of the non-invasive electrophysiology of the cerebellum.

Eccles JC, Ito M & Szentagothai J (1967). The Cerebellum as a Neuronal Machine. Springer-Verlag, Berlin. Albus JS (1971). A theory of cerebellar function. Math Biosci 10, 25-61. Todd NPM, Govender S & Colebatch JG (2017). The inion response revisited: evidence for a possible cerebellar contribution to vestibular-evoked potentials produced by air-conducted sound stimulation. J. Neurophysiol 117, 1000–1013. Todd NPM, Govender S & Colebatch JG (2018). The human electrocerebellogram (ECeG) recorded non-invasively using scalp electrodes. Neurosci Lett 682, 124–131. Todd NPM, Govender S, Keller PE & Colebatch JG (2023) Electrophysiological activity from over the cerebellum and cerebrum during eye blink conditioning in human subjects. Physiol Rep 11, e15642.

PL01

Enhancing student experience and graduate outcomes through inclusive physiology

David Greensmith[1]

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

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

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

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

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

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

PL02

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

Indira Raman[1]

[1]Northwestern University, Evanston, United States

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

PL03

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

Andrew Murray

undefined

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

PL04

“The Place of Physiology in the Neuroscience of Memory”

Richard G M Morris[1]

[1]*Edinburgh Neuroscience, The University of Edinburgh, Edinburgh, United Kingdom*

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

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

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

PL05

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

Anant Parekh[1]

[1]National Institutes of Health, USA, Durham, United States

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

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

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

PL06

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

Neil Herring[1]

[1]University of Oxford, Oxford, United Kingdom

William Bayliss and Ernest Starling are famous as pioneers in cardiovascular physiology but are also responsible the discovery of the first hormone (from the Greek “setting in motion”), the intestinal signalling molecule and neuropeptide secretin in 1902[1]. My research group focuses on neuropeptides and neuromodulators that influence cardiovascular autonomic control as potential biomarkers in disease and tractable targets for therapeutic intervention. Acute myocardial infarction (AMI) and chronic systolic heart failure (CHF) result in high levels of cardiac sympathetic stimulation, which is a poor prognostic indicator. Whilst beta-blockers improve mortality in these conditions by preventing the action of the neurotransmitter noradrenaline, a substantial residual risk remains. Recently, we have identified the sympathetic co-transmitter neuropeptide-Y (NPY) as being released during AMI, leading to larger infarcts[2] and life-threatening arrhythmia[3] in both animal models and patients. Moreover, in patients with severe CHF, local cardiac NPY levels correlate with mortality[4]. I will present recently published and unpublished data demonstrating that peripheral venous NPY levels are associated with heart failure hospitalisation and mortality after AMI[5], and all cause and cardiovascular mortality in CHF, even when adjusting for known risk factors (including BNP). We have investigated NPY expression in human and rat stellate ganglion and cardiac tissue and used human induced pluripotent stem cell (hiPSC) cardiomyocytes to manipulate NPY neurochemistry using state-of-the-art imaging techniques, establishing the receptor pathways responsible for NPY signalling. We propose NPY as a new mechanistic biomarker in AMI and CHF patients and aim to determine whether specific NPY receptor blockers can attenuate the development of heart failure.

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

SA01

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

Mark Boyett[1], Sunil Jit Logantha[2], Cali Anderson[3], Claire Wilson[2], Sana Yaar[3], Yanwen Wang[3], Munir Hussain[1], Henggui Zhang[3], George Hart[3], Morten Bækgaard Thomsen[6], John Cidlowski[7], Robert Oakley[7], Alicia D'Souza[3]

[1]University of Bradford, Bradford, United Kingdom, [2]University of Liverpool, Liverpool, United Kingdom, [3]University of Manchester, Manchester, United Kingdom, [4]University of Manchester, Manchester, United Kingdom, [5]University of Manchester, Manchester, United Kingdom, [6]University of Copenhagen, Copenhagen, Denmark, [7]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, United States, [8]National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, United States

In mammals, there is well known to be a day-night rhythm in the electrical activity of the heart (in heart rate, PR interval, QRS duration and QT interval) and arrhythmogenesis[1]. This has previously been attributed to short-term regulation of ionic conductances in the heart by the autonomic nervous system, but this explanation has been challenged[1]. Instead, recent studies of the mouse have provided an alternative explanation.

In the sinoatrial node, we have shown that there is an intrinsic mechanism that can explain or at least contribute to the day-night rhythm in heart rate: there is a day-night rhythm in ion channels, including the pacemaker channel HCN4, and block of HCN4 abolishes the day-night rhythm in heart rate *in vivo* and *in vitro*[2]. We have shown that there is a functioning circadian clock in the sinoatrial node that could be driving the day-night rhythm in ion channel gene transcription: in the case of HCN4 at least, knockout of the clock gene *Bmal1* abolishes the day-night rhythm in *Hcn4*[2]. However, data are emerging for a system of transcriptional 'combinatorial regulation' in which a specific combination of transcription factors is obligatory for gene transcription: there is a day-night rhythm in the sympathetic nervous system and chronic b-adrenergic receptor blockade also abolishes the day-night rhythm in ion channel transcripts (including *Hcn4*)[3]. For the atrioventricular node, a similar picture is emerging: there is day-night rhythm in ion channel transcripts and a functional circadian clock[4] and genetic knockout of *Bmal1* blunts the day-night rhythm in the PR interval (unpublished data). Again the picture is similar for the ventricles with a day-night rhythm in ion channel transcripts and a functional circadian clock[3]; the day-night rhythm in ion channel transcripts is suggested to be responsible for the well-known vulnerability to ventricular tachyarrhythmias at the start of the awake period. Once again there is evidence of combinatorial regulation: genetic knockout of *Bmal1* abolishes the vulnerability to ventricular tachyarrhythmias at the start of the awake period[5]; chronic b-adrenergic receptor blockade abolishes the day-night rhythm in ion channel transcripts[3]; and RU486 (an antagonist to the glucocorticoid receptor, Nr3c1; of interest because there is a day-night rhythm in plasma corticosteroid) again abolishes the day-night rhythm in ion channel transcripts as well as the vulnerability to ventricular tachyarrhythmias at the start of the awake period (unpublished data). ATAC-seq has shown a day-night rhythm in accessibility to certain genes (chromatin has to be made accessible for transcription to take place) and in many cases of genes showing a day-night rhythm in accessibility there is a consensus binding site for Nr3c1 (a transcription factor as well as a receptor) (unpublished data). Genetic knockout of Nr3c1 also abolishes the

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

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

SA02

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

Sami Al Othman[1], Luca Soattin[1], Matthew Smith[1], Min Zi[1], Elizabeth Cartwright[1], Mark Boyett[2], Alicia D'Souza[1,3]

[1]University of Manchester, Manchester, United Kingdom, [2]University of Bradford, Bradford, United Kingdom, [3]Imperial College London, London, United Kingdom

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

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

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

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

SA03

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

Stefanie Fenske[1,2,3]

[1]Center for Drug Research, Department of Pharmacy, Ludwig-Maximilians-Universität München, Munich, Germany, [2]German Center for Cardiovascular Research (DZHK), Partner Site Munich Heart Alliance, Munich, Germany, [3]Institute of Cardiovascular Physiology and Pathophysiology, Biomedical Center Munich, Munich, Germany

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

SA04

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

Colleen Deane[1], Craig Willis[2], Bethan Phillips[3], Philip Atherton[3], Lorna Harries[4], Ryan Ames[4], Nathaniel Szewczyk[5], Timothy Etheridge[4]

[1]University of Southampton, Southampton, United Kingdom, [2]University of Bradford, Bradford, United Kingdom, [3]University of Nottingham, Nottingham, United Kingdom, [4]University of Exeter, Exeter, United Kingdom, [5]Ohio University, Ohio, United States

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

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

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

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

SA06

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

Cátia Lourenço[1,2,3], João Gonçalves[1,2,3], João Laranjinha[1,2,3]

[1]Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal, [2]Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [3]Center for Innovative Biomedicine and Biotechnology, Coimbra, Portugal

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

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

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

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

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

SA07

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

Jonathan Moore[2]

[1]Bangor University, Department of Sport & Exercise Sciences, College of Human Sciences, Bangor, United Kingdom, [2]Department of Sport & Exercise Sciences, College of Human Sciences, Bangor University, Bangor, Gwynedd, United Kingdom

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

SA08

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

Emma O'Donnell[1]

[1]Loughborough University, Loughborough, United Kingdom

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

SA09

Blood pressure control during exercise: implications for hypertension

Benjamin Chant[1], Thomas Hinton[2], Angus K. Nightingale[1,3,4], Julian Paton[5], Emma Hart[1], Zoe Adams[1]

[1]School of Physiology, Pharmacology, and Neuroscience, University of Bristol, Bristol, United Kingdom, [2]School of Physiology, Pharmacology, and Neuroscience, University of Bristol, Bristol, United Kingdom, [3]Bristol Medical School, University of Bristol, Bristol, United Kingdom, [4]Department of Cardiology, Bristol Heart Institute, University Hospitals Bristol National Health Service (NHS), Bristol, United Kingdom, [5]Department of Physiology, University of Auckland, New Zealand, United Kingdom

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

SA10

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

Catherine Notarius[1]

[1]Division of Cardiology, University Health Network and Sinai Health; University of Toronto, Toronto, ON, Canada

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

SA11

Viroporins: structure, function and potential as antiviral targets

Stephen Griffin[1]

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

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

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

SA12

Role and purpose of microbial rhodopsins in giant viruses.

Dmitrii Zabelskii[1], Kirill Kovalev[2], Alexey Alekseev[3,4], Dmitry Bratanov[5], Valentin Gordeliy[5,6]

[1]European X-Ray Free-Electron Laser Facility GmbH, Schenefeld, Germany, [2]European Molecular Biology Laboratory, Hamburg, Germany, [3]Advanced Optogenes Group, Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen, Göttingen, Germany, [4]Cluster of Excellence "Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells" (MBExC), University of Göttingen, Göttingen, Germany, [5]Institute of Biological Information Processing (IBI-7: Structural Biochemistry), Forschungszentrum Jülich, Jülich, Germany, [6]University Grenoble Alpes, CEA, CNRS, IBS, Grenoble, Grenoble, France

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

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

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

References: Bratanov D, Kovalev K, Machtens J-P, et al (2019) Unique structure and function of viral rhodopsins. Nat Commun 10:4939. https://doi.org/10.1038/s41467-019-12718-0 Ernst OP, Lodowski DT, Elstner M, et al (2014) Microbial and Animal Rhodopsins: Structures, Functions, and Molecular Mechanisms. Chem Rev 114:126–163. https://doi.org/10.1021/cr4003769 Volkov O, Kovalev K, Polovinkin V, et al (2017) Structural insights into ion conduction by channelrhodopsin 2. Science 358:eaan8862. https://doi.org/10.1126/science.aan8862 Yutin N, Koonin EV (2012) Proteorhodopsin genes in giant viruses. Biol Direct 7:34. https://doi.org/10.1186/1745-6150-7-34 Zabelskii D, Alekseev A, Kovalev K, et al (2020) Viral rhodopsins 1 are an unique family of light-gated cation channels. Nat Commun 11:5707. https://doi.org/10.1038/s41467-020-19457-7

SA13

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

Alexandria Miller[1], Patrick Houlihan[1], Ella Matamala[2], Deny Cabezas-Bratesco[2], Gi Young Lee[3], Ben Cristofori-Armstrong[1], Tanya Dilan[1], Silvia Sanchez-Martinez[1], Doreen Matthies[1], Rui Yan[1], Zhiheng Yu[1], Dejian Ren[3], Sebastian Brauchi[2], David Clapham[1]

[1]Janelia Research Campus/HHMI, Ashburn, VA, United States, [2]Physiology Institute and Millennium Nucleus of Ion Channel-Associated Diseases, Universidad Austral de Chile, Valdivia, Chile, [3]Department of Biology, University of Pennsylvania, Philadelphia, PA, United States

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

SA14

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

Nisha Charkoudian[1]

[1]*US Army Research Institute of Environmental Medicine, Natick, MA, United States*

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

SA15

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

Gabrielle Giersch[1]

[1]US Army Research Institute of Environmental Medicine, Natic, United States

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

Funded by USAMRDC; Author views are not representative of the Army/DoD.

SA16

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

Rebekah Lucas[1]

[1]*School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom*

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

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

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

SA17

Female thermal sensitivity across the life span: a hot journey

Davide Filingeri[1]

[1]THERMOSENSELAB, Skin Sensing Research Group, School of Health Sciences, The University of Southampton,, Southampton, United Kingdom

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

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

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

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

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

[1] Ebi et al (2021) Hot weather and heat extremes: health risks. The Lancet, 398 (10301). [2] Vargas et al (2023) Prioritize research on human behaviour during extreme heat. Nature Human Behaviour, 1-2. [3] Filingeri (2016) Neurophysiology of skin thermal sensations. Comprehensive Physiology, 6 (3), 1429-1491. [4] Hutchins et al (2021) Female (Under) Representation in

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

SA18

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

Sandra Villanueva[1,2], Tabata Apablaza[1,2], Bryan Muñoz[2,3], Carlos A. Flores[1,2]

[1]Facultad de Medicina y Ciencia, Universidad San Sebastián, Valdivia, Chile, [2]Centro de Estudios Científicos, Valdivia, Chile, [3]Universidad Austral de Chile, Valdivia, Chile

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

Mice were bred in the C57Bl6/J background and maintained in the Specific Pathogen Free mouse facility of Centro de Estudios Científicos (CECs) with access to food and water ad libitum. We used 6 and 8-week-old wild-type and *Cftr*$^{\Delta F508/\Delta F508}$ animals. The trachea was splitted in two sections: the upper trachea containing the submucosal glands and the rest of the lower trachea, down the cricoid cartilage, sliced in 5µm paraffin sections in the transverse and frontal plane respectively. Ionocytes were identified by immunofluorescence against the FOXI1 transcription factor. All values were expressed as mean±S.E.M. All animal procedures were approved by the institutional IACUC (CECS-2022-03).

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

Preliminary analysis of *Cftr*$^{\Delta F508/\Delta F508}$ tissues indicated that there were no differences in the amount of FOXI1+cells when compared to wild-type lower tracheas (2.0 ± 0.7 vs 1.9 ± 0.1 FOXI1+cells mm^{-1} basal lamina, respectively, n=2 each group). Unexpectedly, we observed that FOXI1+cells were lower in issues of 6-week-old than in those obtained from 8-week-oldwild type mice (2.0 ± 0.5 (n=2) vs 4.0 ± 0.6 (n=5) FOXI1+cells mm^{-1} basal lamina, respectively, p= 0.078; Rank Sum Test).

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

SA19

Morphological, molecular and functional analysis of airway epithelial cell types

Arianna Venturini[1], Vito Terlizzi[2], Luis JV Galietta[1]

[1]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy, [2]Department of Pediatric Medicine, Meyer Children's Hospital IRCCS, Cystic Fibrosis Regional Reference Center, Florence, Italy

The airway epithelium, the first barrier against pathogens, is endowed with active antimicrobial mechanisms and consists of different cell types, from the most abundant ciliated, goblet and basal cells to rare pulmonary neuroendocrine and tuft cells. Recently, different studies have revealed the existence of a new rare cell population, named ionocytes (1-2), characterized by a high expression of the CFTR chloride channel and the transcription factor Forkead Box I1 (FOXI1). In cystic fibrosis (CF), impaired CFTR function results in dehydration of airway surface, mucus accumulation and bacterial colonization. The airway epithelium is also the entry site for a variety of viruses, like SARS-CoV-2, who enters the cells by binding to the angiotensin-converting enzyme 2 (ACE2) (3). Interestingly, CF patients, who are particularly sensitive to respiratory viral infections, do not seem to be at risk of severe COVID-19.

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

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

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

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

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

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

1. Plasschaert LW et al. (2018). Nature 560, (7718):377-381. 2. Montoro DT et al. (2018). Nature 560, 319–324. 3. Wu C et al. (2023). Cell 186, (1):112-130.e20. 4. Bezzerri V et al. (2023). Nat Commun, 14: 132.

SA20

Pulmonary Neuroendocrine Cells: Rare, but not Dispensable

Xin Sun[1]

[1]*University of California San Diego, La Jolla, United States*

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

SA21

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

Holly Shiels[1]

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

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

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

SA22

Pacing intracellular Ca2+ signals in exocrine cells

David Yule[1]

[1]*University of Rochester, Rochester, United States*

The major physiological function of exocrine acinar cells from the pancreas and salivary gland is the secretion of proteins and fluid which are initiated by changes in cytosolic Ca^{2+} following neurotransmitter or hormone exposure. It is established that the spatiotemporal characteristics of the Ca^{2+} signal are vitally important for the appropriate stimulation of secretion and these properties are often disrupted in disease states. Experiments performed in isolated tissue have documented the complexity of these signals including sub-cellularly restricted signals, intra and inter-cellular Ca^{2+} waves, and apparent pacing of signals within individual acinar clusters by initiator cells. Whether these characteristics are mirrored *in vivo* was not known. To address this question, we have generated mice expressing the genetically encoded Ca^{2+} indicator GCamp6f specifically in acinar cells and developed an imaging platform to study the characteristics of Ca^{2+} signals *in vivo* in anesthetized mice by multi-photon microscopy. In submandibular salivary acinar glands (SMG), we show that stimulation of intrinsic nerves to the gland result in rapid oscillatory Ca^{2+} signals following ACh release. These events are positively correlated with fluid secretion. These signals appear to initiate in specific cells within individual acini and propagate to neighboring cells. In pancreas, Ca^{2+} signals were observed following neural stimulation that were dependent on ACh release. Ca^{2+} signals as a function of elevated serum cholecystokinin were observed in fasted animals that were augmented in terms of the number of responding cells and peak response following feeding. Both nerve stimulation and CCK induced Ca^{2+} oscillations in pancreatic acini, but with markedly distinct temporal and spatial characteristics. We speculate that initiating cells in each gland are more directly stimulated, either by direct neural innervation or by proximity to the vasculature or alternatively represent cells most sensitive to secretagogue by virtue of receptor number. In total, these studies define the physiological characteristics of Ca^{2+} signals *in vivo* and the platform will be useful in future investigation of disruption of Ca^{2+} signaling in disease states of exocrine tissue.

SA23

Origin of rhythmicity in the bladder and urethra

Bernard Drumm[1], Caoimhin Griffin[1]

[1]Smooth Muscle Research Centre, Dundalk Institute of Technology, Dundalk, Ireland

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

SA24

Peristaltic pacemakers of the upper urinary tract

Nathan Grainger[1]

[1]*University of Nevada, Reno School of Medicine, Reno, United States*

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

SA25

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

Sophie Allen[1,2], Leigh Breen[1,2,3]

[1]School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom, [2]National Institute for Health Research, Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and University of Birmingham, Birmingham, United Kingdom, [3]MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, University of Birmingham, Birmingham, United Kingdom

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

Reference 1- Allen SL, Marshall RN, Edwards SJ, Lord JM, Lavery GG & Breen L. (2021). The effect of young and old ex vivo human serum on cellular protein synthesis and growth in an in vitro model of ageing. Am J Physiol Cell Physiol 321, C26-C37. Reference 2 - Allen SL, Seabright AP, Quinlan JI, Dhaliwal A, Williams FR, Fine NHF, Hodson DJ, Armstrong MJ, Elsharkaway AM, Greig CA, Lai Y-C, Lord JM, Lavery GG & Breen L. (2022). The Effect of Ex Vivo Human Serum from Liver Disease Patients on Cellular Protein Synthesis and Growth. Cells 11. Reference 3 - Kalampouka I, van Bekhoven A & Elliott BT. (2018). Differing effects of younger and older human plasma on C2C12 myocytes in vitro. Front Physiol 9.

SA26

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

Sophie Joanisse[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom

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

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

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

1. Ekenros L, Papoutsi Z, Fridén C, Dahlman Wright K, Lindén Hirschberg A. Expression of sex steroid hormone receptors in human skeletal muscle during the menstrual cycle. Acta Physiol (Oxf). 2017 Feb;219(2):486-493. 2. Tiidus PM. Can oestrogen influence skeletal muscle damage, inflammation, and repair? Br J Sports Med. 2005 May;39(5):251-3. 3. Gras, F., Brunmair, B., Quarré, L. et al. Progesterone impairs cell respiration and suppresses a compensatory increase in glucose transport in isolated rat skeletal muscle: a non-genomic mechanism contributing to metabolic adaptation to late pregnancy?. Diabetologia 50, 2544–2552 (2007). 4. Mamchaoui, K., Trollet, C., Bigot, A. et al. Immortalized pathological human myoblasts: towards a universal tool for the study of neuromuscular disorders. Skeletal Muscle 1, 34 (2011).

SA27

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

Brian Carson[1]

[1]*Health Research Institute, University of Limerick, Limerick, Ireland*

Background:

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

Methods:

Changes in cell behavior and adhesion properties were monitored by measuring impedance via interdigitated microelectrodes using the xCELLigence system. MPS was measured by puromycin incorporation using the SUnSET technique, intracellular signalling measured by western blot, and myotube thickness by microscopy. To establish the ability of the bioassay to measure the humoral effect of MPS in response to protein feeding, media was conditioned by *ex vivo* human serum from fasted, and protein-fed conditions. To evaluate MPS in response to protein feeding *in vivo,* acute MPS (5 h) was assessed by measuring stable isotope deuterium oxide (D_2O) incorporation into m. vastus lateralis skeletal muscle following consumption of sustainable proteins compared with a non-essential amino acid (NEAA) formulation.

Results:

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

Conclusion:

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

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

SA28

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

Sabine Uijl[1]

[1]*Utrecht University, Utrecht, Netherlands*

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

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

SA29

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

Louise Robson[1]

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

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

SA30

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

Bruno Grassi[1], Giovanni Baldassarre[1], Caterina Ursella[1], Lucrezia Zuccarelli[1]

[1]Department of Medicine, University of Udine, Udine, Italy

In most activities related to work or leisure the energy for muscle work substantially comes from oxidative metabolism. Impairments of this metabolism can significantly affect exercise tolerance and performance, may significantly affect the patient's clinical picture and quality of life, and represent an important predictor of mortality. Near-infreared spectroscopy (NIRS) can offer insights into the physiological and pathophysiological adaptations to conditions of increased O_2 needs which involve, in an integrated manner, different organs and systems of the body. In terms of patient evaluation, NIRS allows to determine the evolution of the functional impairments, identifies their correlations with clinical symptoms, evaluates the effects of therapeutic or rehabilitative interventions, and allows to to gain pathophysiological insights[1,2].

Strengths and limitations of NIRS have been discussed in recent reviews[1,3]. Skeletal muscle fractional O_2 extraction (SMFOE), the main variable evaluated by NIRS, is conceptually homologous to arterial-venous O_2 concentration difference, and is the result of the dynamic balance between O_2 utilization and O_2 delivery in the tissue under consideration[1,3]. The reduced peak SMFOE during an incremental exercise identified and quantified the incapacity to increase O_2 extraction (one of the key pathophysiological mechanisms of these diseases) in patients with mitochondrial myopathyes or McArdle disease[2]. SMFOE allowed, in these patients, insights into the mechanisms responsible for the positive effects of exercise training,[2] and in McArdle patients into the pathophysiology of the “second wind”[2]. Impairments of oxidative metabolism, expressed as reduced SMFOE peak, were described in several other pathological conditions[1] and after exposure to bed-rest/microgravity and/or hypoxia[4].

The slope of the linear SMFOE increase at intermediate work rates, during an incremental exercise, allowed inferences in the adequacy of O_2 delivery in patients with chronic heart failure[5] or in heart transplant recipents[6].The plateau of SMFOE at high work rates has been the object of active research in terms of its associations with variables such as critical power, maximal lactate steady state, respiratory compensation point[7].

SMFOE during the rest-to exercise transition was utilized to evaluate the adequacy of the adjustment of microvascular O_2 delivery *vs.* that of O_2 uptake, which was impaired in patients with chronic heart failure[1], metabolic myopathies[2], in subjects exposed to nicrogravity/bed-rest[8].

During a transient muscle ischemia obtained by cuff inflation, the rate of deoxygenation determined by NIRS indicates muscle $V'O_2$[3]. By adopting rapid inflation-deflation protocols during the recovery from exercise, NIRS allowed to determine muscle $V'O_2$ off-kinetics, mirror image of [PCr] kinetics and a classic index of functional evaluation of oxidative metabolism[9]; studies have been performed in patients[9], healthy subjects[9-10], subjects exposed to microgravity/bed-rest[11]. A modification of the rapid inflation-deflation protocol allowed to specifically investigate peripheral O_2 diffusion[12]. The rate of reoxygenation following a transient muscle ischemia evaluates the microvascular response to an ischemic stress (“reactive hyperemia”)[3]. Insights into peripheral O_2 diffusion can be obtained by analysis of changes of the

total (oxygenated + deoxygenayed) Hb signal, reflecting changes in capillary hematocrit[3]. Exciting new perspectives (simultaneous measurements of microvascular blood flow, SMFOE and regional oxidative metabolic rate) have been raised by diffuse correlation spectroscopy[13].

1. Grassi B, V Quaresima. J Biomed Optics 21(9): 091313, 2016. 2. Grassi B et al. Med Sci Sports Exerc 51: 2183-2192, 2019. 3. Barstow TJ et al. J Appl Physiol 126: 1360-1373, 2019. 4. Salvadego D et al. J Physiol 596: 3341-3355, 2018. 5. Mezzani A et al. Int J Cardiol 167: 2189-2195, 2013. 6. Lanfranconi F et al. Med Sci Sports Exerc 38: 1374-1383, 2006. 7. Keir DA et al. Med Sci Sports Exerc 50: 2375-2378, 2018. 8. Porcelli S et al. J Appl Physiol 109: 101-111, 2010. 9. Adami A, HB Rossiter. J Appl Physiol 124: 245-248, 1018. 10. Zuccarelli L et al. J Appl Physiol 128: 534-540, 2020. 11. Zuccarelli L et al. J Physiol 599: 4183-4829, 2021. 12. Pilotto A et al. J Physiol 600: 4153-4168, 2022. 13. Quaresima V et al. J Appl Physiol 127: 1328-1337, 2019.

SA31

Measuring cerebrovascular function in humans in response to dietary interventions

Catarina Rendeiro[1,2]

[1]School of Sport, Exercise & Rehabilitation Sciences, University of Birmingham, B15 2TT, Birmingham, United Kingdom, [2]Centre for Human Brain Health (CHBH), University of Birmingham, Birmingham, United Kingdom

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

We have conducted randomized, counterbalanced, double-blind, placebo controlled, within-subject acute studies in healthy young adults to investigate the effects of one single dose of cocoa flavonoids (flavanols) on prefrontal cortical oxygenation using Near Infrared Spectroscopy (NIRS). In our first study (N=18), we showed that flavanol intake leads to faster (approx. 1 min; $p < 0.001$) and greater brain oxygenation ($p=0.030$ *for Oxygenated Haemoglobin,* O_2Hb) in response to hypercapnia (5% CO_2), as well as higher performance in a Stroop Task, only when cognitive demand is high ($p=0.045$). We further observed that only participants who benefitted from flavanol intake during hypercapnia, also demonstrated improvements during cognitive performance (1). More recently we have also shown that cocoa flavanols might be beneficial in the context acute mental stress. During periods of stress, individuals often increase their consumption of unhealthy foods, especially high fat foods, and it is well established that both fat and mental stress alone can negatively impact peripheral vascular function. However, their effects on the cerebral vasculature are less understood. We have firstly demonstrated that a high-fat breakfast (56.5 g fat) impaired prefrontal cortical oxygenation ($p<0.05$ *for* O_2Hb *and Tissue Oxygenated Index, TOI*) during the mental stress episode in healthy young volunteers, in comparison to a low fat-breakfast (11.4 g fat) (N=19). In a follow-up study (N=23), we have further investigated whether a high-fat breakfast administered with an acute dose of cocoa flavanols may prevent fat-induced impairments in cortical oxygenation during stress. We are currently analysing this set of data and will be able to share at the meeting.

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

Gratton G et al., (2020). Scientific Reports. 10:19409.

SA32

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

Frederic Lange[1]

[1]UCL, London, United Kingdom

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

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

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

Secondly, I will show more recent work that has focused on combining diffuse correlation spectroscopy (DCS) with our broadband NIRS device to further extend the capacities of the system, in an instrument called FLORENCE [3]. Indeed, DCS is an established optical modality that enables non-invasive measurements of blood flow in deep tissue by quantifying the temporal light intensity fluctuations generated by dynamic scattering of moving red blood cells. Thus, the addition of DCS gives us access to blood flow information, enabling us to extract information about the cerebral metabolic rate of oxygen ($CMRO_2$). I will show the benefits of accessing all of this information in a clinical context.

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

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

[1] Durduran T et al. (2010). Reports Prog. Phys. 73(7), 076701. [2] Bale G et al. (2019). J. Cereb. Blood Flow Metab. 39(10), 2035–2047. [3] Tachtsidis I et al. (2021). Opt. InfoBase Conf. Pap.(December 2021), 1–4. [4] Lange F et al. (2019). IEEE J. Sel. Top. Quantum Electron. 25(1), 1–12. [5] Re R et al. (2016). Biomed. Opt. Express 7(2), 264.

SA33

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

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

undefined

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

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

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

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

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

SA34

The relationship between respiratory mechanics and neural control of respiratory muscles

Anna Hudson[1,2,3]

[1]Flinders University, Adelaide, Australia, [2]Neuroscience Research Australia, Sydney, Australia, [3]University of New South Wales, Sydney, Australia

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

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

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

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

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

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

De Troyer A et al. (2005). Physiol Rev 85, 717-756. Domnik NJ et al. (2020). Front Med 7, 483. Hudson AL et al. (2017). J Physiol 595, 7081-7092. Hudson AL et al. (2019). Exerc Sport Sci Rev 47, 157-168. Nguyen DAT et al. (2020). J Appl Physiol 128, 1262-1270.

SA35

Respiratory mechanics, breathlessness and exercise limitation in health and disease

Rebecca D'Cruz[1]

[1]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom

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

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

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

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

Dyspnea. Mechanisms, assessment, and management: a consensus statement. American Thoracic Society. Am J Respir Crit Care Med 1999; 159: 321–340 Jolley CJ, Moxham J. A physiological model of patient-reported breathlessness during daily activities in COPD. Eur Respir Rev 2009;18:66-79 Jolley CJ, Luo YM, Steier J, et al. Neural respiratory drive in healthy subjects and in COPD. Eur Respir J 2009;33:289-97 D'Cruz RF, Suh ES, Kaltsakas G, et al.

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

SA36

The effects of ageing on the respiratory physiology of exercise

William Sheel

undefined

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

SA37

The mechanisms of emmetropization

Frank Schaeffel[1,2]

[1]Institute of clinical and molecular Ophthalmology Basel (IOB), Basel, Switzerland, [2]Ophthalmic Research Institut, University of Tuebingen, Tuebingen, Germany

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

SA38

Aspects of Retinal Signalling in the Myopic Eye

Victoria Stapley[1], Roger Anderson[1,2], Kathryn Saunders[1], Padraig Mulholland[1,2]

[1]Centre for Optometry & Vision Science, Biomedical Sciences Research Institute, Ulster University, Coleraine, United Kingdom, [2]National Institute for Health & Care Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom

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

Aim: To investigate spatial and temporal summation in physiological myopia

Method: Spatial summation was investigated in 24 myopes (mean: -4.14DS, range: -0.50DS, -9.75DS) and 20 non-myopic controls (mean: +0.71DS, range: +1.75DS, -0.25DS) by measuring achromatic contrast thresholds for six stimuli varying in area (0.01–2.07 deg^2, 200ms). The effects of refractive error induced variations in retinal image size (RIS) were considered by correcting refractive error separately with (i) trial lenses placed at the anterior focal point (constant RIS in mm for all participants), and (ii) contact lenses (RIS increases in line with eye length). Temporal summation was investigated in a similar cohort (24 myopes, mean: -4.65DS, range: -1.00DS, -11.25DS; 21 controls, mean: +0.87DS, range -0.25D, +2.00D) by measuring achromatic contrast thresholds for six stimuli varying in duration (1.1 – 187.8ms, 0.43° diameter). RIS was kept constant. The upper limit of complete summation ('Ricco's Area' (RA) and 'Critical Duration' (CD) for spatial and temporal summation respectively) was estimated from the data with iterative two-phase regression analysis. Retinal temporal summation was also measured objectively using electrophysiology and analysing how the amplitude of response changed for stimuli of constant energy (3cd/m^2) but varying duration (0.5-100ms).

Results: With spectacle correction, RA was significantly larger (p=0.02, Mann Whitney U-test) in the myopes compared to controls (myopes median: -0.92 log deg^2, IQR: -1.10, -0.78; controls median: -1.14 log deg^2, IQR: -1.29, -1.07). However, for contact lens correction, there was no significant difference in RA (p=0.44) between groups (myopes median: -1.19 log deg^2, IQR: -1.28, -0.96; controls median: -1.14 log deg^2, IQR: -1.24, -0.87). There was also no significant difference in CD between groups measured psychophysically (myopes median: 44.3ms, IQR: 26.5, 51.2; controls median: 41.6ms, IQR: 27.3, 48.5), nor objectively with the electroretinogram.

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

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

SA39

Associations between myopia risk polymorphisms and retinal electrophysiology

Zihe Xu[1], Xiaofan Jiang[1,2], Christopher Hammond[1], Pirro Hysi[1], Omar Mahroo[1,2]

[1]King's College London, London, United Kingdom, [2]University College London, London, United Kingdom

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

SA40

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

Christopher Hammond[1], Pirro Hysi[1]

[1]Section of Ophthalmology, King's College London, London, United Kingdom

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

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

SA41

Structural insights into the mechanism of glycine transport and inhibition

Azadeh Shahsavar[1], Peter Stohler[3], Gleb Bourenkov[5], Iwan Zimmermann[4], Martin Siegrist[3], Wolfgang Guba[3], Emmanuel Pinard[3], Steffen Sinning[2], Markus A. Seeger[6], Thomas R. Schneider[5], Roger J. P. Dawson[4], Poul Nissen[2]

[1]University of Copenhagen, Copenhagen, Denmark, [2]Aarhus University, Aarhus, Denmark, [3]F. Hoffmann-La Roche, Basel, Switzerland, [4]Linkster Therapeutics, Zurich, Switzerland, [5]European Molecular Biology Laboratory, Hamburg, Germany, [6]University of Zurich, Zurich, Switzerland

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

SA42

The extended SLC Atlas: towards a unified view

Gergely Gyimesi[1], Matthias A Hediger[1]

[1]Membrane Transport Discovery Lab, Department of Nephrology and Hypertension and Department for BioMedical Research, Inselspital, University of Bern, Bern, Switzerland

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

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

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

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

to a more unified view of the complete cellular “SLC-ome”, essential for a thorough understanding of fundamental physiological and pathological processes.

Gyimesi G, Hediger MA. "Systematic in silico discovery of novel solute carrier-like proteins from proteomes." PLoS One. 2022;17: e0271062. doi:10.1371/journal.pone.0271062

SA43

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

Catriona Anderson[1]

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

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

SA44

The Role of SLC transporters in Host-Tumour Metabolic Interactions

Susumu Hirabayashi[1]

[1]MRC London Institute of Medical Sciences, London, United Kingdom

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

TEST

Textual Narratives: The subconstructive paradigm of consensus in the works of Gibson

Kim Wilson[1]

[1]*c, c, Cambodia*

If one examines the subconstructive paradigm of consensus, one is faced with a choice: either reject capitalist deconstruction or conclude that the establishment is fundamentally a legal fiction, given that Foucault's critique of the subconstructive paradigm of consensus is invalid. Therefore, Debord suggests the use of cultural neodialectic theory to analyse and modify class.

Bataille uses the term 'predialectic deconstructivist theory' to denote the role of the participant as artist. It could be said that Baudrillard promotes the use of subtextual discourse to deconstruct capitalism.

The premise of cultural neodialectic theory implies that the raison d'etre of the reader is significant form. However, if predialectic deconstructivist theory holds, the works of Gibson are postmodern.

Dietrich[1] holds that we have to choose between the subconstructive paradigm of consensus and semioticist theory. Therefore, Lyotard uses the term 'predialectic deconstructivist theory' to denote the bridge between society and class.

1. Dietrich, K. ed. (1981) *The subconstructive paradigm of consensus and predialectic deconstructivist theory.* Schlangekraft

2. McElwaine, N. O. G. (1977) *The Context of Rubicon: Predialectic deconstructivist theory in the works of Stone.* O'Reilly & Associates

3. Scuglia, C. ed. (1981) *Semanticist narrative, the subconstructive paradigm of consensus and rationalism.* Schlangekraft

4. Hubbard, K. B. (1976) *Reassessing Constructivism: Predialectic deconstructivist theory and the subconstructive paradigm of consensus.* O'Reilly & Associates