PCA001

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

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

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

The silencing of *Sesn2* resulted in a decrease in the phosphorylation of eNOS at the activatory (38.68±3.19% of control; p=0. 0002) and inhibitory (55.68±17.35% of control; p=0. 0008) sites compared to control. This decrease in eNOS phosphorylation was driven by a significant reduction in its protein expression in *Sesn2*-silenced cells compared to controls (49±27.03% of control; p=0.0294). Similarly, mRNA expression of *eNOS* was reduced in cells deficient for *Sesn2* (61.9±10.4% of control; p<0.0001). Interestingly, inhibiting the proteasome with MG132 did not reverse the effects of *Sesn2* silencing on eNOS expression. These findings suggest that Sesn2 plays a critical role in regulating eNOS expression and phosphorylation, likely through a mechanism independent of proteasomal degradation.

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

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

PCA002

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

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

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PCA003

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

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

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PCA004

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

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

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

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

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

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

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

PCA005

Circulating neuropeptide-Y dynamics during exercise in heart failure

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Introduction:

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

Method:

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

Results:

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

Conclusion:

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

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PCA006

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

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

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

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

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

PCA007

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

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

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

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

Clyde Biosciences tested the CiPA28 acute cardiotoxic reference compounds on the CellOPTIQ™ platform against Axol's ventricular cardiomyocytes. The cardiomyocytes were grown for 6 days in multiwell format and then transitioned to a serum-free media and loaded with a voltage sensitive dye. Optical measurement of voltage changes allowed the assessment of the effect of each of the 28 compounds (min. n=5), which have varying levels of known TdP risk. hERG block was detected in Axol cardiomyocytes with a range of compounds, as evidenced by action potential duration at 90% repolarisation (APD_{90}) prolongation, APD triangulation and early afterdepolarisations (EADs). For example, clear hERG block was detected at even the lowest conc. of the classic hERG blocker dofetilide (0.3nM) through QT-prolongation (500ms to 550ms) and increased triangulation and the Ca^{2+}-channel blocker nifedipine produced a shortened FPD (500ms to 300ms) while the low TdP risk anti-histamine loratidine only had minimal effects. In addition, the predictive power of Axol cardiomyocytes was reinforced when those compounds with multiple ion channel effects and varying risk profiles, such as verapamil, azimilide and ranolazine, modes of action and relative risks were

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

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

PCA008

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

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Introduction

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

Method

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

Results

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

Conclusions

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

PCA009

The influence of acute dietary nitrate supplementation on endothelial resistance to ischemia reperfusion injury in postmenopausal women

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With postmenopausal women expected to reach a population of 1.2 billion by 2030, there is a great need for low-risk, non-estrogen therapies for cardiovascular disease prevention. Evidence suggests that relative to the early follicular phase, women in the late follicular phase are protected against endothelial ischemia-reperfusion (IR) injury when estradiol concentrations are highest.[1,2] This suggests that the concurrent loss of estrogen following menopause may impair recovery from endothelial ischemia-reperfusion injury (ex. heart attack, bypass surgery).[3] Consumption of beetroot juice and other nitrate-rich foods (celery, spinach, lettuce, etc.) is an effective non-pharmaceutical intervention to increase systemic bioavailability of the vasoprotective molecule, nitric oxide, through the exogenous nitrate-nitrite-nitric oxide pathway. The purpose of this randomized, placebo-controlled, double-blind crossover clinical trial was to determine if a single dose of dietary nitrate supplementation, in the form of beetroot juice, can improve endothelial resistance to IR injury in postmenopausal women at two distinct stages of menopause. We hypothesized that a single dose of nitrate-rich beetroot juice would improve endothelial resistance to IR injury to a greater extent in early- compared to late-postmenopausal women. Early- (1-6 years following their final menstrual period (FMP), n=12) and late- (>6 years FMP, n=12) postmenopausal women consumed a single dose of nitrate-rich (600 mg/140 mL) and nitrate-depleted (placebo, 0 mg/140mL) beetroot juice. Study visits were separated by a washout period of at least two weeks. Whole arm endothelial IR injury was induced by inflating a pneumatic cuff (250 mmHg) for 20 minutes followed by 15 minutes of reperfusion. Brachial artery flow-mediated dilation (FMD, duplex ultrasound) was measured at baseline, acutely (90 minutes post-juice consumption), 15-, and 30 minutes after IR injury for each drink. Analyses with general linear models (SPSS) revealed a significant ($p<0.05$) time*treatment interaction effect for FMD. Pairwise comparisons revealed that FMD was significantly lower 15-minutes post-IR in comparison to all other time points with nitrate-depleted beetroot juice (Early- $FMD_{placebo}$=2.553.48%, Late- $FMD_{placebo}$=1.322.06) and was lower than post-IR with nitrate-rich beetroot juice (Early- $FMD_{nitrate}$=3.924.15%, Late- $FMD_{nitrate}$=3.242.67%, $p=0.014$). There was no significant interaction effect of menopausal stage. These results suggest that a single dose of dietary nitrate supplementation is sufficient to increase endothelial resistance to whole-arm IR injury to a similar extent in women at both stages of postmenopause. Our observations emphasize the endothelial protective benefits of dietary nitrate supplementation for postmenopausal vascular health.

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PCA010

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

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

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

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

This report reliably indicates that clinicians and adult patients should not differentiate between radiofrequency and cryoballoon ablation based on clinical efficacy and total complication rate but can reliably discriminate based on phrenic nerve injury rate and total procedure duration. Future high-quality, multicentre, randomised controlled trials with more subgroup classifications (like age, race, gender, paroxysmal versus non-paroxysmal atrial fibrillation) will produce data that applies to more specific patient groups. More rigorous and transparent reporting of study design and bias risk reduction is required to improve future study validity.

PCA011

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

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

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

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

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

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PCA012

Estrogen ameliorates cardiac function by reducing GRK2-mediated β1AR internalization during acute stress

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Introduction

Stress is an inevitable response to internal and external stimuli that can impair cardiac function, such as stress cardiomyopathy, arrhythmia, and sudden death [1]. Stress response is significantly regulated by the sympatho-adrenomedullary system, resulting in an increased secretion of catecholamines, which activates of β-adrenoceptors (βAR) [2]. During stress, GRK2, also known as β-adrenoceptor kinase 1 (βARK1), predominately translocates to the cell membrane to catalyze the phosphorylation of an already activated βAR, the main receptor that governs the inotropic and chronotropic effects of the heart. Evidence suggests that the female sex hormone estrogen could improve cardiac function during stress by regulating βAR-Gs/Gi signaling pathway [3]. However, the interplay between estrogen and GRK2 is not well understood.

Aim

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

Methods and results

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

WT mice were divided into sham-operated and ovariectomy groups. These mice and isolated adult cardiomyocytes were subcutaneously administered with isoproterenol (ISO) and estrogen (E2). The results showed that **(1)** Estrogen enhanced cardiac function by measuring ECG and cardiomyocytes' shortening amplitude, and improved APD (action potential duration), I_{Na}, I_{to}, $I_{Ca\text{-}L}$ of hESC-CMs by patch clam in acute stress. **(2)** Estrogen reduced total GRK2 and membrane GRK2 content in the myocardium during acute stress in mice and hESC-CM by western blot and immunofluorescence. **(3)** By inhibiting or overexpressing GRK2, the results showed that estrogen enhanced the contractile function and electrophysiological indexes of cardiomyocytes via inhibiting GRK2 in acute stress. **(4)** Estrogen reduced GRK2-mediated internalization of β_1AR in myocardium during acute stress by Immunoprecipitation and immunofluorescence.

WT and GPER -KO mice were also categorized into groups based on the subcutaneous administration of ISO. hESC-CM were subjected to GPER siRNA transinfection, then cells were pre-treated with ISO and estrogen. The results showed that **(1)** GPER attenuated GRK2-induced reduction in cardiac function during acute stress by measuring ECG and cardiomyocytes'shortening amplitude. **(2)** GPER reduced GRK2 content in the myocardium membrane and β_1AR internalization in acute stress by immunofluorescence.

Statistical analysis

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

Conclusion

Exciting new findings from our study demonstrate that estrogen has a powerful protective effect on the heart by reducing the content of GRK2 in the myocardium and preventing the internalization of β_1AR during acute stress. These findings not only shed light on the complex mechanisms underlying estrogen's beneficial effect on heart function but also highlight GRK2 as a crucial target for treating stress-induced heart disease. By targeting GRK2, we can potentially avoid the side effects of estrogen and develop more effective therapies for women with estrogen deficiencies or elderly patients.

Ethical standards

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

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PCA013

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

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

All work was undertaken in accordance with local ethical regulations and approval from the University of Bordeaux and in accordance with the European Parliament Directive 2010/63/EU. Surgical plane anesthesia was induced in Sheep (n=5; 50-65 kg) with 10 mg/kg sodium pentobarbital and maintained under isofluorane (2% in 100% O_2) prior to euthanasia by an intravenous injection of 2000 mg sodium pentobarbital. Hearts were quickly excised and perfused with cardioplegia and heparin. LV wedges were cannulated at the ostia and coronary-perfused with Tyrode. With the endocardial surface of the heart exposed, LV wedges were electrically stimulated (1.34-1.79 Hz) at the His Bundle by external electrodes. A suture was looped underneath the midpoint of a randomly selected single free-running Purkinje fibre and attached to a force transducer. Thus, raising the force transducer stretched the Purkinje fibre and indicated the timing and level of extending force applied. Endocardial Purkinje fibres were mechanically stimulated by applying a mean extending force of 4.86 ± 0.17 g for 10s, with 10s rest between each stretch. A pseudo-ECG was recorded and the effect of stretch on the number of ectopic excitations was tested by a Wilcoxon signed-rank test.

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

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

The stretch of individual Purkinje fibres led to an increase in the number of ectopic activations in preparations that were already showing ectopic activity. This suggests that Purkinje fibre stretch has the potential to cause electrical destabilisation in compromised tissue. This mode of investigation has the potential to investigate the role of Purkinje fibres as a source for stretch-induced ectopics or in the maintenance of arrhythmias. However, more detailed electrical mapping of ectopic initiation sites and their conduction is necessary.

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PCA014

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

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

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

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

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PCA015

Peripheral vascular function in white European and black African descent individuals

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

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

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

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

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

PCA016

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

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Background: Whether the adverse effects of glucocorticoids on left ventricular (LV) function are exacerbated by the consumption of a high-fructose diet, as a model of metabolic syndrome (MetS), is uncertain. This study aimed to determine whether the presence of MetS exacerbates glucocorticoid-induced changes in LV function. **Methods:** Sprague Dawley rats were divided into control, glucocorticoid (GC), high-fructose (HF), and glucocorticoid+high-fructose (GC+HF) groups. HF and GC+HF rats received 20% fructose solution in drinking water and GC and GC+HF rats received 10mg/kg intraperitoneal injections of methylprednisolone daily for 10 weeks. After 10 weeks, echocardiography was used to assess LV function and cardiac collagen content was determined by picrosirius-red staining. **Results:** Relative wall thickness (RWT) was increased in GC compared to control rats ($p=0.001$). Heart weights and LV weights indexed to body mass and RWT were increased in GC+HF compared to control rats ($p=0.04$, $p=0.009$, and $p=0.03$ respectively). Lateral e' was reduced in GC and GC+HF rats compared to control ($p=0.001$ and $p=0.005$ respectively) and HF ($p<0001$ and $p=0.0001$) rats. E/e' was increased in GC and GC+HF rats compared to control ($p<0.0001$ and $p=0.004$ respectively) and HF ($p<0.0001$ and $p=0.02$ respectively) rats. Cardiac collagen content was increased in GC and GC+HF rats compared to control rats ($p=0.001$ and $p<0.0001$ respectively). **Conclusion:** Administration of glucocorticoids caused concentric remodelling and impaired diastolic function by reducing LV relaxation and increasing filling pressures. The administration of glucocorticoids in a model of MetS caused concentric hypertrophy. However, the MetS did not exacerbate the diastolic dysfunction induced by the administration of glucocorticoids.

PCA017

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

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

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

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

Results: HRpeak was significantly higher in BFR (164.45 ± 15.03) compared with noBFR (143.45 ± 14.69, $p < 0{,}001$, $d = 5.30$), whereas HRR60% was significantly lower in BFR (0.19 ± 0.07) compared with noBFR (0.26 ± 0.06, $p = 0.013$, $d = 0.98$). There were no significant differences in any other HRR indices.

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

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

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

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PCA018

Mechanical stimulation of Piezo1 channels using high throughput automated patch clamp

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

Methods: Here we show that optimization of pipetting parameters coupled with the possibility to increase the number of cells per well in the NPC-384 chip of the SyncroPatch 384 lead to Piezo1-mediated currents activated by solely mechanical stimulation (M-Stim). Mouse and human Piezo1 channels expressed in HEK293 cells and untransfected HEK293 cells were tested by M-Stim in the absence and presence of Yoda1. Cells showing stable seal resistance and eliciting inward currents higher than −100 pA that could be inhibited by $GdCl_3$ (non-specific blocker of mechanosensitive channels) were considered Piezo1 responding cells.

Results: Our results strongly suggest that applying solutions on top of the cells at a fast pipetting speed is crucial for activating Piezo1 channels by M-Stim on the SP384. Moreover, stimulating 4 cells simultaneously in one well of a NPC-384 chip increased the current amplitudes at peak by 2-fold in mPiezo1, from −387.8 pA, 95% CI [297, 511.4] (n=148) to −894.3 pA, 95% CI [757.9, 1043] (n=579) and by 1.5-fold in hPiezo1, from −398,8 pA, 95% CI [332,5, 518] (n=174) to −612.8 pA, 95% CI [544.8, 685.5] (n=346) for hPiezo1. No currents were detected from the untrasfected cells. In line with this, the number of responding cells increased significantly from approximately 10% (both mPiezo1 and hPiezo1) to 64.17% ± 3.25% for mPiezo1 and to 52.75% ± 1.60% for hPiezo1 when using 4-hole chips. The number of responding cells was close to 100% when using 4-hole chips in combination with Yoda1.

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

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PCA019

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

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

Aims/Objectives: This study aimed to confirm, by blocking nitric oxide synthesis, if the relaxation of lauric acid involves other pathways aside from the nitric oxide pathway. Also, the inhibitory effect of lauric acid on calcium sensitivity was assessed as a possible alternative relaxation pathway, by evaluating its influence on Ca^{2+}- induced contraction.

Method: Ethical approval was obtained from the Ahmadu Bello University Animal Care and Use Committee and the rats were handled in compliance with the World Medical Association Declaration of Helsinki. Corpus cavernosum tissues from five euthanized male Wistar rats were extracted and mounted in an organ bath filled with Krebs solution. Using KCl and Phenylephrine as contractile agents in separate experiments to mimic sympathetic stimulation and depolarization respectively, relaxation responses to cumulative concentrations of lauric acid were evaluated in the control condition and in the presence of N-nitro-L-arginine methyl ester (L-NAME); a nitric oxide synthase inhibitor. In another experiment, to evaluate calcium sensitivity, the tissues were bathed in a Ca^{2+}- free physiological solution and pre-incubated with N, N-ethylene glycol tetraacetic acid (EGTA); a calcium chelating agent. Contraction responses to cumulative concentrations of Ca^{2+} in the control condition and in the presence of lauric acid were then evaluated. Results were presented as mean ± S.E.M. Data was analyzed using ANOVA. Values with $p < 0.05$ were considered significant.

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

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

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

Keywords: Lauric acid, Corpus cavernosum, Erectile function

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PCA020

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

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

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PCA021

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

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

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

Results: There were no significant differences between COVID-19 and non-COVID-19 groups for body mass index (26.9 ± 4.2 vs 26.5 ± 3.8 kg/m^2, p = 0.59), body surface area (1.8 ± 0.21 vs 1.8 ± 0.22 m^2, p = 0.217), resting systolic and diastolic blood pressures (systolic: 134 ± 17 vs 131 ± 17 mmHg, p = 0.392; diastolic: 83 ± 8 vs 81 ± 10 mmHg, p = 0.156), and HR, (62 ± 11 vs 60 ± 9 bpm, p = 0.880). At rest, participants in the COVID-19 group demonstrated significantly higher SV (77 ± 18 vs 66 ± 17 mL, p = 0.021) and CO (4.6 ± 1.1 vs 4.1 vs 0.8 L/min, p = 0.017) compared to the non-COVID group. At peak exercise, HR and diastolic blood pressure were significantly higher in the COVID-19 than non-COVID-19 group (HR: 137 ± 23 vs 128 ± 17 bpm, p = 0.037; diastolic blood pressure: 93 ± 12 vs 85 ± 12 mmHg, p = 0.001). There were no significant differences between the groups in peak exercise systolic blood pressure (198 ± 28 vs 197 ± 27 mmHg, p = 0.974), cardiac output (15.1 ± 4.5 vs 16.3 ± 14.0, p = 0.487), or stroke volume (110 ± 31 vs 106 ± 31, p = 0.739).

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

PCA022

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

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

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PCA023

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

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

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

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

Conclusions: We noted postnatal persistence of fetal circulatory adaptation even though detached from *in-utero* hypoxemic state. Greater CABF postnatally may reflect an altered metabolic state, fetal programming, higher prostaglandin levels in FGR, and effects of altered myocardial architecture leading to ↑ O_2 consumption/requirements, possibly a combination of all the above. While coronary perfusion was higher in FGR infants (heart sparing effect), this was not accompanied by improved cardiac function, mimicking fetal age dichotomy as well the dichotomy in the cerebral circulation (increased flow [brain sparing effect] but sub-optimal neurodevelopment). Secondly, FGR cohorts might be unable to further augment CABF as per acute need (already exhausted 'coronary flow reserve'). The failure of myocardium to relax (combined with intrinsic myocardial changes) and the coronary vascular resistance to drop in the face of increasing demand may heighten the risk of cardiovascular disease, when faced with additional workload such as strenuous exercise, obesity or hypertension. Epidemiologic studies

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

PCA024

Quantifying the functional implications of uncertainties in IKr and IKs in human atrial cellular electrophysiology

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

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

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

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

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

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

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PCA025

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

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

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

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

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

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

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PCA026

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

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

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

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

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

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PCA027

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

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

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

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

Discussion: This novel soapmaking practical provided a platform to improve core skills numeracy, analytical and research skills despite the limitations placed on practical laboratory classes. Our results indicate that the soapmaking practical is an effective way to enhance student understanding of skin physiology and experimental processes. Furthermore, the

practical enabled exploration of issues relating to health and safety such as toxicology, allergy and anaphylaxis which are important considerations for industrial manufacturing and helped to prepare medical sciences students for the demands of their future careers.

PCA029

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

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

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

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

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

A novel method of design was implemented to facilitate this process, the design sprint methodology (Grabill J, et al. 2022). This involved 3 phases of activity. The discovery phase involved two independent researchers holding one-to-one interviews with assessment experts in

the field externally, a variety of academic staff, current students and alumni. These 'lived experience' insights were then used in the second phase.

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

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

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

PCA030

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

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The murder of George Floyd, the degree awarding gap between Black and White students currently stands at 18.5%[1] and the School of Medicine's commitment to the British Medical Association charter to prevent and address racial harassment in 2020[2], collectively highlighted a need to critically appraise our course. The aim of the initiative was to determine whether we were serving our diverse student community with the appropriate content towards building an inclusive course curriculum and cocreating it with them.

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

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

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

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

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

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

PCA031

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

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

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

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

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

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

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

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

PCA032

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

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Introduction

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

Aim/Objective

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

Methods

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

Results

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

Conclusion

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

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

PCA033

Reduced maternal progesterone level increases apoptosis in rodent placenta

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

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binding sites favoring RNA polymerase II elongation. Nucleic Acids Res, 41(12), 6072-6086. https://doi.org/10.1093/nar/gkt32

PCA034

Effects of Incubating Temperature Manipulation on Sex Determination in Korat Chickens

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

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PCA035

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

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

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

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

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

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

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PCA036

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

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Introduction and Objectives:

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

Methods:

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

Results:

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

Conclusions:

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

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PCA037

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

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Introduction

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

Method

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

Results & Conclusions

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

PCA038

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

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

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

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

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

elderly secretory cells were shed, and cultures suffered greater epithelial damage with age. Dysfunctional repair pathways were stimulated, and there was an increase in basaloid-like cells that are associated with fibrosis markers and greater viral spread. We hypothesized that SARS-CoV-2 infected nasal epithelial cells undergo reprogramming by these mechanisms in an age-dependent manner and that these processes contribute to COVID-19 pathogenesis by delaying disease resolution and enhancing viral spread.

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

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

PCA039

Peripheral chemoreceptor sensitivity is elevated in patients with long COVID

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Post-COVID-19 syndrome (long COVID) describes ongoing symptoms for 12 or more weeks following infection with SARS-CoV-2. Respiratory-related symptoms including breathlessness [1], exercise intolerance and ventilatory dysfunction [2] are common in long COVID. Further, cells at the primary site of oxygen sensing (carotid bodies) are susceptible to SARS-CoV-2 infection [3]. We aimed to determine whether long COVID patients have altered carotid chemoreceptor sensitivity, by assessing resting hypoxic ventilatory response and ventilatory efficiency during exercise. Fourteen long COVID patients (3 male, 42 ± 12 years) and eight control participants (did not develop long COVID post-infection) gave written informed consent to participate in an ethically approved study (NHS REC). The control group was supplemented by six healthy individuals who undertook the same experimental procedures in the same laboratory, prior to the pandemic (in total; 2 male, 35 ± 13 years, *P*=0.1195 for age versus patient group). Participants did not have pre-existing respiratory or cardiovascular disease and resting lung function (spirometry) was similar between the groups. Transient hypoxia was achieved by supplementing inspired air with 100% N_2 for 5-8 short periods, lasting 5-30 seconds, reducing S_PO_2 to nadirs of between 65 and 99%. Breath-by-breath minute ventilation (V_E), tidal volume and breathing frequency, and beat-to-beat heart rate and blood pressure, were monitored during N_2 supplementation and for one minute afterwards. The 95th percentile of these variables and the nadir S_PO_2 were determined for each hypoxic period and entered into a simple linear regression, where the slope defined response to hypoxia (peripheral chemoreceptor sensitivity). The V_E response to hypoxia was greater in long COVID patients versus controls (-0.44 ± 0.23 versus -0.17 ± 0.13 L/min/S_PO_2, Figure; *P*=0.0007, independent samples T-test), demonstrating greater ventilation for a given fall in S_PO_2. This difference was caused by a larger tidal volume response, as breathing frequency response was similar. Heart rate and blood pressure responses were also unchanged (Figure). Participants performed a maximal exercise test (upright cycle ergometer, ramp protocol to volitional exhaustion). Long COVID patients had a lower peak VO_2 versus controls (18.6 ± 4.7 versus 26.7 ± 7.4 ml/kg/min, *P*=0.0015), despite reaching a similar peak V_E (66.4 ± 22.4 versus 71.5 ± 29.2 L/min, *P*=0.6012), respiratory exchange ratio (1.27 ± 0.09 versus 1.28 ± 0.11, *P*=0.7483), and percentage of predicted heart rate (92 ± 9 versus 97 ± 6 %, *P*=0.1326) to the control group. V_E/VCO_2 slope (ventilation for a given expired CO_2 volume) was greater in the long COVID group versus controls (37.8 ± 4.4 versus 31.4 ± 4.8, *P*=0.0008), indicating reduced ventilatory efficiency in the long COVID group. Furthermore, V_E/VCO_2 slope was correlated with hypoxic ventilatory response, such that enhanced chemoreflex sensitivity was associated with poorer ventilatory efficiency during exercise (r=0.54, *P*=0.0037, Pearson's correlation, n=28). These data demonstrate that long COVID is associated with high peripheral chemoreflex sensitivity

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

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PCA040

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

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Rationale: The rate at which oxidative ATP provision can meet the energy demand of exercise is a determinant of contractile dysfunction [1]. Evidence suggests that females experience less contractile dysfunction than males for the same duration and intensity of exercise [2], however whether this is caused by bioenergetic differences or differences in the contractile properties of exercising muscle is unknown. Therefore, this study compared the pulmonary oxygen uptake ($\dot{V}O_2$) kinetics during moderate and heavy intensity exercise in males and females.

Methods: Sixteen healthy adults (8 of each sex, 27 ± 5 years) completed three experimental visits. First, participants completed a submaximal incremental exercise test (+25 W every 5 minutes) to identify lactate threshold (LT), then a maximal incremental exercise test (25 $W.min^{-1}$) to exhaustion to identify $\dot{V}O_{2peak}$ and power at $\dot{V}O_{2peak}$ (P_{max}). Visits two and three involved three six-minute cycling bouts at 80% of LT (moderate intensity), interspersed with six minutes of unloaded pedalling, and one 30-minute bout at a work rate 30% between LT and P_{max} (heavy intensity).

Data from the final two visits were filtered and linearly interpolated (1s intervals), then pooled to form a dataset of six moderate and two heavy intensity transitions. The first 20 s of each transition was removed. Thereafter, three minutes of pre-transition data and six (moderate) or two (heavy) minutes of post-transition data were fit with a mono-exponential curve to obtain the parameters of the phase II kinetics. The $\dot{V}O_2$ slow component was also quantified for the heavy intensity bouts.

Results: Absolute $\dot{V}O_{2peak}$ was greater in males (3.47 ± 0.58 vs 2.49 ± 0.44 $L.min^{-1}$, p=0.002), however relative values were not statistically different (46.2 ± 6.6 vs 40.5 ± 6.7 $ml.kg^{-1}.min^{-1}$, p=0.111). Males achieved greater power outputs at $\dot{V}O_{2peak}$ and LT (p≤0.023), meaning power outputs for subsequent bouts were 30% greater compared to females.

The primary amplitude of the moderate intensity transition was not different between male and females (24 ± 3 vs 24 ± 5 %$\dot{V}O_{2peak}$, p=0.949). The time constant was also not different (27.9 ± 7.5 vs 24.8 ± 6.6s, p=0.385). Similarly, in the heavy intensity domain, neither the primary amplitude (43 ± 5 vs 38 ± 7 %$\dot{V}O_{2peak}$, p=0.179) or time constant (28.8 ± 7.9 vs 27.2 ± 7.1s, p=0.633) were different. Likewise, the amplitude of the $\dot{V}O_2$ slow component was not different between sexes (12 ± 7 vs 11 ± 3 %$\dot{V}O_{2peak}$).

Conclusion: No sex differences were observed in the $\dot{V}O_2$ response to exercise in the moderate or heavy intensity domains, implying there was no sex difference in the bioenergetic stress experienced. Combined with evidence of no hormonal effect [3] on these parameters, this suggests females should not be excluded from studies of cardiopulmonary responses to

exercise. Deoxyhaemoglobin kinetics recorded via near infrared spectroscopy of the *vastus lateralis* will also be shared.

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PCA041

Role of concurrent endurance and strength training on disease expression of patients with hypertrophic cardiomyopathy

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INTRODUCTION: Recommendation on physical exercise in patients with hypertrophic cardiomyopathy (HCM) has evolved from a global stressor to a stimulus whose variables are controllable. Our group has recently reviewed and analysed the impact of medications, invasive procedures and also exercise in HCM, reaching the conclusion that physical exercise emerges as a coadjuvant therapy which is safe and associated with benefits on functional capacity[1,2]. There are several clinical gaps on how to properly stimulate HCM patients with concurrent training to achieve the best dose-response, particularly regarding the optimal intensity or volume of the endurance component, and the type of exercises of the strength training.

AIM AND OBJECTIVES: To examine the impact of a concurrent strength and endurance training on functional capacity by cardiopulmonary exercise test (CPET), serum biomarkers and clinical (ECG, advanced cardiac imaging) related variables in patients with HCM, and the safety training protocol.

METHODS:40 adults HCM patients with obstructive and non-obstructive phenotype (1:1) and 20 healthy age and gender-matched controls will follow a 12-week training program supervised by sport scientists and cardiologists (Fig 1). All participants will be informed of the details of the aims and the protocol and should signed a dedicated consent form. The protocol has been approved by the local Ethical and Research Committee (2021-10-4-HCVA). All participants will undergo an initial and final assessment including anthropometry, quality of life survey, CPET, cardiac examination, ECG, echocardiogram, strength of the upper and lower body, muscle oxygenation, hemogram and blood tests including serum biomarkers analysis before and after the 12-week concurrent endurance and strenght-adapted (Fig 2) training protocol.

EXPECTED RESULTS: The training protocol proposed here will probably improve those previously proposed in terms of the selection and distribution of sessions and exercises, as well as the choice of intensity and adequate volume. In this sense, it is expected to achieve an increase in functional capacity substantially higher than those that have already been documented. Taking into account the results published by our group[1,2], an average improvement of 4.33 mL kg^{-1} min^{-1} is observed with the protocols used up to now, which represents an increase of 19.5% in functional capacity. Numerous studies establish a threshold value of functional capacity at 7 METs, from which functional HCM patients are considered functional. Therefore, an increase exceeding the threshold and reaching ~26.6 mL $kg^{-1}\cdot min^{-1}$ of VO_{2max}.would make the average of trained HCM patients progress from "non-functionality" to the

functional condition. In addition, training is expected to increase the daily caloric expenditure of patients, thus promoting the achievement of a negative energy balance that facilitates weight loss and improve BMI, which have an independent impact on HCM[3].

CONCLUSION: Therefore, in conclusion, it is expected that, as a result of training, patients will improve their symptomatic status and functional class by a safe protocol of training.

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PCA042

The exercise-mediated metabokine Beta-aminoisobutyric acid is an exercise mimetic driving skeletal muscle metabolic and functional adaptation

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Background: Skeletal muscle integrates many of the systemic signals, which contribute to the adaptive remodeling, and beneficial effects of exercise. One mechanism through which muscle mediates the systemic effects of exercise is through muscle-derived hormones known as myokines. We identified the metabolite β-aminoisobutyric acid (BAIBA) as an exercise-mediated small molecule myokine. BAIBA is secreted from muscle in response to increased expression of the transcriptional co-regulator PGC-1α, a master regulator of the muscle adaptive response to exercise. However, how BAIBA functions to regulate the adaptive responses of skeletal muscle to exercise remains poorly understood.

Methods: 8-week-old male C57BL/6J mice (n=20) were randomly assigned to receive either a chow diet or a chow diet supplemented with 100mg/kg/day of β-aminoisobutyric acid (BAIBA) in their drinking water for 6 weeks. At the end of the study, the right soleus muscles were assessed *in situ* for force and fatigability; and for oxygen consumption using Oxygraph-2K high-resolution respirometers. The expression of genes associated with fibre-type was evaluated using RT-qPCR in fully differentiated primary human myotubes. The role of PPARδ was investigated using siRNA techniques. Two way ANOVA was performed for statistical analysis. P-value nominal significance will be $p< 0.05$.

Results: We show that BAIBA improves muscle metabolism, exercise efficiency and performance in mice. Oxygen consumption (VO_2) and energy expenditure are increased in BAIBA-treated mice. Furthermore, BAIBA increased soleus *in situ* muscle contractile force ($p=0.0004$), fatigue resistance ($p=0.0063$), mitochondrial number and function. We found that BAIBA drives muscle fibre-type switching to an oxidative phenotype. BAIBA increased expression of PPARδ ($p=0.0001$, n=4/group), and genes determining muscle fibre-type, including Myosin Heavy Chain 7 (MYH7) (type I muscle) ($p=0.0001$ n=4/group), Myosin Heavy Chain 2 (MYH2) (type IIA intermediate muscles) ($p=0.0046$, n=4/group) in fully differentiated primary human myotubes. BAIBA regulates specific fibre-type gene expression in human myocytes through PPARδ.

Conclusion: Our findings demonstrate that BAIBA is a key paracrine myokine, which, in part, regulates the effects of exercise to improve muscle function with resultant effects on exercise performance.

Keywords: BAIBA, skeletal muscle adaptation, exercise performance, PPARδ, oxidative phenotype, fibre-type switching.

PCA043

The impact of the time of day on metabolic responses to resistance exercise in healthy adults: a randomised controlled trial

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The impact of the time of day on metabolic responses to resistance exercise in healthy adults: a randomised controlled trial

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Introduction:

Resistance exercise has many health benefits, including stimulation of glucose metabolism. However, the optimal time of day to perform resistance exercise is still unknown. The current study sought to determine the impact of the time of day on metabolic responses to resistance exercise in healthy adults in a randomised controlled trial.

Methods

We recruited 17 participants, inclusion criteria age between 18-45 years old with body mass index (BMI) >23.0kg/m². Exclusion criteria included having undergone surgery for weight loss, had prior history of heart, lung, cancer, kidney, endocrine, or liver disease. Participants were randomised into either a control, exercise in the morning (8:00-10am) or exercise in the evening (4:00-8:00pm) group. Those in the exercise group performed 8 resistance exercises (1 set to failure) 3 times over a one-week period, at their allocated time of day. Interstitial glucose responses were measured using a flash glucose monitoring (FGMs) for a one-week period prior to any exercise (habitual activity) and during the one-week exercise period with data compared between groups in time periods 6h post-exercise time and over 24h periods (both on exercise and non-exercise days), and over a 7-day period (exercise and non-exercise weeks).

Results

Participants were randomised to morning exercise n= 5; evening exercise; n= 6 or control group n=6. No time (exercise or no exercise days), group (control, morning or evening) or time*group interactions were seen for with mean glucose or glucose variability (measured as glucose standard deviation (SD)) in the 6h post-exercise period (all p>0.05). When comparing the 24h period no time (p=0.909), group (p=0.334) or time*group (p=0.911) interactions were seen for mean glucose. No time (p=0.537) or group (p=0.510) effects were seen for SD but a significant time*group interaction (p=0.008) was seen. Post-hoc tests revealed a lower (p=0.012) SD on exercise (0.65(0.05)) compared to no-exercise days (0.74(0.10)) within the

evening group. No other differences were seen in post-hoc tests. Comparing data over a 7-day period no time, group or time*group (all $p>0.05$) interactions were seen for mean glucose and SD (all $p>0.05$).

Conclusion

The current data is part of an ongoing study, but our preliminary data indicates that there is no effect of the time of day mean glucose levels, although exercise may reduce measures of glucose variability.

PCA044

The effects of beta-lactoglobulin (BLG) versus whey protein isolate (WPI) on blood aminoacidemia and muscle protein synthesis in older males

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Background: Maximising the muscle anabolic response to dietary protein is crucial for muscle mass maintenance in older individuals, due to the presence of anabolic resistance contributing to sarcopenia (1). Within dietary protein, leucine is thought to be the most potent amino acid (AA) for stimulating muscle protein synthesis (MPS; 2, 3). Supplementing leucine-enriched essential AA and/or whey protein (WP) has previously been shown to be as anabolic as larger doses of protein (4), thus may negate the need for large protein meals, especially in clinical settings. Beta-lactoglobulin (BLG) is the most abundant WP in bovine milk and has a high content of branched chain amino acids (BCAA), especially leucine (5). We, therefore, investigated the impact of BLG *versus* WP isolate (WPI), at a suboptimal protein dose, on blood aminoacidemia and MPS in healthy older males.

Methods: In this double-blind cross-over trial, 10 healthy older males (69 ± 1 years, 181 ± 2 cm, 92 ± 5 kg) were randomised to receive either i) BLG (1.5 g leucine) or ii) WPI (1 g leucine), with supplements matched for protein content (10 g). A primed, constant intravenous infusion of [1, 2 $^{13}C_2$] leucine was used to determine MPS at baseline and following feeding. Muscle biopsies were taken at 1.5 h after commencement of stable isotope tracer infusion (i.e., 0 h), immediately before a bolus feed (3 h; i.e., BLG or WPI) and 3 h post-feed (6 h). Serial arterialised blood samples were obtained to quantify plasma AA concentrations. Data are presented as mean ± SEM. MPS data were analysed using a two-way ANOVA (supplement x time) with a Šídák correction. Plasma AA data were analysed using a mixed-effects analysis (supplement x time) with a Šídák correction. All data analysis was performed using GraphPad Prism (GraphPad Software Inc, San Diego, CA). The alpha level of significance was set at $p < 0.05$.

Results: Blood plasma BCAA, leucine, isoleucine and valine concentrations significantly increased following feeding in response to BLG and WPI before returning to baseline, except plasma leucine which remained elevated in both groups. Peak plasma leucine concentrations were significantly greater following BLG (BLG: 422 ± 17 μM *versus* WPI: 364 ± 20 μM, $p = 0.0013$). Conversely, peak isoleucine concentrations were significantly greater following WPI (269 ± 34 μM *versus* BLG: 165 ± 14 μM, $p < 0.0001$). Myofibrillar MPS increased significantly following feeding (BLG; fasted: 0.048 ± 0.006 %/h, fed: 0.101 ± 0.012 %/h, $p < 0.0001$ *versus* WPI; fasted: 0.042 ± 0.006 %/h, fed: 0.075 ± 0.006 %/h, $p = 0.0032$), with a strong trend to BLG stimulating greater MPS than WPI post-feed ($p = 0.052$).

Conclusion: BLG exhibited greater peak leucinaemia compared to WPI, with both BLG and WPI significantly stimulating MPS following feeding. There was a strong trend to BLG stimulating greater MPS compared to WPI which may be physiologically significant, especially in larger trials. We conclude BLG containing a suboptimal dose of protein leads to an anabolic response to feeding in healthy older men.

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PCA045

A healthy lifestyle and indoor air quality on cognitive performance of elementary school pupils

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Since the Covid-19 pandemic in 2020, indoor ventilation rates in classrooms became a major topic. However, the research on the effect of the indoor environment on occupants' health and cognition is much older. Studies have shown that high concentrations of carbon dioxide (CO2) are negatively associated with the cognition performance of occupants (Du et al., 2020). Air pollution can also cause adverse health reactions (Fuentes-Leonarte et al., 2009). Elementary school children are a particularly vulnerable group because they are still in their growth phase and their school performance early on determines their success in higher education and the labour market later on. Past research found a negative relationship between classroom air quality and school performance (Wargocki et al., 2020). However, there are individual differences in the response to poor air quality which are still unknown. Overall health could be an important factor moderating individual resilience.

Therefore, this study examines if unhealthier school children in terms of overweight and higher sick leave days than their peers are less resilient towards the detrimental effects of indoor CO2 on test scores. Data from two field studies have been merged. The first study found a negative effect of indoor CO2 on test scores for pupils in schools in the south of The Netherlands (Palacios et al., 2022). From this dataset, 5 schools took part in an intervention study aiming to improve children's dietary behaviour and physical activity levels, which successfully reduced BMI levels (Bartelink et al., 2019). Thus, the health data of 1,149 children could be connected to their test scores and the indoor CO2 concentration of 58 classrooms. The scores of a nationally standardized test have been collected in January 2019 and 2020.

Linear regression models with various fixed effect specifications on the testing period, test domain and classroom were conducted. Standard errors were clustered on classroom and period levels to account for observation dependency within a classroom. Preliminary results show that higher indoor CO2 concentrations and being more often on sick leave are negatively associated with lower test scores ($p < .05$). Additionally, being overweight is negatively associated with test scores ($p < .05$), however, it partially mitigates the negative effect of CO2 ($p < .05$), contradicting our hypothesis. Being part of an intervention school offsets the negative effects of CO2 on test scores ($p < .001$). This countereffect is significant for the test domains of math and reading ($p < .01$) compared to spelling tasks. The regression models corrected for indoor temperature and relative humidity levels, indoor fine particles concentration, children characteristics (age, sex, absence other than due to sickness, parental socioeconomic status) and learning environment characteristics (class size, noise levels). In conclusion, the individual health of a child determined by BMI and sick leave days does not reduce the negative effect of CO2 on academic performance. However, an intervention aiming at the physical activity level

and dietary behaviour can partially offset the negative impact of poor air quality on school performance.

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PCA046

An exercise-responsive candidate obesity gene with sexual dimorphism

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Obesity increases the risk for diabetes and cardiovascular disease. Genetic predisposition exacerbates environmental drivers of obesity such as energy dense diets and sedentary lifestyle. We have exploited divergently selected Fat (23% fat as bodyweight) and Lean (4% fat as bodyweight) lines of mice originating from a common base population to identify genes underlying divergent adiposity. A stratified approach using quantitative trait loci (QTL; heritable genetic intervals segregating with adiposity in Fat x Lean F2 populations), metabolic tissue transcriptomics and comparative cross-species bioinformatics identified candidate obesity and leanness genes in adipose tissue (Morton *et al.*, 2011, 2016). Using a similar approach, we have identified novel muscle-expressed genes that segregate with adiposity. A specific phospholipase A2 isoform (we name here PlaX), positioned in the Found in obesity (Fob)-1 QTL, exhibited ~5-fold elevated mRNA levels in the skeletal muscle of Fat mice compared to Lean mice.

Our aim in this study was to characterise PlaX's expression and regulation in skeletal muscle across different muscle beds, in response to exercise, and between sex.

To better understand the role of PlaX in skeletal muscle, PlaX expression was measured in different skeletal muscle beds in 8-week old male C57BL/6J mice. Relative mRNA expression of PlaX was detected in soleus, gastrocnemius, EDL and quadriceps muscle, and preferentially expressed in gastrocnemius and EDL muscle [$P<0.05$; (n=4/group)]. To test the translational relevance of our findings, PLAX expression was measured in human skeletal muscle. PLAX expression was measured in vastus lateralis biopsies from female and male participants with normal glucose tolerance (NGT) and those with Type 2 Diabetes (T2D) by microarray (NGT-female: n=45, NGT-male: n=50, T2D-female: n=40 and T2D-male: n=50). PLAX mRNA abundance was significantly higher in female skeletal muscle (q-value<10e-15), with no difference between participants with T2D and NGT. We thus hypothesised that PlaX expression may be regulated by sex hormones. To test this, quadriceps muscle from 8-weeks old gonadectomised male C57BL/6 mice supplemented with vehicle, male sex hormone (dihydrotestosterone, DHT; 100µg/day) or female sex hormone (estradiol, E2; 2µg/day) by a subcutaneous minipump for 3 weeks were analysed to quantify PlaX expression. Relative PlaX mRNA abundance did not differ between the male or female sex hormone supplemented gonadectomised mice. However, PlaX expression was significantly increased after gonadectomy surgery [$*P<0.05$; (n=9/group)]. Expression levels were not rescued after sex hormone supplementation in male mice. Given the beneficial effects of exercise upon skeletal

muscle, we then aimed to determine whether PLAX expression is altered in human skeletal muscle in response to exercise. PLAX expression was analysed in MetaMex, an application to perform meta-analysis of skeletal muscle response to exercise (Pillon *et al.*, 2020). PLAX expression was significantly downregulated in vastus lateralis biopsies of young male and female healthy subjects after acute aerobic exercise (log2FC=-0.23, FDR=0.038).

Our genetic strategy has identified a novel potential skeletal muscle driver of obesity that appears to display sexual dimorphism and is downregulated in response to exercise.

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PCA047

Altered skeletal muscle mitochondrial morphology in late middle-aged humans, despite maintained mitochondrial function and content

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Introduction: Ageing is typified by a decline in maximal oxygen uptake (VO_{2max}) (Conley et al., 2000*a*; Conley et al., 2000*b*), which can limit physical function and daily life activities. This decline in VO_{2max} is related to a lower skeletal muscle mitochondrial function and loss of muscle mass (Conley et al., 2000*a*; Conley et al., 2000*b*). However, data in humans are inconsistent, with many studies showing no difference in mitochondrial function between young and old or middle-aged groups (Rasmussen et al., 2003). Most human studies have relied on assessments of maximal mitochondrial function (i.e. enzyme activities or phosphocreatine recovery kinetics), which may conceal subtle changes in mitochondrial ultrastructure and function occurring prior to an overt reduction in maximal mitochondrial oxygen consumption.

Objectives: To determine aerobic exercise capacity, mitochondrial respiration, density, and ultrastructure in late middle-aged and younger adults, and systematically assess the interrelationships between VO_{2max} and muscle mitochondrial measures.

Methods: 12 healthy young (27 ± 5 years, 8 males) and 10 late middle-aged adults (55 ± 6 years, 5 males) were recruited. All participants performed a maximal ramp incremental test on a cycle ergometer to determine VO_{2max}. On a separate day, muscle biopsies were obtained from the *vastus lateralis* muscle. Mitochondrial respiration was assessed in permeabilized fibres using high-resolution respirometry. Transmission electron microscopy was to study mitochondrial density and ultrastructure. Succinate dehydrogenase (SDH) activity was assessed via histochemistry in sections.

Results: VO_{2max} was lower in the late middle-aged compared to the younger group (34±5 vs. 45±7 $mL.kg^{-1}.min^{-1}$, $P<0.001$). Despite this, maximal oxidative phosphorylation capacity (old = 99±27 vs. young = 99±17 pmol $O_2.s^{-1}.mg^{-1}$, $P=0.95$) and mitochondrial area density (old = 6.2±1.5 vs. young = 6.0±0.5%, $P = 0.86$) did not differ between groups. SDH activity was lower in old versus young subjects (old = 0.97±0.18 vs. young = 1.18±0.20 $*10^{-5}$ $\Delta A_{660}.\mu M^{-1}.s^{-1}$, $P=0.02$), an effect that appeared to be confined to the most oxidative fibres (old = 1.23±0.22 vs. young = 1.65±0.39 $*10^{-5}$ $\Delta A_{660}.\mu M^{-1}.s^{-1}$, $P =0.008$) and not the least oxidative fibres (old =

0.71±0.22 vs. young = 0.75±0.13 *10^{-5} $\Delta A_{660}.\mu M^{-1}.s^{-1}$, *P*=0.69). Late middle-aged participants displayed smaller (old = 66±5 vs. young = 95±17 nm^2, P=0.001), but more numerous (old = 0.71±0.13 vs. young = 0.94±0.22 mitochondria.μm^{-2}, P=0.03) mitochondria when compared to younger participants. The area of individual mitochondria correlated negatively, and the number of mitochondria per unit area of muscle correlated positively with age, respectively (Figure 1). Various indices of mitochondrial function were correlated with VO_{2max} in the younger but not the late middle-aged group (Figure 2).

Conclusion: These data demonstrate that late middle-aged individuals had smaller, more numerous mitochondria for the same mitochondrial density and maximal respiration. The lack of correlations between mitochondrial measures and VO_{2max} in this group suggests that VO_{2max} is more likely constrained by O_2 delivery-related processes in late middle-aged humans. The extent to which these mitochondrial structural alterations predispose skeletal muscle to ageing remains to be determined.

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PCA048

Effect of spermidine administration on denervation-induced skeletal muscle atrophy

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Introduction

In modern society, skeletal muscle atrophy is a serious problem. It is well known that in many types of skeletal muscle atrophy, autophagy, which is a degradation mechanism, is reduced and waste products accumulate (Sakuma et al., 2016). Spermidine (SP) is a kind of polyamine that is abundantly contained in foods such as soybeans, and known as an autophagy inducer (Madeo et al., 2019). Previous studies showed that spermidine administration during exercise suppresses pharmacological skeletal muscle atrophy (Fan et al., 2017). However, it is unclear whether SP administration suppresses denervation-induced skeletal muscle atrophy.

Aims

The aims of this study was to investigate the effects of SP on denervation-induced skeletal muscle atrophy and autophagy-related proteins.

Methods

All the experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba, Japan (22-397). Male Institute of cancer research mice aged 7 weeks were used in this study (n = 4). After 1 week of acclimation, sciatic nerve transection-induced denervation (Den) was performed on the right leg of the mouse to induce skeletal muscle atrophy. A sham operation was performed on the left leg as a control. After the operation, spermidine 10 mg/kg BW was administered by intraperitoneal injection every other day. Tibialis anterior (TA) was collected two weeks after surgery. Two-way analysis of variance (ANOVA) was performed using the GraphPad Prism 8 (GraphPad, Inc.), and significance was set at $P < 0.05$ for all cases.

Results and discussion

We performed immunohistochemical staining of Laminin-α2 using frozen sections of TA. Significant main effects of Den and SP on muscle fiber size were identified (Fig. 1A). We evaluated protein expression levels by western blotting. Only a significant main effect of Den

was identified on the protein expression related to autophagy (P62,LC3-Ⅰ,LC3-Ⅱ) (Fig. 1B,2AB) and Protein synthesis (p-RPS6)(Fig. 2C). These data suggest that SP suppress denervation-induced skeletal muscle atrophy independent of autophagy pathway. SP has also been reported to relieve inflammation and oxidative stress, so further research is needed (Madeo et al., 2019).

Conclusion

SP suppressed denervation-induced skeletal muscle atrophy but did not affect key autophagy-related proteins.

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PCA049

Assessment of Cardiopulmonary Fitness and Physical Activity in Health Science Students: a Cross-sectional Study

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Background: Low levels of physical activity and cardiopulmonary fitness can have a negative impact on health and wellbeing, increasing the risk of chronic diseases and premature mortality. However, levels of physical activity in young people, including health science students, is usually low. Addressing this issue is important as health science students potentially affect the health of wider population.

Aims: This study aimed to assess the levels of cardiopulmonary fitness and physical activity among health science students at Karnali Academy of Health Sciences Jumla, a remote mountainous district of Nepal.

Methods: A cross-sectional study was conducted among the certificate and undergraduate level health science students of Karnali Academy of Health Sciences. All consenting apparently healthy students were included in the study, while those with conditions that might affect physical activity were excluded. Cardiopulmonary fitness was assessed by calculating VO2max from the Queen's College Step Test. Physical activity levels were measured using the International Physical Activity Questionnaire, short form, to obtain the MET value. Descriptive (proportion, median, interquartile range) and inferential (Wilcoxon Rank-sum, Spearman correlation) statistics were used for data analysis using GNU-PSPP software. Multiple linear regression analysis was performed to determine the predictors of VO2max.

Results: A total of 107 health science students (56 females) from Karnali Academy of Health Sciences in Jumla, Nepal were included in the study. Their age ranged from 18 to 37 years (median age 20). The median VO2max of all students was 40.05 (IQR 35.68 – 50.85) ml/kg/min, with males having significantly higher value [51.69 (IQR 45.81 – 57.57)] than females [36.37 (IQR 34.90 – 38.58)] ($p<0.001$, Wilcoxon Rank-sum test). The median weekly physical activity score was 1030 MET-minutes/week, with males reporting higher levels [1436 (962 – 2670)] than females [678 (414 – 1103)] ($p<0.001$, Wilcoxon Rank-sum test). Moreover, only 20.5% of the students met the WHO's recommended levels of physical activity. There was a significant moderately positive correlation between VO2max and total MET value per week (Spearman rho = 0.504, $p<0.001$), but a negative correlation with body adiposity ($p<0.02$). Physical activity level ($p<0.001$), sex ($p<0.001$), and BMI ($p=0.004$) were significant predictors of VO2max, but age was not a significant predictor ($p=0.254$) according to multiple linear regression analysis.

Conclusion: Health science students at Karnali Academy, Jumla, have average levels of cardiopulmonary fitness, but a significant proportion did not meet the recommended levels of physical activity. The findings highlight the need for targeted interventions to improve the health and wellbeing of these students, especially among females, who showed significantly lower levels of physical activity than males. The education system should encourage physical activity and promote healthy lifestyle behaviors among students, as this could have significant implications for their future health and wellbeing, and potentially benefit the wider population.

However, it is important to note that this study is limited to a single center and is a cross-sectional study, therefore, causal associations between variables cannot be made. Future research should focus on identifying effective interventions that can promote physical activity and healthy lifestyle behaviors among health science students in this region.

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PCA050

Withania somnifera (L.)Leaf Extracts Therapeutic properties of Wound Healing in Experimental Male Rats

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Introduction: *Withania somnifera* (L.), is one of the important medicinal plants and have a wide range of medicinal properties and bioactive compounds.

The present study aimed to investigate chemical profile and wound healing activity of methanol:choloroform(1:1) extract of *Withania somnifera* leaves on coetaneous wounds in the experimental male rats model.

Methods: Phytochemical screening for methanol:choloroform(1:1) extract of

Withania somnifera leaves was carried out using different standard methods to show the chemical profile of active components in extract.

Wound healing assay:

20 Male rats weighing 150 to 200 g were housed under normal conditions of light, room temperature and humidity.

The dorsal skin was shaved and cleaned with bethedine under anesthesia, and one open full-thickness wound that approximately 1.5 × 1.5 cm long was incised up to the level of subcutaneous adipose tissue by means of a surgical blade.

After the wounding process, each mouse was housed in a sterilized cage and given autoclaved food with redistilled water in order to prevent bacterial infection.

. The first group was left without treatment (control group), the second was treated with Povidone iodine (standard drug), the third group received 100 mg concentration of methanol:chloroform extract of *W. somnifera* and the forth group received 200 mg concentration of methanol:chloroform extract of *W. somnifera*. The wounds in the control and experimental groups were treated topically twice daily.The area of wounds was measured every day, from the first day to 12th day.

Results: The results present study revealed that Phytochemical screening for methanol:chloroform (1:1) extract of *Withania somnifera* leaves indicated the presence of various phytoconistituants like alkaloids, flavonoids, tannins, phenols, terpenoids and steroids.

application of methanol:chloroform (1:1) extract of *W somnifera* leaves at two concentrations 100 and 200 mg improved wound healing at all times beginning from first day of treatment to 9th day when the wounds were completely healed compare to wounds treated with standard drug (Povidone iodine) which were not completely healing after 12th day of treatment. Therefore methanol:chloroform (1:1) extract of *W. somnifera* leaves at two concentrations 100 and 200 mg showed significantly wound healing activity more than that of Povidone iodine ($p<0.05$).

The concentration of 200 mg from extract showed high significant of wound healing was not significantly compare to the effect of 100 mg of extract ($p<0.05$). Interestingly, during this study w the zone of wounds in rats treated with plant extracts returned to normal appearance and the hair had grown faster compared to positive and negative control

Conclusion: Methanol:chloroform (1:1) extract of *W.somnifera* leaves at two concentrations 100 and 200 mg showed significantly wound healing activity in experimental rats model more than that of Povidone iodine ($p<0.05$)

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PCA052

The metabolic cost of inspiratory muscle training in healthy adults

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Introduction: Inspiratory muscle training (IMT) is an intervention used in various clinical settings, including in critical care to ameliorate the effects of ventilator-induced diaphragm dysfunction. Metabolic oxygen consumption during IMT has not previously been explored. A recent study by our group explored the metabolic cost of IMT in mechanically ventilated individuals. We sought to supplement this with an understanding of the response of the healthy respiratory system to IMT.

Methods: The study conformed to the principles of the Declaration of Helsinki. Maximum inspiratory pressure (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.

IMT was applied at 4cmH_2O ("sham") and at 30%, 50% and 80% of each participant's PImax using an inspiratory threshold loading device (POWERbreathe Plus IMT). Sham training was delivered using an inverted Philips Threshold PEP device. Breath-by-breath oxygen consumption (VO_2) was measured using the Beacon Caresystem (Mermaid care A/S, Noerresundby, Denmark). Pressure at the airway opening was measured continuously using a port in the bacterioviral filter attached between the facemask and the IMT device.

IMT was applied for twelve breaths at each load with five minutes of tidal breathing between loads; the order of loads was randomised. VO_2 was recorded as a mean of the two minutes immediately following each IMT dose (recording during IMT bouts was found to be unfeasible due to the substantial negative pressures generated). Tension-time index of the respiratory muscles (TTmus) was calculated as mean airway pressure divided by PImax, multiplied by the respiratory duty cycle (PI/PImax x Ti/Ttot). Friedman's ANOVA was used to examine whether VO_2 differed with IMT dose, with Dunn's post hoc testing (using Bonferroni correction for multiple comparisons) for differences in VO_2 at individual doses. Linear mixed effects modelling (LMM) was used to quantify the relationship between IMT dose and VO_2 and between TTmus and VO_2.

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. Distribution of VO_2 differed significantly with IMT dose ($p<0.001$). Baseline median (IQR) VO_2 (4.42 (4.81 – 6.50) ml/min/kg) was not significantly different to sham (4.90 (4.11 – 5.03), $p=0.305$) or 30% (4.38 (3.69 – 5.23), $p=1.000$) but 50% (4.64 (4.09 – 5.28)) and 80% (5.09 (4.81 – 6.50)) IMT doses were significantly higher ($p=0.043$ and $p<0.001$ respectively). VO_2 at 30% and 80%, sham and 80%, and 50% and 80% doses were significantly different ($p<0.001$, $p=0.004$ and $p=0.043$ respectively). LMM showed a significant dose-response relationship between IMT dose and VO_2 (slope (95% confidence interval): 0.013 (0.009 – 0.018)ml/min/kg per %PImax increase in IMT dose, $p<0.001$). VO_2 was

also significantly related to TTmus: slope (95% CI) 3.74 (2.67 – 4.81)ml/min/kg per unit increase in TTmus ($p<0.001$).

Conclusion: Oxygen consumption during inspiratory muscle training exhibits a significant positive dose-response relationship. There is also a significant positive relationship between VO_2 and respiratory muscle effort relative to capacity. Examining the metabolic cost of breathing may offer an option to guide prescription of IMT.

PCA053

Phase angle, hydration and quality muscle index. Standardisation parameters of muscle quality in professional football players

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Introduction

Traditionally, muscle quality has been defined as the strength generated by muscle mass. Nowadays, we are able to look more deeply into the physiological and body composition mechanisms that determine muscle quality[1].

Intracellular water content (ICW) has been shown to be a useful indicator of strength-related muscle quality in the elderly, as this is the population group that is the most compromised at the water level[2]. In high-performance sport, ICW has been found to be closely related to physical performance[3]. Phase angle (PhA) has also become an objective indicator of cellular health and functionality[4].

For this reason, the aim is to obtain bioelectrical impedance (BIA) values of both PhA and muscle quality related to hydric parameters.

Methods

The sample of this longitudinal study was 33 professional football players (Table 1), from the first team of Watford FC (England). The measurements were taken during the competitive stage of the 22/23 season (year 2022).

Table 1. Characteristics of the players assessed. n=33.

Variable	Mean ± SD
Age (years)	26,33 ± 4,26
Weight (kg)	80,90 ± 8,69
Height (cm)	183,16 ± 7,96

Players were assessed by multifrequency BIA model Tanita MC-780 MA under fasting conditions, without having exercised in the previous twelve hours and having emptied the

bladder before the test. A total of 247 measurements were obtained where the water component and muscle quality were analysed using the AIC/ACT index (intracellular water/total body water) and lower limb PhA, both at 50 KhZ. Injured players were excluded from the total sample.

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

Results

In our group, we obtained mean reference values for the AIC/ACT index of 0.661 ± 0.017, and for the lower limb PhA of 7.8° ± 0.72°. Approximately 70% of the players are in the AIC/ACT (x±SD) range of 0.644-0.678 and PhA of 7.08-8.52º, below which we estimate muscle quality deficits in relation to hydration and muscle quality. Approximately 96% of our players are in the interval of AIC/ACT (x±2SD) of 0.627-0.695 and PhA of 6.36-9.44º, below which we estimate as low muscle quality.

The evolution of this index and PhA during the 22/23 season is shown in table 2.

Table 2. BIA measurements season 22/23 (year 2022) n=33.

	ICW/TBW and PhA LL	
Period	**July – August** **Preseason**	**September to December**
Number of measures	153	94
ICW/BTW **Mean ± SD**	0,658 ± 0,018	0,660 ± 0,016
PhA LL **Mean ± SD**	7,84º ± 0,89º	7,96º ± 0,66º

LL: Lower limbs. Frequency BIA at 50 kz

Conclusions

We consider the muscle quality index and the PhA to be very useful for analysis by the technical and health staff of a professional football club. Our data on the 70% range can serve as a reference, below which there is a deficit in muscle quality. However, it would be advisable for each club to obtain the average values of the index and PhA in order to be able to compare and analyse their athletes.

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PCA054

Supplementation with quercetin: cortisol and immunoglobulin values in professional soccer players

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Introduction

Quercetin is one of the most widely consumed flavonoids in the human diet and is widely distributed in fruits and vegetables[1]. It is the most potent remover of Reactive Oxygen Metabolites (ROMs) and enhances the body's antioxidant capacity by modulating glutathione levels[2].

This flavonoid has been shown to promote post-exercise recovery[3], reduce oxidative stress and muscle damage, and accelerate recovery after intense exercise[4].

Methods

Longitudinal study with 27 players from Udinese Calcio of Italy (Table 1), in which two evaluations were carried out, November 2021 and March 2022.

The impact of quercetin supplementation on post-match recovery was studied, measuring cortisol and salivary immunoglobulin (IgA) values at 60-72 hours post-match.

Table 1. Characteristics of the evaluated players n=27.

Variable	Mean ± SD
Age (years)	25,24 ± 6,8
Weight (kg)	86,33 ± 9,76
Height (cm)	184,5 ± 8,3

The competition started on 22nd August. From August to November the players did not take quercetin and from December to March they took 500 mg quercetin the day after each of the official matches. In the first and second evaluations, IgA and cortisol values were measured 60-72 hours post-match in order to compare the values obtained at both times.

The players performed the cortisol and IgA tests on an empty stomach, always supervised by the nutritionist. Once the sample was obtained, it was analysed immediately. To assess salivary cortisol and IgA we used the SOMA Bioscience system, a non-invasive, fast and easy-to-use system.

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

Results

The average values obtained are shown in table 2.

Table 2. Cortisol and IgA values after quercetin supplementation.

	1st assessment **30th November 21**	**2nd assessment** **22nd March 22**
Cortisol (µg/ml)	3,84 ± 1,45	3,29 ± 2,17 *
IgA (µg/ml)	304,93 ± 206,1	125,72 ± 74,76 **
Number of matches	16	19
*Significant differences between November and March. * $p < 0.05$, ** $p < 0.01$.		

We found that post-match quercetin administration significantly reduced cortisol values and very significantly reduced IgA values, which could be related to better recovery, as argued in another research.

Conclusions

The administration of 500 mg quercetin the day after the match is a good recovery strategy in football, as it improves the immune response and adaptation to the stress produced by the competitive event, which we believe leads secondarily to injury prevention.

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PCA055

Risks, Comorbidities, and Diagnostic Assessment Tools of Chronic Obstructive Pulmonary Diseases (COPD): A Systematic Review and meta-analysis

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Background:

Chronic obstructive pulmonary disease (COPD) is a respiratory condition associated with chronic and/or acute respiratory insufficiency. According to the Global Initiative for Chronic Obstructive Lung Disease, COPD classification is based on a combination of spirometry variables, symptom scores, and history of exacerbations/ hospitalizations. Recent epidemiological studies have shown that COPD often coexists with other diseases. Although some comorbidities arise independently of COPD, others are causally related, either through shared risk factors (i.e smoking, aging), genetic factors, or the low-grade inflammation characteristic of COPD. The distinction between comorbidities and systemic manifestations of COPD is unclear. Cardiovascular disease, metabolic syndrome, osteoporosis, depression, anxiety, and lung cancer are all highly prevalent comorbidities that exist regardless of COPD severity. Cachexia, skeletal muscle dysfunction, and anemia among many others may be viewed as systemic manifestations of COPD. Cross-sectional studies have shown that comorbidities are more common in persons with advanced COPD, thus resulting in poorer clinical outcomes. Hence, there is an overall growing need for improved characterization of COPD interplay with comorbidities and its association with long-term outcomes.

Methods:

Literature was searched in several electronic databases including Embase, Google Scholar, Ovid SP, MEDLINE-PubMed, and the European Respiratory Journal. The electronic databases were systematically searched till 31 Dec 2022. Thirteen studies with a total of n= 8,333,023 COPD and non-COPD subjects met the precise inclusion criteria. To pool the results, meta-analyses of odds ratios (ORs) were carried out with subgroup and sensitivity analyses under the random effects model.

Results:

The thirteen studies (n=4,110,161 COPD and n=4,222,862 non-COPD control patient data) were used for meta-analysis. The average age of COPD patients included in the studies ranged from 40.9 to ≥ 85 years of whom 52.3 to 83.8%. were male. The prevalence of cardiovascular comorbidities (OR 1.93, 95% confidence interval, CI 1.73-2.15; P value <0.00001), cerebrovascular incidents (OR 1.88, 95% confidence interval CI 0.72–4.95; P value <0.00001), hypertension (OR 1.62, 95% confidence interval, CI 1.47-1.78; P<0.00001), diabetes (OR 1.50, 95% confidence interval, CI 1.24-1.82; P value <0.00001) and osteoporosis (OR 1.90, 95% confidence interval CI 1.37–2.64; P value <0.00001) were all considerably higher in COPD patients in comparison to non-COPD patients when adjusted for covariance. Additionally, men suffering from COPD were significantly more likely to develop cardiovascular comorbidities (OR 1.17, 95% confidence interval CI 0.98–1.39; P value <0.00001) and pulmonary malignancies

(OR 2.15, 95% confidence interval CI 0.48–9.60; P value <0.00001) than their counterparts. In contrast, women with COPD had a significantly higher risk of developing neurotic disorders, depressive disorders, and other non-psychotic mental disorders, than their counterparts.

Conclusion:

COPD is associated with a significantly higher prevalence of comorbidities including diabetes, hypertension, cardiovascular disease, cerebrovascular diseases, and osteoporosis. Risk factors such as male gender, older age, current smoking, and obesity all exacerbate the risk of comorbidity development. These findings should be considered in COPD research, control strategies, and prevention.

Keywords: chronic obstructive pulmonary disease, lung cancer, mortality, cardiovascular disease, cerebrovascular incidence,

PCA056

The Impact of Wim Hof Breathing on Cardiovascular, Respiratory and Metabolic Response to Maximal Aerobic Capacity Test in Healthy Adults

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Introduction: Wim-Hof breathing (WHB) combines periods of hyperventilation (HV) followed by voluntary breath holding (BH) at low lung volumes until one can hold it. It is increasingly used by recreational and professional athletes to improve physical performance. **Aim:** The purpose of our study was to compare the acute metabolic, cardiovascular, and respiratory response to maximal aerobic capacity test (MACT) performed after WHB to MACT response after spontaneous breathing. **Methods:** Fourteen healthy recreational athletes participated in our crossover study, designed in accordance with the Declaration of Helsinki and approved by the national Ethics Committee. After baseline measurements, the MAPT was performed with a randomly selected breathing pattern applied immediately prior to exercise: spontaneous breathing (control) or WHB. For WHB, participants followed the breathing instructions of the mobile application of the WHB method: HV (30 deep breaths at 0.32 Hz), BH, and deep inhalation held for 15 seconds, repeated three times. MAPT consisted of a 1-minute warm-up at 30 W followed by gradual cycling with a power increase of 30 W per minute to exhaustion. The Cosmed Quark PFT was used to measure cardiac, respiratory, and metabolic parameters before and during MAPT: heart rate, end-tidal partial pressure of carbon dioxide and oxygen (PetCO2 and PetO2), oxygen consumption (VO2), carbon dioxide production (VCO2), pulmonary ventilation (VE), workload and derived parameters such as the ratio of VO2 to workload before reaching the anaerobic threshold (VO2/WR slope), ventilatory equivalents, oxygen pulse ect. Participants rated their perception of exertion during MAPT using the Borg scale (RPE). ANOVA for repeated measures was performed in SPSS, and $p < 0.05$ was considered evident for significant differences between the two trials. **Results:** Analysis showed positive effects of WHB practice prior to MAPT on VO2/ WR slope and RPE. We found lower VO2/ WR slope in WHB compared to control ($p=0.016$) and lower RPE in WHB compared to control ($p=0.015$). WHB decreased PetO2 ($p=0.032$), PetCO2 ($p=0.002$), and respiratory quotient ($p=0.01$) prior to exercise compared to spontaneous breathing. No significant differences were found in other measured parameters, including peak oxygen consumption and peak power, between the control and WHB trial. **Conclusions:** Our results suggest that athletes may benefit from performing WHB pre- exercise as less physical exertion is perceived compared to no WHB, at least when performing graded exercise of short duration. A lower VO2/ WR slope after WHB may be either a positive or negative adaptation to WHB: it may represent better oxygen uptake efficiency or lower oxygen availability in active muscles. However, none of the possible adaptations were associated with differences in peak aerobic power and peak oxygen consumption, suggesting that they are shortlasting or limited to aerobic metabolism. Measurement of the slope of oxygen uptake efficiency (OUES) or blood lactate concentration during MAPT would be useful to address potential adaptation to WHB.

PCA057

Relationship between Body Mass Index and Emotional Eating among Indian and Indian and Indonesian Population: A cross-sectinal study

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Introduction: Obesity is a major social issue with increased prevalence globally over the decades. Emotional eating has been considered as one of the factors leading to weight gain and subsequent obesity. Literature shows potentially addictive properties of hyperpalatable foods, and the existence of food addiction. This study aims to classify individuals as a function of the relation between food intake and emotions in Indian and Indonesian population using Emotional Eater Questionnaire (EEQ) developed by Garaulet et al[1].

Method: Voluntarily consenting 69 Indians (25 males, 44 females) and 71 Indonesian adults (23 males and 48 females) in age range 20-45years were selected to observe and compare the relationship between emotional eating behaviour. The subjects had statistically matched body mass index (BMI). They answered EEQ as a google form that included demographic variables and eating attitude-related questions. Subjects diagnosed with hypertension, diabetes, cancer, renal or heart diseases, endocrinal disorders and patients having any severe mood disorder on medication were excluded. The BMI were measured using Quetelet Index in kg/m^2 and subjects were classified as non-obese (BMI < 23 kg/m^2) and obese (BMI > 23 kg/m^2).

Results: Among Indians, 12 males and 24 females and among Indonesians 16 males and 25 females were found to be obese. BMI of the subjects was compared statistically intra and inter categorically using Spearman correlation. Depending on their EEQ scores, the subjects were categorized as non-emotional (EEQ score 0-5), low-emotional (EEQ score 6-10), emotional (EEQ score 11-20) and high-emotional (EEQ score >21) eaters. Obtained results indicate that subjects with higher BMI have higher EEQ scores. Spearman coefficient showed significant association between the various categories of EEQ and had higher BMI. MannWhitney test was done to compare scores of EEQ between Obese and Non-obese subjects which showed a strong, positive correlation, which was statistically significant (p< 0.00001) for both Indian as well as Indonesian subjects. One way ANOVA applied between EEQ categories and BMI was also found to be statistically significant (p<0.05) for both nationalities.

Discussion: Overeating and obesity stems from many biological factors engaging both central and peripheral systems in a bi-directional manner involving food and emotions. It was observed

that the proportion of emotional eating is more in obese than in non-obese subjects. Pleasure associated with food consumption leads to Dopamine production, causing activation of brain reward pathways that overrides other signals of satiety and hunger. Thus, a gratification habit through a favorable food leads to overeating and obesity. Obtained results support the hypothesis that emotional eating is one behavioral mechanism between over-eating and subsequent development of obesity.

Conclusion: Emotional eating behavior can be quantified using EEQ score in Indian and Indonesian population. Participants with higher BMI showed higher EEQ scores suggesting emotional eating as one of the contributing factors of obesity. Identifying and developing quantifiable measures to assess behavioral adaptations associated with emotional eating could provide a means of holistic approach to obesity management and prevention.

1. Garaulet et al. Validation of a questionnaire on emotional eating for use in cases of obesity; the Emotional Eater Questionnaire (EEQ) (Nutr Hosp. 2012;27:645-651)

PCA058

Musculoskeletal limitation is prevalent in preoperative cardiopulmonary exercise testing and favours use of submaximal metrics: implications for surgical risk assessment

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Background: Cardiopulmonary exercise testing (CPET) is used to determine cardiorespiratory fitness (CRF) in patients prior to major surgery given its capacity to predict post-operative survival [1]. Low CRF is attributed to 17% of postoperative mortality, presents greater risk than traditional measures of cardiovascular disease, and unfit patients experience a 5-fold greater mortality hazard [2]. CPET is preferably conducted to the limit of tolerance and primary metrics include, anaerobic threshold (AT), peak oxygen consumption ($\dot{V}O_2$ peak), and ventilatory equivalent for carbon dioxide at AT ($\dot{V}_E/\dot{V}CO_2$-AT) [3]. In non-surgical populations, musculoskeletal (MSK) conditions affect up to 1 in 3 people and are associated with a two-fold likelihood of being physically inactive [4]. In this study we observed a large cohort of patients who underwent CPET prior to colorectal surgery to identify the prevalence of MSK limitation. We then compared to what extent potential inability to provide authentic maximal CPET effort may inform metrics used for surgical risk stratification in this population.

Methods: A consecutive sample of 640 patients scheduled for elective colorectal surgery who attended CPET testing were retrospectively examined. CPET was conducted in accordance with consensus clinical guidelines [5] using cycle ergometry (Lode, Gronigen, The Netherlands) and a Medgraphics Ultima metabolic cart (MedGraphics™, Gloucester, UK). Patients exercised to their limit of tolerance to provide information for prognostic and diagnostic utility. The Medgraphics Breeze™ software automatically determined $\dot{V}O_2$ peak and respiratory exchange ratio (RER). The AT was manually interpreted using the V-slope method and $\dot{V}_E/\dot{V}CO_2$-AT calculated. Immediately following test termination patients were asked why they stopped, and responses used to stratify groups by prevalence of MSK pain (MSK+ or MSK-). Following confirmation of distribution normality (Shapiro *W* Wilks tests), data were analysed using independent samples *t*-tests for continuous data, or χ^2 tests for frequency counts. Data are expressed as mean ± SD and significance established at $P < 0.05$.

Results: Not all patients completed CPET. Seventy seven of 619 patients (12%) who completed CPET reported MSK pain as the reason for terminating prematurely, whilst 13 of the 21 (62%) patients unable to perform CPET were prevented in doing so by MSK limitations. Patients who reported MSK pain as the cause for premature test termination exhibited lower $\dot{V}O_2$ peak whereas the submaximal AT metrics were comparable (Table 1).

Conclusions: Exercise limitation due to MSK pain is prevalent in patients undergoing preoperative CPET. Patients unable to CPET have high risk of postoperative mortality and MSK limitation is often what prevents them from performing a test. Of MSK+ patients able to CPET, reduced $\dot{V}O_2$ peak is likely, and caution should be applied to this metric. Clinicians should consider metrics, such as the AT, that do not require maximal effort and investigate alternatives like the oxygen uptake efficiency slope allowing opportunity to account for disruption by early termination of exercise in these patients.

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PCA059

The venoarteriolar reflex revisited – a pilot study

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The venoarteriolar reflex (VAR) is one of the several physiological responses that contribute to maintain hemodynamic stability, especially in dependent regions of the body. VAR consists on arteriolar constriction in response to an increase in venous transmural pressure, as occurring in postural changes, therefore preventing the formation of edema. A recent study has demonstrated, in the lower limb, the existence of a vascular response in the contralateral limb that occurs simultaneously to VAR. However, it remains unclear whether this contralateral response is also observed in the upper limb. This study aimed to quantify the microvascular changes to a postural modification designed to evoke VAR in the upper limb. Ten young healthy subjects (23.4 ± 4.9 y.o.; 6 females, 4 males) participated in this study after giving informed consent. After acclimatization, subjects performed a postural modification while sitting upright, as follows – 7 min with both hands at heart level (baseline phase), 5 min with one random hand (test) placed 40 cm below the heart (challenge phase), and 7 min in the initial position (recovery phase). Local blood flow and skin temperature were measured in the second finger of both hands. Galvanic skin response was measured in the third and fourth fingers of the unmoved (control) hand in order to assess sympathetic cutaneous activity. These variables were compared between the different phases of the protocol with the Wilcoxon signed rank test ($p<0.05$). During the challenge phase a significant decrease in local blood flow was observed for the test hand. Similarly, there was a decrease in blood flow in the control hand, although not statistically significant. Skin temperature did not show any statistical differences between phases, guaranteeing that blood flow changes were not related to thermoregulatory phenomena. Also, no significant changes were found for galvanic skin response, suggesting that the sympathetic nervous activity is not involved in the observed vascular responses. These results suggest that the contralateral vasoconstrictor response to VAR, previously demonstrated in the lower limb, is also detected in the upper limb. The specific nature of this contralateral response should be investigated in future studies.

PCA060

Cardiovascular autonomic dynamics - an insight from the spectral organization of electrodermal activity signals

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Suprasystolic limb occlusion (SLO) is an important manoeuvre for blood pressure measurement, being also used as a challenge for the assessment of endothelial activity. It is commonly carried out by inflating a pneumatic pressure cuff above systolic pressure for a certain period of time. Although very useful, the magnitude and duration of occlusion are critical experimental aspects because of the mild discomfort it may cause to the subject. This perceived discomfort is likely to increase the activity of sympathetic nervous system and to have a systemic impact in hemodynamics. This study aims to further expand the knowledge on the physiological response to SLO by exploring the dynamics of autonomic regulation. Ten healthy male subjects (mean 20.2 ± 2.3 y.o.) participated in this study after giving informed written consent. After acclimatization, subjects performed a standard SLO protocol on a random upper limb while sitting upright, as follows: 10 min resting with both arms at heart level, 5 min arm occlusion (200 mmHg) and 10 min recovery in the initial position. Photoplethysmography (PPG) signals were acquired from the second finger of the occluded and non-occluded arms, with the latter being used for pulse rate variability (PRV) analysis, a surrogate of the well-known heart rate variability (HRV). The power of the high (HF), low (LF) and very low frequency components of the PPG signals were determined in all phases, as well as the LF/HF ratio. The electrodermal activity (EDA) was also acquired from the third and fourth fingers of both hands. The PPG and EDA signals were then decomposed with the wavelet transform in order to obtain their frequency spectra. During occlusion a significant decrease in the LF/HF ratio was noted, suggesting a decrease in cardiac sympathetic activity. In contrast, a significant increase in EDA was noted in both hands, suggesting an increase in cutaneous sympathetic activity. Also noteworthy, the EDA signals resembled one another very closely during the entire procedure, showing that the mechanical compression of the arm did not affect the magnitude of the signals. The spectral organization of the PPG signals has already been proposed two contain several components - cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent and endothelial NO-independent. The spectra of the EDA signals revealed a high frequency component that was aligned with the PPG cardiac component, together with several low frequency components which were only partially aligned with the sympathetic PPG component. These results suggest that the autonomic response to a SLO manoeuvre is complex and probably organ-dependent. This complexity is apparent from the EDA signal spectra, whose usefulness should be better investigated in the future.

PCA061

Spectral organization of skin temperature signals

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The human cutaneous microcirculation has been considered as a useful “window” to assess microvascular function, especially because of the possibility of using non-invasive technologies. Whenever measuring skin perfusion, it is advisable to measure skin temperature as well, in order to control the interference of thermoregulatory phenomena due to changes in ambient temperature. When performing measurements in an environment of stable external temperature, changes in skin temperature should reflect changes in skin perfusion. In fact, depending on the sensitivity of the sensors, it is possible that changes in skin temperature may reflect physiological mechanisms regulating perfusion. However, the spectral organization of continuous skin temperature signals remains poorly understood. Therefore, this study aimed to compare the frequency spectra of skin perfusion and skin temperature signals obtained from healthy subjects. Twenty healthy subjects (21.0 ± 3.0 y.o., both sexes) participated in this study after giving informed consent. After acclimatizing to room conditions, subjects performed one of two protocols while sitting upright – 10 subjects were subjected to a suprasystolic limb occlusion protocol (SLO, 5 min baseline, 5 min occlusion of a random arm, 5 min recovery); the other 10 subjects performed a postural modification (5 min with both hands at heart level, 5 min with a random hand placed 40 cm below heart level, 5 min recovery in the initial position). In both protocols, two variables were quantified in the index finger of the tested (occluded or lowered) limb - skin perfusion was quantified in the distal phalanx with a photoplethysmography (PPG) sensor, and skin temperature was quantified in the middle phalanx with a negative temperature coefficient (NTC) thermistor. Nonparametric statistics were used for comparisons between phases and signals ($p<0.05$). In the SLO protocol both perfusion and temperature decreased significantly due to the mechanical compression of the brachial artery. In the hand lowering protocol, however, perfusion decreased significantly due to the venoarteriolar reflex, but not significant changes were observed for skin temperature. These results show that the NTC thermistor was less sensitive to the physiological challenges than the PPG sensor. Both signals were decomposed with the wavelet transform to obtain their respective frequency spectra. The spectral organization of the PPG signal has already been proposed to contain several components - cardiac, respiratory, myogenic, sympathetic, endothelial NO-dependent and endothelial NO-independent. The spectra of the temperature signal revealed components in the same frequency intervals as the PPG signal. In fact, the dominant frequency of each observed component was generally coincident between signals, although appreciable differences in terms of skewness and kurtosis were identified for the regions of cardiac and respiratory components. These results suggest that skin temperature signals might have the same physiological origins

than PPG signals and, consequently, might be useful to explore the dynamics of perfusion regulation.

PCA062

Study of the impact of body surface area on functional exercise capacity and disease progression in patients of silicosis

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Introduction: Silicosis is one of the oldest occupational lung diseases, occurring gradually over a period of 12-15 years in workers exposed to silica dust. However, there are very few studies identifying anthropometric variables associated with silicosis and their impact on disease progression. It would be of value to consider the physical characteristics of individuals as risk factors for developing occupational lung disease.

Aim: The objective of present study was to study the association between body surface area (BSA), pulmonary function indices and 6 minute-walk distance(6MWD) in patients of silicosis.

Materials and Methods: A cross-sectional study was conducted on 102 male patients diagnosed with silicosis. Height and weight were measured to calculate BSA. The subjects were divided into three groups on basis of BSA (square metre) - Group I with BSA< 1.6 sq. m., Group II with BSA =1.6- 1.9 sq. m. and Group III with BSA >1.9 sq. m. Each group was further subdivided into three subgroups according to years of exposure to silica dust, subgroups being Ia, IIa and IIIa (10-15 years of exposure), Ib, IIb and IIIb (15-20 years of exposure) and Ic, IIc and IIIc (>20 years of exposure). Spirometry and 6MWD were performed on all groups and subgroups. Data was expressed as mean and standard deviation. Statistical analysis was done using Epi info V7 software. The outcome variables were Forced expiratory volume in first second (FEV1), Forced vital capacity (FVC), FEV1/FVC ratio and Peak expiratory flow rate (PEFR). Student's t test of significance (ANOVA) was applied to test the difference between means. Level of significance was set at 5%.

Results: Average age of subjects was 43.80±8.8 years. The average duration of exposure to silica dust was 21.25±6.35 years. 6MWD showed no significant changes with years of exposure and BSA. Except for FVC [2.80±0.76, 3.12± 0.51, 2.62 ± 0.69], the total mean of all other pulmonary function indices showed a statistically significant decrease as we move from subgroup 'a' (10-15 years of exposure) to subgroup 'c' (> 20 years of exposure) in each group [FEV1- 2.27±0.69, 2.44± 0.44, 1.91± 0.59 ; FEV1/FVC(%) - 80.14±5.84, 78.13 ± 5.15, 72.17± 10.45; PEFR(L/s) - 5.18±1.27, 6.15 ±1.29, 5.06 ± 1.88 respectively in each subgroup a, b, c].In group III, only FEV1 and FVC showed statistically significant decrease with increase in years of exposure [FEV1-3.05 ± 0.09(IIIa), 2.93 ± 0.66(IIIb), 2.23 ± 0.37(IIIc) ($p<0.001$) & FVC-3.66 ± 0.31(IIIa), 3.5± 0.81(IIIb), 2.90 ± 0.20(IIIc)]. The spirometric indices were higher in group III compared to group I and group II. Statistically significant higher values of FEV1 [2.73 ± 0.37($p=0.03$)] and FVC [3.35 ± 0.44 ($p=0.01$)] were observed in group III patients in all subcategories of exposure.

Conclusion: Patients of silicosis with BSA > 1.9 sq. m. had higher values of pulmonary function indices, irrespective of period of exposure to silica dust. Large body size may be of value in protection from developing occupational lung disease.

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PCA063

Acute cardiovascular responses to a single bout of high intensity inspiratory muscle strength training in smoking and non-smoking adults

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The use of cigarettes and e-cigarettes both increase blood pressure. Inspiratory muscle strength training (IMST) can lower blood pressure in healthy subjects (De Lucia et al, 2018). The aim of this study is to investigate whether IMST affects blood pressure and heart rate variability (HRV), a marker of cardiac autonomic function, in a group of smokers and non-smokers.

15 participants volunteered for this study (11 female). 5 participants smoked both cigarettes and e-cigarettes. Ethical approval was granted by the Science & Engineering Research Ethics committee, University of Plymouth, in accordance with the Declaration of Helsinki. All participants gave informed written consent. Participants completed a health questionnaire including information on exercise and smoking. Anthropometry, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were initially recorded, along with respiratory muscle strength (RMS); using the mean of maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP). 50% of the MIP reading was used to determine the resistance on two different brands of IMST (Threshold and Breather devices). Subjects were seated and five, 1-minute experiments were conducted; 1-minute rest, 1-minute IMST Device 1, 1-minute rest 2, 1-minute IMST Device 2 and 1-minute rest 3. IMST device order was randomised. Blood pressure, oxygen saturation (SpO_2), Borg scale for breathlessness, chest plethysmography and ECG were recorded for all experiments, with a nose-clip being worn. LabChart software and a PowerLab were used for data acquisition. HRV was analysed using time domain (heart rate - HR, standard deviation of the RR interval - SDRR), frequency domain (low and high frequency - LF and HF) and Poincaré analysis (SD1 and SD2). Repeated measures ANOVA (RMANOVA) and SPSS software was used to analyse the data (n=15). The probability of <0.05 was taken a statistically significant. Post-hoc analysis using pairwise comparisons between means was performed.

SDRR, SD1, Borg and DBP were all significantly different across all five interventions (Table 1). SDRR and SD1 were higher with both IMST devices, Borg was only higher with Device 2 (Threshold), while DBP fell with Device 1 (Breather). HR ($p=0.45$), LF ($p=0.95$), HF ($p=0.96$), SBP ($p=0.12$) and SpO_2 ($p=0.18$) did not show any significant changes over the five interventions. No significant differences were noted between smokers (n=5) and non-smokers (n=10).

The main findings from this study are the IMST devices resulted in time domain HRV parameters, breathlessness and DBP to differ. Unlike the work of De Lucia et al. (2018), which showed a drop in both SBP and DBP, our study only showed a drop in DBP. Reasons for this

difference could include the acute duration of IMST and the fact we used a lower percentage of MIP when giving IMST. A low statistical power was most likely the reason no difference in smokers was found in the current study.

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PCA064

High intensity muscle contraction under caloric restriction promotes Irisin secretion in mice

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Introduction

It is well known that combining diet and physical activity are more effective for health than interventions of diet or physical activity alone (Faught *et al.*, 2017). Recent research show that skeletal muscle secretes myokines in response to exercise, which contribute to the adaptation of exercise in other organs (Severinsen & Pedersen, 2020). Therefore, we hypothesized that muscle adaptation by calorie restriction (CR) might enhance myokine responses by exercise. We have already reported that Irisin, one of myokines that contributes to metabolic activation of adipose tissue and weight loss, is secreted in response to muscle contraction (Tanimura *et al.,* 2022), and we combined this model with CR in this study.

Objective

The aim of this study was to investigate that effects of muscle contraction and CR on Irisin secretion.

Methods

All the experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba, Japan (22-397). Male ICR mice aged 7 weeks were used in this study. After 1 week of acclimation, mice in the CR group were given 60% of the average amount of food eaten by each mouse for 1 week acclimation periods. After 2weeks CR or *Ad libitum* (AL), we conducted electrical stimulation (ES) as a model to induce high intensity muscle contractions (Tanimura et al., 2022). Gastrocnemius muscle and blood were obtained immediately after single bout of ES (n = 5-6 in each groups). We used Western blotting as the method of analysis for protein expressions in skeletal muscle and blood. One-way analysis of variance (ANOVA) or Two-way ANOVA were performed using the GraphPad Prism 8 (GraphPad, Inc.), and significance was set at $P < 0.05$ for all cases.

Results and Discussion

Body weight and gastrocnemius mass were significantly decreased in CR groups than AL groups (Fig.1). Thus, CR affected not only weight but also muscle condition. Blood Irisin levels were increased by both CR and ES, and an interaction was observed in the CR+ES group (Fig.2). Therefore, CR enhances Irisin secretion by exercise. One of the upstream molecules of Irisin, PGC-1α, may be involved in this interaction.

Conclusion

CR and muscle contraction promote Irisin secretion synergistically.

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PCA065

The effects of skin hydration levels on local skin wetness perception at the underarm

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Introduction:

The perception of skin wetness plays an important role in behavioural thermoregulation and thermal comfort (e.g. the onset of sweating). Whilst our understanding of the neurophysiology of skin wetness perception (WP) is expanding [1], research is lacking on how the biophysical status of the skin, such as its hydration levels, impacts local wetness sensitivity. Skin hydration levels can vary individually due to intrinsic (e.g. sex, age) and extrinsic factors (e.g. environment). Changes in the skin' stratum corneum hydration could alter the skin's properties leading to increased or decreased wetness sensitivity [2]. This study aimed to investigate the effect of skin hydration levels on local WP and its individual variability.

Method:

Ten male (*n=5)* and female (*n=5)* participants (28.8 ± 7.2y; 171.3 ± 9.5cm; 78.1 ± 18.2kg) took part in two separate experimental trials, during which they underwent a quantitative sensory test (QST) of WP at baseline and following localised overhydration [i.e. OVH; +22 ± 20% from baseline] or dehydration [i.e. DEH; -44 ± 20% from baseline] of the underarm' skin. Participants reported on a 100-mm visual analogue scale the perceived magnitude of WP (anchor points: 0=dry; 100=completely wet) from the short-duration (i.e. 10s) static application of a cold-wet (i.e. 5°C below local skin temperature), neutral-wet (i.e. equal to local skin temperature) and warm-wet (i.e. 5°C above local skin temperature). Before the QST(s), local tactile sensitivity, skin temperature, stratum corneum hydration, and skin surface roughness were measured. Individual participants' perceptual responses to each temperature stimuli were coded as either A) a change in WP (i.e. ≥10-mm difference in WP from baseline to post OVH or DEH); or B) no change (i.e. <10-mm difference). Pearson's chi-squared tests of independence were used to examine the association between changes in WP from baseline and skin hydration status (i.e. OVH or DEH) for each temperature stimulus.

Results:

We found a statistically significant association [X^2 (2)=6.9, p= 0.03] between skin hydration status and changes in WP during neutral-wet stimulation. Specifically, 60% of participants reported an increase in WP following OVH, whilst 30% reported a decrease following DEH (Fig. 1A). A similar trend was observed during cold-wet stimulation [X^2 (2)= 5.4, p= 0.07) whereby 50% of participants reported an increase in WP following OVH, whilst 40% reported a decrease following DEH (Fig. 1B). No significant association was found between changes in WP and skin hydration status during warm-wet stimulation (X^2 (2)= 0.3, p= 0.865)(Fig.1C). Differences in the changes in WP following manipulation in hydration status were not explained by sex (Fig. 1).

Conclusion:

The study found that skin hydration levels may influence WP, although this effect is dependent on stimulus temperature. Furthermore, hydration-dependent changes in WP were observed in ~50% of the sample only. The response to the change in skin hydration state may be divided into subgroups of responders and non-responders, with individual variability modifying the effect of skin hydration levels on WP to a larger extent than sex-related differences.

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PCA066

Effective dose regimen of STZ for STZ-induced diabetes in a rat model

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Introduction: Diabetes mellitus (DM) is a metabolic illness, defined by a high level of blood sugar because of a problem with insulin synthesis, action, or both. Various clinical signs follow DM, majorly hyperglycaemia, polydipsia, polyuria, and polyphagia. Worldwide prevalence is high and predicted to rise to 592 million by 2035. Animal models are used in the study of diabetes due to ethical issues. Streptozotocin (STZ) model is frequently used but has poor dependability due to unexplained acute toxicity and effective dose variability. This research was carried out to determine the effective dose regimen of STZ for inducing diabetes in locally reared Wister rats in Abeokuta.

Methodology: 28 male Wistar rats (160 - 190 g) were randomly divided into 4 groups (n=7) and monitored for 21 days after diabetes induction with STZ: Control (CTR), diabetics: DIA1 (60 mg/kg STZ), DIA2 (60 mg/kg STZ twice at 0 and 24 hours), and DIA3 (60 mg/kg STZ thrice at 0, 24 and 48 hours). Plasma glucose was determined with a glucometer. Body weights, feed intake, and faecal output were weighed with a digital balance, while water intake and urine output were measured with a measuring cylinder. Analyses of the data obtained were performed using a One-way ANOVA and Tukey's test at $p<0.05$ for significance. The ethical rules set forth by the Federal University of Agriculture, Abeokuta's committee on animal care ethics and usage (FUNAAB/COLVET/CREC/2022/02/03) were adhered to.

Results: There was a significant ($p<0.05$) decrease change in the percentage body weight of the diabetics (-15.53±1.2, -26.8±1.2, -28.5±1.9%) compared to the CTR (10.5±2.5 %). Also, there was a significant ($p<0.05$) increase in fasting blood glucose concentrations (135.2±9.0, 273.2±6.5, 257.0±5.3 mg/dL) in the diabetics compared to the CTR (79.3±1.1 mg/dL). Furthermore, water intake (56.9±0.9, 72.1±1.7, 77.8±5.5 mL), feed intake (19.4±0.6, 23.3±1.9, 42.1±2.1 g), voided urine (6.34±0.1, 8.39±0.88, 9.58±0.50 mL) and voided faeces (10.4±0.26, 11.7±0.43, 8.5±0.17 g) in the diabetics increased significantly ($p<0.05$) when compared to the CTR (26.5 ± 0.8 mL, 13.4±0.3 g, 1.84±0.08 mL, and 6.5±0.33 g respectively).

Conclusion: This study showed that the dose regimen of 60 mg/kg STZ administered intraperitoneally twice (24 hours apart) sustained diabetes for 21 days. We recommend that this dose regimen be adopted in STZ-induced diabetic studies in male locally reared Wistar rats in.

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PCA067

Placental Mitochondrial Respiratory Function at High-Altitude: Studies of Growth Restriction in Andean Highlanders

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Objectives:

Chronic exposure to hypobaric hypoxia during pregnancy is associated with low birthweight and suppression of electron transfer system (ETS) proteins in placental mitochondria. Some resistance to growth restriction is seen in native high-altitude populations, which may be associated with placental metabolic changes protecting infant growth. It remains unclear whether such alterations in placental metabolism occur in intrauterine growth restriction (IUGR) pregnancies in highland Andeans at altitude. This study aimed to determine the relationship between fetal growth and placental mitochondrial respiration and enzyme activity in highland Andeans at high altitudes.

Methods:

Placental tissue and umbilical cord blood were collected from 50 Andean maternal-infant pairs living in La Paz, Bolivia (~3850m) after scheduled Cesarean delivery. Within this cohort, 26 had infants that were diagnosed with IUGR on prenatal ultrasound and the other 24 had infants of normal growth. Placental tissue was cryopreserved and analyzed using high-resolution respirometry and a substrate-uncoupler-inhibitor titration to assess for oxidative phosphorylation (OXPHOS) and ETS capacity supported by Complexes I, II, and IV. Spectrophotometric enzyme activity assays (EAAs) were used to measure citrate synthase, hydroxyacyl-CoA dehydrogenase (HOAD), lactate dehydrogenase, and hexokinase activities. Respiratory states and EAAs between groups were compared with a Student's t-test and contextualized with clinical data through simple linear regression or one-way ANOVA. These studies were approved by the University of Colorado IRB (Approval No. 14–2178 and 17-1529) and the Ethics Review Boards for the Caja Nacional de Salud and Hospital Materno-Infantil in La Paz, Bolivia.

Results:

There was no significant difference in mass-specific respiratory capacity in placentas from IUGR and non-IUGR infants in LEAK state, Complex I, Complex II, or Complex IV supported respiration (Figure 1). However, when only term pregnancies were considered, maximal respiratory capacity supported by substrates for Complexes I+II and max ETC were lower ($p<0.05$) in the IUGR cohort (Figure 2). The IUGR placentas also had a 34.5% smaller surface

area ($p<0.01$). Although the umbilical vein PO_2 did not differ by IUGR status, the change in arteriovenous O_2 difference (v-aO_2) between umbilical vein and artery was 56.1% larger ($p<0.01$) in the IUGR cohort when corrected for infant birthweight. Cord blood hemoglobin ($p<0.05$), hematocrit ($p<0.05$), and red blood cells ($p<0.05$) were also higher in the IUGR cohort. There was no significant difference in the activity of any enzyme measured.

Conclusions:

Within highland Andeans at altitude, there was a mild suppression of mass-specific oxidative phosphorylation in the placenta from IUGR pregnancies, and smaller placental surface areas. The mitochondrial suppression may conserve oxygen and thereby compensate for the reduced surface area for gas/nutrient exchange in the IUGR cohort. It is possible that the influence of altitude or pre-term status has masked the full effect of metabolic changes in IUGR pregnancies. The elevated change in v-aO_2 per birthweight in the IUGR cohort suggests a fetal hypoxia response despite a similar PO_2 in the umbilical vein.

PCA068

Programming of impaired hepatic drug metabolism during pregnancy in sheep: Effects of hypoxic pregnancy and antioxidant treatment in fetuses and adolescent offspring

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Intro: Fetal growth restriction (FGR) affects 10% of pregnancies globally. FGR results from chronic fetal hypoxia which promotes oxidative stress *in utero* and increases the risk of chronic disease in later life (1). These chronic diseases generally require long-term drug treatment where tight control of drug-plasma concentrations within a therapeutic range is essential. Hepatic cytochrome P450 (CYP) enzymes metabolise 70-80% of all clinical drugs – with CYP3A metabolising more than 30% – and their activity is impacted by acute and chronic changes in oxygen and glucose availability (2). Understanding programmed changes in hepatic CYP activity can improve therapeutic outcomes; however, programming of hepatic drug metabolism in hypoxic pregnancy has not been investigated. We have shown that hepatic CYP3A activity is reduced in 21d old lambs born FGR (3), although what is driving this alteration is unknown. Mitochondria-targeted antioxidant treatment (MitoQ) in FGR animal models protects against programmed hypertension in adult offspring (4). Whether this protection extends to the programming of altered CYP activity in hypoxic pregnancy has not been investigated.

Aim: To determine if hypoxic pregnancy impairs hepatic CYP activity in offspring and if maternal MitoQ treatment is protective.

Methods: At 100±1 day of gestational age (dGA; term is 145 days) Welsh mountain ewes carrying singletons were catheterized under general anaesthesia (1.5-2.5mg/kg IV alfaxalone, Alfaxan; maintained with 1.5-2% isofluorane in 60:40 O_2:N_2O) with analgesic administered prior to surgery (1.4 mg/kg SC carprofen). Ewes were randomly allocated to normoxic (21% O_2: n=34) or hypoxic (isobaric chambers, 11% O_2: n=36) pregnancy with MitoQ (MS010 IV, 6mg/kg) or control (saline IV) treatment from 105-138 dGA. Ewes carrying male fetuses were humanely killed with an overdose of sodium pentobarbitone (0.4ml/kg IV, Pentoject) at ~138 dGA. Ewes carrying female fetuses lambed spontaneously and offspring were humanely killed (as per ewes) at 9 months of age (sexual maturity). Activity of 3 and 7 CYP enzymes in fetal and adolescent offspring respectively was determined in isolated hepatic microsomes using established functional assays (5). Data are presented as mean ± SEM and analysed using two-way ANOVA with the Tukey *post hoc* test.

Results: In 9-month-old lambs, hepatic CYP2B6 and CYP2E1 activity was reduced by 22% (P_{oxygen}=0.0127) and 38% (P_{oxygen}=0.0349) respectively in hypoxic pregnancy with no treatment. MitoQ treatment alone in pregnancy significantly decreased CYP1A2 activity by 12% ($P_{treatment}$=0.0011) and CYP3A activity increased by 22% ($P_{treatment}$=0.0167). Conversely, in late-gestation fetuses, no significant alterations in CYP activity were observed in any group.

Conclusions: The fetal data are consistent with many CYPs not becoming active until after birth. Hypoxaemia in late pregnancy programmed reduced CYP activity in adolescent offspring, although unchanged CYP3A activity was unexpected and contrasts previous work potentially

due differences in the timing and duration of the hypoxic insult. Despite MitoQ treatment offering protective benefits for hypertension in FGR offspring, our data suggests MitoQ programs increased CYP3A activity and decreased CYP1A2 activity. Programmed changes to hepatic CYP activity in adolescent offspring may alter the efficacy and safety of commonly used therapeutics throughout the life-course.

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PCA069

Physiological Changes, Prevention and Management of Musculoskeletal Disorders Across the Lifespan: a Mini-Review

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Musculoskeletal disorders (MSDs) are increasingly common among older adults, and they are often associated with physiological ageing processes, such as muscle loss, decreased bone density, and changes in joint structure and function. This natural ageing process can contribute to MSDs, namely osteoporosis, osteoarthritis, and Sarcopenia. An analysis of the Global Burden of Disease (GBD) in 2019 estimated 1.71 billion people have developed MSDs worldwide. Accordingly, it is compulsory to highlight MSDs as they have critical adverse outcomes, including the risk of fractures, functional decline, frailty, and mortality.

For example, Osteoporosis is a quantitative metabolic bone disease that, on a cellular level, results in an imbalance between osteoclastic bone resorption and osteoblastic bone formation. Traditional pathophysiological concepts include low dietary intake of Calcium or Vitamin D, mainly focusing on endocrine mechanisms; however, recent research goes far beyond this. Mechanisms such as interactions between bone and immunity and cellular senescence attracted a growing area of research interest in this field.

In addition, Sarcopenia, or age-related muscle loss, is a frequent physiological change that contributes to developing MSDs. It leads to a decline in the size and mass of the muscle due to the decrease in the cross-sectional area of the fibres. Even though nearly 25% of 65+ year-olds develop sarcopenia, there is still yet no confirmatory diagnosis tool for it. This has attracted many researchers in the field to conduct meta-analysis using cohort and cross-sectional studies to assess the efficacy of different ways of diagnosis.

Osteoarthritis(OA), or “wear and tear arthritis” is a degenerative joint disease. OA was classified as a leading musculoskeletal cause of impaired mobility of the elderly. Taking this into consideration, accurate molecular and chemical causes or mechanisms that degrade cartilage are vague.

This review aims to i) highlight discovered molecular mechanisms that contribute to osteoporosis and how these mechanisms contribute to its pathophysiology, ii) accentuate possible ways of diagnosing sarcopenia and which is most accurate and reliable according to meta-analyses, and iii) summarise the precise molecular mechanisms involved in OA pathogenesis

PCA070

Isotope-specific effects of lithium on mitochondrial calcium phosphate cluster size distribution and calcium capacity

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Introduction and Aims

Lithium salts are established medications in a variety of mental health conditions. The molecular mode of action if lithium is incompletely understood. Many cellular effects associated with lithium treatment are linked to mitochondrial function. We investigated the effects of lithium on mitochondrial calcium handling due to its obvious role in neuronal signaling.

Methods

We investigated the calcium capacity of mitochondria from murine mouse liver in the presence and absence of lithium, both in its natural composition and as pure 6-lithium and 7-lithium. To achieve this, we utilized high-resolution respirometry and fluorometry techniques. Additionally, we examined the subcellular distribution of lithium isotopes using inductively coupled plasma mass spectrometry and nanoscale secondary ion mass spectrometry. Furthermore, we analyzed the formation of amorphous calcium phosphate, both in the presence and absence of lithium isotopes, using 31-phosphorus nuclear magnetic resonance and dynamic light scattering.

Results

Lithium protected against calcium-induced permeability transition (32.84±11.2 min to onset vs 15.69±7.3 min to onset, errors: SD, n=8, $p>0.001$) and decreased calcium capacity of liver mitochondria (589.8±99.1 nmol/mg vs 635.7±100.3 nmol/mg, errors: SD, n=10, $p<0.01$) at clinically relevant concentrations. Interestingly, brain mitochondrial calcium capacity was increased, not decreased, by lithium (601.3±64.8 nmol/mg vs 463.2±34.5 nmol/mg, errors: SD, n=5, $p<0.01$). Further analyses revealed that 7-lithium was more effective than 6-lithium in altering calcium capacity, whereas 6-lithium was more effective in delaying permeability transition. Interestingly, these effects were not attributed to differences in lithium isotope distribution within cells or subcellular compartments. Instead, our in vitro experiments demonstrated that lithium isotopes had distinct effects on the size distribution of amorphous

calcium phosphate colloids, which is a plausible mechanism underlying the isotope-specific mitochondrial calcium capacities observed in our study.

Conclusion

We identified mitochondrial calcium management as a plausible component of clinical lithium effects and found evidence for a direct interaction of lithium with amorphous calcium phosphate aggregation. The isotope-specificity of lithium effects provide a promising avenue for the development of more effective lithium-based drugs.

PCA071

Inhibitors of focal adhesion complex formation disrupt anabolic responses to amino acid and growth factor provision in immortalised human primary myotubes

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Focal adhesion complexes (FACs) are large multi-protein structures anchored at the plasma membrane of cells to connect the extracellular matrix to the cytoskeleton. Due to this membrane-spanning location, FACs have predominantly been associated with mechanosensing signalling pathways, particularly in skeletal muscle where FAC-related proteins are loading-responsive*(1)*. Recently, however, FACs have also been implicated in the regulation of mechanistic target of rapamycin complex 1 (mTORC1) activity, a primary regulator of cell anabolism, in response to amino acids (AAs) and/or growth factors (GFs)*(2)*. Importantly, however, these investigations were conducted in non-muscle cell types and therefore FACs role in AA and/or GF-mediated mTORC1 activation in skeletal muscle is yet to be elucidated. Our recent data displayed that mTORC1 activation occurs in close proximity to FACs following anabolic stimuli in human skeletal muscle*(3)* suggesting signalling responses to elevated AA/GF availability may centre at these complexes *in vivo*. While this displayed the localisation of mTORC1 activity at FACs, further studies specifically assessing the importance of FACs in the regulation of anabolic responses to AAs and/or GFs are required. Therefore, in this investigation we utilised two pharmacological compounds known to disrupt FACs (Y-27632 dihydrochloride – Rho-associated protein kinase inhibitor (50μM), Cilengitide – integrin antagonist (2.5μM)) and studied their effects on anabolic signalling responses to elevated AA (2x minimal essential medium (MEM) concentrations) or GF (10ng/mL IGF-1) concentrations. Immortalised human primary myotubes (C25 cell line) were proliferated and differentiated before being starved of nutrients (EBSS) for 4h in the presence of each compound, followed by a 1h period of AA/GF stimulation. Myotubes were then collected, lysed and prepared for immunoblotting (n=8 for each condition) for a variety of mTORC1-related signalling targets. Independent t-tests were used to test for statistical significance between each compound and an untreated (control) condition (significance set at $p<0.05$). In response to elevated AAs, Y-27632 reduced $RPS6^{Ser240/244}$ and $RPS6^{Ser235/236}$ phosphorylation compared to control (~36% & ~67% respectively, $p<0.01$) whilst cilengitide reduced $RPS6^{Ser235/236}$ phosphorylation (~34%, $p=0.015$) and elevated $eEF2^{Thr56}$ phosphorylation (~50%, $p=0.012$), all indicative of impaired mTORC1 activity. In response to IGF-1, phosphorylation at both sites on RPS6 was reduced by Y-27632 ($RPS6^{Ser240/244}$ - ~39%, $RPS6^{Ser235/236}$ – 68%, $p<0.01$), whereas cilengitide had no effect. Further markers of mTORC1 activity (p-$4EBP1^{Thr37/46}$), GF signalling (p-AKT^{Ser473}) and autophagy (LC3b II/I ratio) were unaltered by the FAC inhibitors in both conditions. Importantly, abundance of a prominent FAC protein (Talin1) and mTOR itself were also unaltered suggesting the effects of Y-27632 and cilengitide occurred independently of changes to FAC or mTOR content. These results show that intact FACs are required for some, but not all, mTORC1-related signalling responses to elevated AAs and GFs in human skeletal muscle cells, which builds on our current understanding of anabolic regulation in this tissue. Further work will employ immunofluorescent staining to confirm FAC disruption and the SUnSET technique to determine effects of global protein synthesis.

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PCA072

Amylin aggregation and suppression of mitochondrial respiratory capacity in the diabetic heart

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Introduction: The diabetic heart is metabolically inflexible, displaying maintained fatty acid oxidation (FAO) but impaired glucose uptake. Amylin is co-released with insulin by pancreatic β-cells, forming deposits locally and in hearts of patients with Type II diabetes mellitus (T2DM). This contrasts with rodent amylin which has a reduced propensity to aggregate. In HIP rats expressing human amylin, deposits occur in the pancreas, and heart, and T2DM ensues. This is not observed in hyperglycaemic (UCD) rats expressing rodent amylin.

Objective: To investigate if cardiac amylin aggregates exacerbate mitochondrial and metabolic changes in diabetic cardiomyopathy.

Methods: All animal experiments conform to the NIH guide for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee at University of Kentucky. Cardiac mitochondrial respiration was studied in male, wild-type (WT), HIP, and UCD rats (14-16 months) using a protocol optimised for frozen samples. Enzyme activities were measured by spectrophotometric assays, and gene expression by qPCR. Respiratory oxygen fluxes (JO_2) were normalised to cardiac tissue wet weight and analysed by One-way ANOVA (n=10).

Results: In HIP rats, cardiac respiratory capacity was lower than in WTs and UCDs. Complex I (CI), Complex I and II (CI&II), and Complex IV-supported (CIV) respiration rates were 57.3% ($P<0.01$), 49.0% ($P<0.01$), and 34.7% ($P<0.05$) lower, respectively, than in WTs. CI&II and CII-supported rates were 39.7% and 56.5% lower than UCDs ($P<0.05$). In comparison with WTs, *β*-hydroxyacyl CoA dehydrogenase (HOAD) activity was unchanged in UCDs, with a trend towards decreased activity in HIP rats, alongside a trend towards lower expression of other components important for fatty acid oxidation: *Ppara, Cpt1b, Ucp3*.

Conclusion: Cardiac amylin aggregation in HIP rats suppresses mitochondrial respiratory capacity, and potentially fatty acid oxidation, compared with UCDs and WTs.

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PCA073

Gestational diabetic myometrium and its responsiveness to an anti-diabetic plant, Thunbergia laurifolia L., in in vitro and in vivo study.

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Gestational diabetes mellitus (GDM) has a significantly increased risk of spontaneous preterm delivery (SPD) (Boriboonhirunsarn & Tanpong, 2023). Certain medications used in patients without diabetes to control premature contractions should be used with extreme caution in pregnant women with diabetes because they can significantly affect blood sugar control by causing increases in blood glucose concentrations (e.g., terbutaline/brethine) (Peterson et al., 1993). Therefore, there is a need to find complementary therapies. Our main aim was to examine the impact of *Thunbergia laurifolia* L. (TL), a well-established anti-diabetic plant (Kosai et al., 2015), on GDM rat myometrium contractility (*in vitro*) and ultrastructure (*in vivo*). TL leaves were ethanolic extracted, and the effect was tested. The animal care followed the guidelines of the committee of Care and Use of Laboratory Animal Resources, National Research Council of Thailand. The experiment procedures were approved by the Institutional Animal Care and Use Committee, Suranaree University of Technology, Nakhon Ratchasima, Thailand (Approval no. 13/2560). A single dose of streptozotocin (STZ) 60 mg/kg BW was given to induce GDM in pregnant rats on day 5 of gestation. Blood samples were obtained from a tail vein puncture, and glucose levels were monitored two days after STZ to confirm diabetic induction by a glucometer. Diabetes was defined as hyperglycemia exceeding 200-300 mg/dL. For *in vitro* study, GDM rats were humanely killed at term, and myometrial strips were isolated for isometric force measurements. For *in vivo* study, TL was orally and daily administrated at high (500 mg/kg BW) and low doses (50 mg/kg BW) from day 7 of gestation until term, and its effects on blood glucose and myometrial ultrastructure were investigated. The results showed that TL extract significantly inhibited spontaneous uterine contractility in a concentration-dependent manner with IC_{50} of 1.19 mg/ml (n = 5). The spontaneous force was significantly reduced to 76±8% when compared with 100% control (n = 5). The significant reduction was still active, continuing in combination with KCl depolarization and oxytocin-mediated contractility in both groups. Thus, the force was reduced to 79±7% and 74±7% in the presence of KCL and oxytocin when compared with 100% control (n = 5). TL significantly decreased blood glucose levels in GDM (n = 5). Both high and low doses of TL significantly decreased blood glucose levels to 418 ± 29 and 431 ± 11 mg/dL when compared with GDM non-treated control 588 ± 13 mg/dL. Histological study revealed that the muscular layer significantly increased in thickness in both high and low doses of TL compared with GDM control (67.35 ± 2.89%, 62.30 ± 2.26%, and 52.66 ± 2.36%, respectively). Taken together, TL produced an inhibitory effect on GDM myometrium, irrespective of the type of contractility. Along with the effect of reducing blood glucose levels, TL also restores deleterious GDM myometrium by increasing its thickness. Therefore, TL is worth further investigation in human myometrium and has developed as a tocolytic agent to prevent SPD in GDM.

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PCA074

Garlic Oil Improves Small Intestinal Motility in Experimentally Induced Type II Diabetes Mellitus in Female Wistar Rats

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Diabetes mellitus impairs small intestinal motility; however, little studies have demonstrated the effect of garlic oil on small intestinal motility, so .his study evaluated the beneficial effects of garlic oil in type 2 diabetes mellitus. Thirty-six adult female Wistar rats were allocated into: control (C), garlic oil supplemented non-diabetic, diabetic, and garlic oil-treated diabetic groups. Rats were anesthetized with pentobarbitone (40 mg/kg BW), then small intestinal segments were studied for motility parameters and oxidative markers. Nasoanal length, waist circumference, fasting blood glucose level (FBG) and plasma insulin level were determined. Compared to control group, diabetic rats had reduced average force of contraction and motility index in all small intestinal segments, accompanied by decreased average duration of contraction only in jejunum, in addition to hyperglycemia, insulin resistance, prominent oxidative stress and obesity denoted by motility parameters, fasting blood glucose, HOMA-IR, intestinal MDA and waist circumference. Garlic Oil non-diabetic rats had reduced average force of contraction and motility index in all small intestinal segments, despite persistent higher Lee index and waist circumference. However, Garlic oil treated-diabetic rats had improved effects on small intestinal motility in almost all small intestinal segments and controlled the oxidative stress. In conclusion, decreased small intestinal motility was present in DM, mostly by oxidative stress, and in normal rats supplemented with garlic oil. However, garlic oil treatment in diabetic rats resulted in an improvement in small intestinal motility and in a remarkable anti-hyperglycemic effect, mostly due to its antioxidant effect.

PCA075

The effects of pharmacological modulation of pancreatic cell death on acute/chronic pancreatitis and pancreatic fibrosis

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Acute pancreatitis (AP) is a serious pancreatic disease, characterised by sudden onset, severe pain, and overall mortality rates of up to 10%. Unlike its acute form, which is potentially reversible, chronic pancreatitis (CP) worsens over time, leading to atrophy of the pancreatic parenchyma and fibrosis mediated by activated pancreatic stellate cells (PSCs). This often results in impaired digestion, diabetes, and increases the risk of pancreatic cancer. Currently, there is no effective and specific treatment for AP or CP.

The hallmark of pancreatitis is premature activation of digestive enzymes stored in pancreatic acinar cells (PACs). Active enzymes damage neighbouring PACs causing necrotic death and triggering the release and activation of other enzymes, thus promoting a self-perpetuating chain reaction of autodigestion and inflammation.

A potential strategy to break the vicious cycle of pancreatic necrosis and mitigate the disease is to direct PACs towards apoptotic death, which is not normally associated with the release of cellular content. Therefore the aim of this study was to test the therapeutic benefit of pharmacological modulation of cell death by Bcl-2 inhibitors in mouse models of acute and chronic pancreatitis.

All animal experiments on C57BL6J mice were approved by our Local Ethics Committee, approval numbers: 106/2020, 312/2021. In both models, the mice were divided into equal groups (n=6). AP was induced by seven hourly intraperitoneal injections of caerulein (50 μg/kg), while control mice were only given saline. In the CP model, mice received caerulein administrations twice a week for eight weeks. In addition, mice received one of the tested Bcl-2 inhibitors or an appropriate vehicle. The efficacy of therapy was evaluated by histological scoring (H/E or Sirius Red staining) and by comparing the extent of necrosis and apoptosis (IHF for cleaved caspase-3) in the pancreata of the experimental animals.

Our results show that 1 h treatment with caerulein induced 20.24±2.43% of necrosis and 9,67±2,35% of apoptosis in freshly isolated mouse PACs; and two Bcl-2 inhibitors reduced caerulein-elicited necrosis to 6.4±1.67% ($p<0.001$) or 7.44±3.84% ($p<0.001$), while increasing apoptosis to 15,06±2,67% ($p<0.001$) or 17,13±1,44% ($p<0.001$).

Bcl-2 inhibition significantly improved histological score in the AP mouse model. The overall histological severity score for the AP group was 14.25±2.04 points, and Bcl-2 inhibition reduced it to 6.67±2.14 points ($p<0.001$) and 10.25±2.25 points ($p=0.005$). The inhibitors not only

decreased necrosis from 29.22±11.21% (of the tissue area) in the AP group to 1.75±2.06% ($p<0.001$) and 10.81±4.24% ($p<0.001$) in the treatment groups, but also increased apoptosis from 0.7±0.69 (apoptotic cells per mm^2 of the tissue) in AP to 26.87±9.63 ($p=0.002$) and 58.16±41.83 ($p=0.0027$). In contrast, long-term therapy did not show efficacy in the CP model, and one of the inhibitors exacerbated caerulein-induced fibrosis in the organ.

Short-term Bcl-2 inhibition has the potential to shift acinar cell death from necrosis to apoptosis and thus could find its application as a therapeutic strategy in severe cases of AP. Since one of the Bcl-2 inhibitors is currently approved for the treatment of leukaemia, its use should be carefully considered in patients with concomitant CP.

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PCA076

Exploring the link between pro-inflammatory cytokines and carotid body dysfunction in metabolic diseases

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Introduction and aim: Metabolic diseases are regarded as a leading cause of mortality and morbidity worldwide. Carotid bodies (CBs), which were traditionally considered oxygen-sensitive organs, are also metabolic sensors that play a role in the development of metabolic disorders [1-3]. In metabolic disease states the CB activity is increased and the abolishment of its activity improves metabolic function [1-3]. Inflammatory cytokines are among the factors that can contribute to CB dysfunction in dysmetabolic states [4]. Indeed, the presence of inflammatory cytokines and their corresponding receptors in the CB have been reported [1,4]. In this study, we investigated the contribution of CB to the ventilatory responses induced by pro-inflammatory cytokines, as well as the effect of pro-inflammatory cytokines on CB function.

Methods: Two groups of Wistar rats were used: a control group fed with a standard diet (CTL) and a high-fat (HF) group fed with a diet rich in lipids (60% energy from fat) for 3 weeks. The animals were anesthetized with pentobarbital sodium (60mg/kg i.p.) and at the end of the experimental protocol were killed with an overdose of the same anesthetic. The effect of IL-6 on ventilation was tested by administering IL-6 (0.5 and 5 ng/ml) into the femoral vein in CTL animals with and without carotid sinus nerve (CSN) resection and on the release of adenosine from isolated CBs [5]. To quantify adenosine release, CBs were incubated in normoxia (20% O2 + 5% CO2, balanced N2) in the presence of erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA; an inhibitor of adenosine deaminase, 2.5 μM) for 10 min, followed by incubation during 30 min with IL-6 (1ng/ml) in normoxic conditions. Adenosine levels were quantified by HPLC with UV detection. TNF-α levels and the receptors for IL-1β and IL-6 of CTL and HF animals were evaluated by immunohistochemistry. One-way ANOVA with Dunnett's comparison test and Student's t-test was used. Experiments followed the 2010/63/EU European Union Directive and were approved by NMS Ethics Committee and the Portuguese Authority for Animal Health.

Results: In control animals, IL-6 enhanced basal ventilation, an effect abolished by CSN resection. Moreover, in CTL animals, IL-6 increased by 87% ($p<0.05$) the adenosine release from the CB. HF diet intake for 3 weeks, increased by 80% ($p<0.01$) and 46% ($p<0.01$), respectively, the TNF-α immunoreactivity and IL-1β receptor in CB, compared to CTL animals. Additionally, the immunoreactivity for the IL-6 receptor remained unaltered.

Conclusion: We conclude that the CB plays a crucial role mediating IL-6's effect on ventilation, an effect that can involve the stimulation of adenosine release from the CB. Additionally, HF diet intake promotes inflammation within the CB. Taken together, these

results suggest that pro-inflammatory cytokines may contribute to CB dysfunction in metabolic diseases.

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PCA078

): Effects of glyphosate-based herbicide exposure on adiposity and liver histopathology in female mice at reproductive age submitted or not to bilateral ovariectomy

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Women display augmented risk to obesity and hepatic steatosis, after bilateral oophorectomy. Some environmental chemicals (EC) can worsen these conditions. The glyphosate-based herbicides (GBHs) are an EC and studies have showed that may predispose to various chronic diseases. Here we evaluated the effects of GBH exposure on glucose tolerance, adiposity, and liver histopathology in female mice, at reproductive age, submitted or not to bilateral ovariectomy. For this, 80-days old *C57Bl/6* female mice received a subcutaneous injection of 2 mg/kg meloxicam and an intraperitoneal (IP) injection of 3 mg/kg acepromazine. After 30 minutes, females were anesthetized via IP (10 mg/kg xylazine plus 100 mg/kg ketamine), and randomly ovariectomized (OVX) or sham-operated. After 30 days, sham and OVX females were underwent to a daily gavage of distilled water [vehicle; SHAM0 (n=9) and OVX0 (n=7) groups, respectively], containing or not, 0.5 [SHAM0.5 (n=9) and OVX0.5 (n=9) groups, respectively] or 50 mg/kg GBH [SHAM50 (n=9) and OVX50 (n=9) groups, respectively] for 60 days. Afterward, glucose tolerance, body weight (BW) and plasma lipids, and aspartate (AST) and alanine (ALT) aminotransferases were evaluated. Retroperitoneal fat pads and the liver were weighted and processed for histology. Results were analyzed using two-way ANOVA followed by Tukey test ($p < 0.05$). Experimental procedures were in accordance with brazilian's ethical standards and approved by the animal use committee (Process: 0477634/2021). OVX mice exhibited increased retroperitoneal fat stores (4.6 ± 0.6 mg/gBW) with hypertrophic adipocytes (53.1 ± 3,5 µm), but similar BW (22.6 ± 0.6 g) of SHAM0 (2.5 ± 0.2 mg/gBW, 34.5 ± 1,2 µm and 20.8 ± 0.6g, respectively). GBH exposure did not change these parameters in OVX0.5, OVX50, SHAM0.5 or SHAM50 female mice. OVX0 and SHAM0 females displayed similar fasting glycemia (101.1 ± 4.4 and 87.7 ± 3.4 mg/dL), triglyceridemia (34.9 ± 1.3 and 42.4 ± 2.8 mg/dL), cholesterolemia (94.0 ± 3.1 and 100.3 ± 6.2 mg/dL), AST (36.8 ± 4.6 and 27.5 ± 3.4 U/mL) and ALT plasma levels (19.7 ± 2.7 and 15.3 ± 1.2 U/mL), respectively. Glucose tolerance did not differ among OVX0 (9834 ± 344 mg/dL.min-1) and SHAM0 (8702 ± 287 mg/dL.min-1). GBH exposure at 0.5 or 50 mg/kg/day did not modify all these plasma biochemical parameters in OVX and SHAM mice. Liver weight was not altered in OVX0 (39.7 ± 0.9 mg/g BW) and SHAM0 females (42.3 ± 1.2 mg/g BW), or by the exposure of these mice to 0.5 (OVX0.5: 40.7 ± 0.9 and SHAM0.5: 39.6 ± 1.3 mg/g BW) and 50 mg/kg GBH (OVX50: 41.6 ± 0.6 and SHAM50: 41.9 ± 1.2 mg/g BW). But liver samples of OVX0 mice exhibited mild hepatic microvesicular steatosis. GBH increased hepatic steatosis only in SHAM0.5 females, since 44.4% and 11.11% of their liver samples exhibited moderate and mild steatosis, respectively. Therefore, GBH exposure, at the doses and time used here, not modified adiposity or plasma biochemical parameters in OVX or SHAM females. But at the dose of 0.5 mg/kg/day, GBH may change hepatic lipid metabolism in females with regular ovarian cycle.

no aplie

PCA079

Effect of nutraceutical combination as an integrative approach on the kidney function in the high-fat diet-induced obese Wistar rats

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Introduction: Globally, Obesity is found to be a separate risk factor for kidney damage, according to research using rat models. Nutraceuticals have garnered considerable interest in obesity research due to their role in etiopathogenesis. In addition to their potential nutritional, safety, and therapeutic effects, they provide various health benefits, including the prevention and treatment of diseases. Nutrition is also recommended as a therapy for managing CKD by KDOQI guidelines.

Aim: The study aims to investigate whether the developed nutraceutical combination (NuTC) is effective in preventing high fat diet (HFD) -induced chronic kidney disease in obese Wistar rats, by assessing physiological, biochemical, and histopathological indices.

Methodology: The protocol was approved by the Institutional Animal ethical committee (IAEC/KMC/66/2019). Obesity was induced in male Wistar rats by feeding with a high-fat diet for 11 months and studied the effect of change in nutrition on kidney functions. Rats were randomly assigned to one of three diets containing regular chow diet (Standard control- NC), HFD infused with lard (Disease control- DC), and NuTC(intervention group) (6 animals in each group). The diet used for intervention was made using locally available barley, fish oil, moong bean, flax seed, and foxtail millet. Blood samples were collected for renal functional measurements (urea and creatinine), and kidneys were examined for histological evaluation to study the variation across 0-90 days. Jamovi 2.3.21 is used to analyze the data statistically.

Results: After 90 days, the rats in the HFD group had significantly higher body weight compared to NC ($p< 0.001$) and HFD+NuTC ($p <0.001$) groups. Similarly, the rats in the HFD group had significantly higher U (77.26±4.89) and Cr (1.34±0.38) in mg/dL compared to NC and HFD+ NuTC (42.41±2.87, 0.488±0.04) respectively. Thus, compared to the HFD group, the intervention group had a significant decrease in body weight and renal functions ($p<0.001$). The NC group showed normal renal articulator (Figure 1a); the HFD diet group showed renal cortex with cellular glomeruli with endocapillary congestion and adjoining tubular system showing luminal hyaline cast with congestion of stromal arterioles (Figure 1b); and HFD+NuTC group showed renal cortex with cellular glomeruli with endocapillary congestion and adjoining tubular system appearing unremarkable with congestion of the stromal arterioles (Figure 1c).

Conclusion:

Pharmaceutical therapy is the most common modality to reduce the CVD burden in CKD. Although pharmaceuticals aim to treat oxidative stress, inflammation, and nephropathies in CKD, they have inherent limitations, as they exhibit adverse side effects. Components of the

nutraceutical prepared in our study also contain omega-3 fatty acids, protein, fiber, vitamins, and minerals that help recover from kidney damage due to obesity. Overall our study observed a statistically significant ($p < 0.05$) reduction in body weight and an improvement in renal function tests in NuTC group compared with the DC group.

To conclude, the combination of nutraceuticals developed shows evidence of improvement in both obesity and renal functions in high-fat diet-induced obese Wistar rats.

PCA080

The effect of L-type amino acid transporter 1 overexpression in response to leucine administration in C2C12 muscle cells

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Introduction:

Skeletal muscle mass is regulated by the net balance between muscle protein synthesis and breakdown. Essential amino acids are the substrates of muscle protein and activate muscle anabolism strongly (1). Leucine, one of the essential amino acids, has the strongest anabolic capacity and is known to exert this after being transported into muscle cells through L-type amino acid transporter 1 (LAT1) (1-3). However, the role of LAT1 in skeletal muscle protein synthesis and hypertrophy is not fully understood.

Aims:

Here, we made murine C2C12 myoblasts overexpressing the LAT1 and investigated the role of LAT1 in muscle growth and muscle protein synthesis response after leucine administration.

Methods:

LAT1 overexpressing cells (LAT1-OE) and control cells were made by transferring the LAT1 gene or vehicle into C2C12 myoblasts using in vitro electroporation (n = 6 for each group). One day after the electroporation, the cells were cultured in DMEM containing 2% horse serum and induced differentiation into myotubes for 6 days. After the differentiation, the myotubes were administered with 5 mM leucine. Data were analyzed using t-test or two-way ANOVA (LAT1-OE × Leucine). If an interaction was observed, Sidak multiple-comparison test was performed.

Results:

The LAT1-OE myotubes showed a lower fusion index and embryotic myosin heavy chain expression compared to the control myotubes ($P < 0.05$). The LAT1-OE myotubes showed lower mTORC1 activity (indicated by the expression of phosphorylated $p70S6K^{Thr389}$) compared to the control myotubes (main effect of LAT1-OE, $P < 0.05$), but mTORC1 activation induced by leucine administration was not influenced (main effect of leucine, $P < 0.05$).

Conclusion:

These results suggest that the expression of LAT1 negatively regulates muscle growth but does not have a negative effect on the response to leucine administration.

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PCA081

31P-NMR reveals muscle metabolic abnormalities after exercise-induced muscle damage

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Introduction. Exercise-induced muscle damage (EIMD) is particularly prevalent after unaccustomed eccentric contractions and is characterised by muscle weakness and soreness lasting several days (Armstrong, 1984). Previous studies using ^{31}P magnetic resonance spectroscopy (MRS) found metabolic muscles abnormalities at rest with increased inorganic phosphate (Pi) and phospho-diesters (PDE) contents alongside reduced phosphocreatine (PCr) and adenosine triphosphate concentrations (ATP) (McCully *et al.*, 1988). However, it is unclear if muscle metabolism differs during and after recovery from exercise of damaged muscles, and if changes are associated with perceived effort during exercise. Therefore, the aim of the present study was to investigate whether phosphorous metabolism changes with EIMD and whether changes are related to perceived effort during exercise.

Methods. Twenty physically active, healthy volunteers (age 23.4±4.0 years; BMI 23.6±2.4 Kg/m^2; training hours 5.4±3.3 (mean ±SD)) were eligible and provided written, informed consent to take part in the study. Participants visited the laboratory for a familiarization session, the first (baseline) experimental session and the follow-up session 48h after baseline (48h EIMD). EIMD was induced by repetitive high-intensity eccentric single-leg extension contractions. Assessments at baseline and 48h after EIMD were implemented in both legs. A visual analog scale (VAS_{SQ}) assessed knee-extensors soreness and strength was assessed by single-leg maximal voluntary knee extension isometric contractions (MVC). Both thighs were imaged for quadriceps cross-sectional area (Qcsa) and ^{31}P MRS at rest, during 3-min sustained isometric contractions and for 2-mins of resting recovery (Sleigh *et al.*, 2016). Data between control and EIMD leg were analysed using two-way repeated measures ANOVA. The area under the curve (AUC) and differences for the kinetic changes (slopes) were determined in EIMD leg for Pi/PCr and PCr and were compared with a paired t-test.

Results. EIMD was evident at 48h from the significant time, leg, and time x leg interactions for MVC, Qcsa, and VAS_{SQ} (all $p<0.002$; Table 1). EIMD leg showed 18±4% reduction of MVC, 2.80 ± 0.04% increase of Qcsa, 20.8±0.1% increase in RPE, and 8267±1734% increase of VAS_{SQ} at 48h compared with baseline (all $p<0.001$). Resting PCr, Pi, Pi/PCr and ATPγ all showed significant effects of Time (all $p\leq0.010$). Resting PCr values were marginally (-2.3%) lower at 48h compared with baseline for both the EIMD and control legs (effect of time $p=0.010$; time x leg interaction $p=0.446$). Significant time x leg interaction for Pi, Pi/PCr and ATPγ (all $p\leq0.002$) was found, as values for Pi and Pi/PCr were higher and ATPγ was lower in the EIMD leg at 48 h compared with baseline, but control leg values for all these measurements remained

unchanged over 48h (Table 1). Changes in RPE from baseline to 48h were significantly associated with changes to resting Pi, Pi/PCr and ATPγ only in the EIMD leg. Increased slopes from resting to exercise were found for Pi/PCr for EIMD leg, giving greater area under the curve (all $p<0.01$; Figure 1) but no changes were found for exercise-to-recovery slopes.

Conclusion: Our results suggest that EIMD changes muscle metabolism at 48h, affecting both resting and exercising muscle as well as perceptions of effort.

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PCA082

Quantifying the superabundance of mitochondria in the sensory terminals of mammalian muscle spindles.

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Mitochondria are unusually abundant in sensory terminals of mammalian muscle spindles; e.g., Landon (1966). We present the first quantitative morphological studies on these mitochondria, aiming to better understand their role in mechanosensory function. 3 adult C57/Bl6SJ mice were killed by Schedule 1 methods (Animals (Scientific Procedures) Act 1986 incorporating European Directive 2010/63/EU). Muscle-spindle-rich portions of deep masseter were removed in physiological saline, fixed (cacodylate buffer, 2.5 % glutaraldehyde/4 % paraformaldehyde; 4°C) for 24 hrs, postfixed (3 % potassium ferrocyanide and 4 % OsO_4), liganded with thiocarbohydrazide and 2 % OsO_4, block stained (1 % uranyl acetate and lead aspartate), and embedded in hard Epon. Sections (90 nm thick, transmission EM) were used to estimate the proportion of sensory-terminal volume occupied by mitochondria (V_V) and the areas of mitochondrial membranes per unit volume of mitochondria (S_V). Digital images (15000x magnification) were overlain with a 0.5mm sampling grid (ImageJ), for stereological analysis (Howard and Reed, 1998). Additional blocks were examined with serial block-face scanning EM (SBF-SEM) for 3-D reconstruction (Reconstruct; Fiala, 2005).

Mean V_V (% ± s.e.) for sensory terminals from the 3 mice were: 53.03 ± 4.38; 63.90 ± 7.37; and 47.88 ± 5.66; giving an overall average of 54.94 ± 3.49%, when estimated by stereology. To cross-validate this estimate, virtual reconstruction of 575 mitochondria in a sensory-terminal loop within a 15mm-long segment of a bag_2 fibre was made using SBF-SEM, revealing the mitochondria to be unbranched, more or less ovoid, with mean volume 0.358mm^3 ± 0.015 s.e. (range, 0.015-3.57), highly skewed towards the smallest values (median = 0.260). Total mitochondrial volume was 209.41mm^3 and terminal-loop volume was 376.79mm^3, giving V_V of 55.58%, remarkably close to overall average V_V estimated by stereology. Mean surface area of the mitochondria was 2.14mm^2 ± 0.06 s.e., also skewed towards the smallest values (median = 1.80). Surface area: volume ratio was much less skewed, with mean 7.80mm^{-1} ± 0.11 s.e. and median = 7.04. Overall values of S_V for the sensory-terminal mitochondria were: cristae, 13.67mm^{-1} ± 0.58 s.e.; outer membrane, 4.47mm^{-1} ± 0.22 s.e.; and inner membrane, 4.17mm^{-1} ± 0.23 s.e. These values correspond to absolute areas per mean mitochondrial volume of: 4.90mm^2; 1.60mm^2; and 1.49mm^2, respectively.

These data show that volume proportion of mitochondria in the sensory terminals is extraordinarily high, at about 55%. By comparison a 21mm-long sarcomeric segment of a chain fibre, also reconstructed using SBF-SEM, contained 13.1% mitochondria by volume. These mitochondria were elongate, often branched, with mean volume 0.444mm^3 ± 0.086 s.e. and mean surface area 4.557mm^2 ± 0.763 s.e. Using a similar reconstruction technique, Bleck *et al.*, (2018) reported that small (approximately 1000mm^3) volumes of glycolytic and oxidative extrafusal muscle fibres contained about 4 and 10% mitochondria by volume, and even the highly energetic cardiac muscle contained just about 34% in mouse. The functional importance of this superabundance in sensory terminals has yet to be determined, but it may underlie the

observation that sensory ataxia is a commonly listed symptom in many mitochondrial genetic diseases (Ghaoui and Sue, 2018).

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PCA083

Quantifying the predictive value of brain oxygenation and neurometabolism for dementia and cognitive impairment: a study protocol and preliminary data

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Introduction

Dementia is associated with vascular and metabolic abnormalities, several of which may arise prior to prodromal stages (Toth et al., 2017). Detecting these abnormalities may thus be vital for early intervention. Unfortunately, current detection tools are inadequate: there are no objective, accessible, and effective biomarkers for dementia. Near-infrared spectroscopy (NIRS) is a non-invasive neuroimaging method which uses near-infrared light to quantify brain oxygenation and neurometabolism (the latter via broadband NIRS). Anatomical information can be incorporated with high-density NIRS to produce volumetric reconstructions of brain oxygenation, termed 'high-density diffuse optical tomography' (HD-DOT). Metabolic and structurally accurate oxygenation information, provided by broadband NIRS and HD-DOT, may be crucial to understanding dementia, but this has not been explored. A study was thus developed to evaluate the diagnostic value of measures of brain oxygenation and metabolism by interrogating resting state and functional differences in dementia.

Methods

This observational study will aim to recruit twenty participants with Alzheimer's Disease, Dementia with Lewy Bodies, Mild-Cognitive Impairment, and healthy controls (total n = 80). Clinicaltrials.gov ID: NCT05460143. Participants will have two NIRS scans. The first will use HD-DOT (Lumo; Gowerlabs Ltd.) with 34 sources and 48 detectors. The second will use broadband NIRS (miniCYRIL; Bale et al., 2014) using 1 channel. Cortical atrophy can lead to the misattribution of changes in optical absorption to functional activity. Therefore, AD/DLB patients will have a structural Magnetic Resonance Imaging scan for accurate image reconstruction. During the HD-DOT scan, participants will undergo data collection in resting state, and during activation in the following: Boston naming task (BNT; Kaplan et al. 1983), implicit memory task, mismatched negativity task, visual stimulation paradigm (inverting checkerboard), and a naturalistic motor task. During the broadband NIRS scan, participants will undergo data collection in resting state and in a hyper/hypocapnia paradigm. Statistical models integrating signal and functional connectivity metrics from broadband NIRS and HD-DOT, behavioural and clinical data, and structural data will be created to evaluate the diagnostic value of these metrics. The results from piloting a subsection of these paradigms (n=2) are presented here (one dataset for visual stimulation was removed due to low optical coupling), with further data to follow when the study begins in April.

Results

Visual stimulation elicited a clear, delayed rise in oxygenated haemoglobin (HbO; Figure 1; peak ~13.2s from stimulus onset) with a concurrent decrease in deoxygenated haemoglobin (HbR) in the contralateral hemisphere to the stimulated hemifield. The BNT yielded an immediate rise in HbO (Figure 2) only evident in longer source-detector separations, more likely to sample the brain.

Conclusion

A robust response to visual stimulation was observed, whereas the BNT may be insufficiently demanding. These results show promise that NIRS can detect differences between people with dementia and controls. Future work in these populations will test this hypothesis.

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PCA084

Carotid sinus nerve resection prevents cognitive dysfunction and increased LTP levels in the hippocampus of prediabetic rats

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undefined

Introduction: Type 2 diabetes (T2D) is an established risk factor for the development of neurodegenerative diseases as Alzheimer's (AD) and Parkinson's Disease (PD)(1). The carotid bodies (CBs), peripheral chemoreceptors classically defined as O2 sensors, have been recently described to have a role in energy and glucose homeostasis and its dysfunction associated with the development of dysmetabolic states (2). In agreement with the tole of CB in dysmetanolic states, the abolishment of CBs activity, via the resection or neuromodulation of its sensitive nerve, the carotid sinus nerve (CSN), prevented and reverted dysmetabolic features of prediabetic and T2D animal models (3). Herein, we evaluated if the modulation of CB through the CSN resection may prevent the impact of metabolic dysregulation on cognition and synaptic plasticity.

Methods: Male wistar rats (8-10 weeks of age) fed a high fat-high sucrose (HFHSu) (60% lipid rich diet plus 35% sucrose in drinking water), or a standard (CTL) diet for fifteen weeks. After this period animals were randomly assigned to CSN resection or sham surgery. Metabolic profile and behavior were evaluated at 14 (before surgery) and 20 weeks of diet (5 weeks post-surgery). After final behavioral assessment, electrophysiological recordings in hippocampal brain slices were performed to evaluate synaptic function and plasticity, by recording fEPSPs in hippocampus CA1 area, evoked by Schaffer-colateral stimulation. We also assessed whether the induction or the maintenance of long-term potentiation (LTP) is altered between experimental conditions. Experiments followed the 2010/63/EU Directive and were approved by the NMS Ethics Committee and the Portuguese Authority for Animal Health. Significance between the means was calculated by one-way ANOVA with multiple comparison tests. Differences were considered significant at $p<0.05$.

Results: HFHSu animals exhibited insulin resistance and glucose intolerance, and CSN resection reversed these phenotypes ($p<0.05$). Behaviorally, HFHSu-animals: 1) spent 62% less time interacting with the novel/own object in the novel object recognition (NOR) test ($p<0.05$); 2) sniff 91% less time novel/own scent and take 80% more time to identify the novel block in block test ($p<0.05$) and 3) exhibit 43% less alternative behavior in the y-maze test in comparison with CTLs. ($p<0.05$). All these effects were prevented by CSN resection ($p<0.05$), except for the Y-maze test. Electrophysiological recordings showed no alterations in baseline synaptic transmission nor in PPF (pair pulse facilitation) but showed that in HFHSu sham rats, LTP expression is increased ($p<0.05$). This effect was not observed in CTL animals and was completely reversed in HFHSu-CSN denervated rats ($p<0.05$).

Conclusions: Altogether, we showed that HFHSu diet promoted peripheral dysmetabolism leading to cognitive functions impairment, and that CSN resection was able to restore cognitive performance and synaptic plasticity function. These results show that the modulation of CB

activity could be used as a therapeutic approach to prevent neurodegenerative diseases associated with T2D.

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PCA085

Differentiated neurons are damaged by organophosphate and carbamate pesticides by non-cholinergic mechanisms

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Organophosphate (OP) and carbamate compounds are widely employed pesticides that are toxic to target species through targeted inhibition of acetylcholinesterase (AChE). However, their toxicity to off-target species including humans remains a health and environmental concern. The neurotoxicity of a 24-hour exposure to the OP pesticides chlorpyrifos-oxon (CPO) and azamethiphos-oxon (AZO), and the carbamate pesticide, aldicarb, were investigated using undifferentiated and differentiated SH-SY5Y neuroblastoma cells. Pesticide concentration-response curves for cell viability were undertaken using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assays. Concentration-response curves for pesticide inhibition of cellular AChE activity were also generated and the production of reactive oxygen species (ROS) was monitored using a 2',7'-dichlorofluorescein diacetate (DCFDA) assay. Pesticides reduced cell viability and neurite outgrowth in a concentration-dependent fashion, from a threshold pesticide concentration of ≥10 μM. Neurotoxic potency was in the order AZO > CPO > aldicarb for undifferentiated cells but CPO> AZO > aldicarb for differentiated cells, and this toxic potency of CPO reflected its more extensive induction of ROS and generation of carbonylated proteins that were characterized by Western blotting. Hence, the relative neurotoxicity of OP pesticides and aldicarb in part reflects non-cholinergic mechanisms that likely contribute to tissue injury.

PCA086

The effect of low grade insular glioma on air hunger

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Background: Functional brain imaging studies of dyspnoea consistently report activation of insular cortex (Evans *et al.*, 2002; Banzett *et al.*, 2000). The precise role and importance of the insula in dyspnoea perception cannot be discerned from brain imaging studies alone, alternative approaches are needed to establish a structure-function relationship. We had the opportunity to study patients with insular gliomas and hypothesised that sensitivity to experimentally induced air hunger would be diminished.

Method: Three patients with low grade insular glioma and three healthy age matched controls underwent incremental hypercapnic air hunger (AH) tests. This involved 1min increments of 1.3% in inspired CO_2 raising end tidal PCO_2 up to 55mmHg while ventilation was constrained to their baseline level (mean±sd 10.7±2.3 and 9.7±0.5 L/min in patients and controls, respectively). Participants rated their air hunger on a 10cm visual analogue scale (VAS) every 15 seconds throughout the test.

Results: The three glioma patients (aged 38-45 years, one female) produced a substantially lower hypercapnic air hunger stimulus response slope compared to age matched healthy controls (aged 34-41 years, one female); mean±sd slopes were 2.27±2.6 vs 23.1±11mmVAS/mmHg PCO_2. This difference approached significance (p=0.08). The lowest slope in the healthy control group was 14.3mm/mmHg PCO_2. The mean±sd AH threshold was the same for both patient and control group (43±4.5 versus 43±3.6mmHg PCO_2). During debrief, patients comments suggested that it was specifically the affective component of breathlessness that was diminished. The one patient who has had surgery to remove the glioma continued to show a reduced sensitivity when re-tested post surgery(6.7mm/mmHg) with a slight rightward shift in threshold for AH.

Conclusion: This data suggests that low grade insular glioma generates insensitivity to hypercapnic air hunger, particularly the affective component. If a fully powered study verifies these findings, this will add support for targeting this region for symptomatic relief of intractable dyspnoea in patients with incurable cardiopulmonary conditions.

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PCA087

Pulsating pressure enhances transport of fluid through a macromolecular matrix: support for a flow of cerebrospinal fluid along the basement membranes of brain capillaries.

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Cerebrospinal fluid (CSF) delivers molecules to the vicinity of brain cells and may carry away others, such as amyloid-beta. In the cortex, CSF flows down peri-arteriolar space: its subsequent pathway is uncertain (see Hladky & Barrand, 2022). Protein marker molecules in CSF are observed to accumulate in the pericapillary basement membranes of brain capillaries (e.g., Rennels et al. 1985; Fig. 3C in Iliff et al. 2012) but it is improbable that a constant physiological pressure gradient could drive adequate CSF along a pericapillary pathway. The accumulation is slower if blood pulsation is reduced (e.g., Rennels et al. 1985). We have found only one publication on the effect of experimental pressure pulsation on transport through a porous medium of any kind: McMaster and Parsons (1938) on the movement of Evans Blue through rabbit ear tissue. We asked if a pulsating pressure could substantially increase fluid flow through a hydrogel mimicking basement membrane. We constructed a chamber with a central rod 20 mm long and 4 mm in diameter inside a silicone rubber tube i.d. 5 mm, wall thickness 0.5 mm (Fig. 1). This was filled with agarose gel 1% in NaCl 0.15M. A raised reservoir of 0.15M NaCl provided a static pressure of 20, 30 or 60 cm H_2O. Flow was measured by weighing the efflux and care was taken to avoid menisci in the circuit. As described for other porous media, the flow was not proportional to the pressure gradient: when pressure was increased from 30 to 60 cm H_2O, flow increased by a factor of 2.92 (SEM = 0.13, n = 17 tests), significantly more than 2 ($P<0.0001$, two-tailed t-test). The pressure could be modulated (without changing the mean pressure) by changing the air pressure applied to the external face of a rubber membrane bounding the vestibule upstream of the gel. The flow Q during a period of pulsation (lasting typically 15 - 40 min) was divided by the mean of the flows before and after to give the ratio Q(pulsation)/Q(no pulsation). With pressure heads of 20, 30 or 60 cm H_2O, pulsation at 3 Hz with an amplitude of no more than 5.5% increased the flow (two-tailed t-test, $P< 0.001$, $n > 30$). With a pressure head of 20 cm and pulsation of 5.5% the increase ($P = 0.018$) was by a factor of 1.39, SEM = 0.10, n = 5. This increase is much smaller than that described by Hale & Coles (2022): we suggest that in those experiments the main effect of pulsation occurred at the menisci of the bubble used to measure flow along a polyethylene microburette, and not in the gel itself. The effect now reported seems too small to account for physiological transport of CSF along capillary basement membrane. However, if the dimensions were scaled down to those of a real capillary, and an optimum hydrogel were used, there might be a bigger effect.

Hale MG & Coles JA (2022) Biorxiv doi: https://doi.org/10.1101/2021.12.30.474515 Hladky SB & Barrand MA (2022). Fluids Barriers CNS 19.9. Iliff JJ et al. (2012). Sci Transl Med 4,147ra111 McMaster PD & Parsons RJ (1938). J Exp Med 68, 377-400. Rennels ML et al. (1985). Brain Res 326, 47-63

PCA088

From dendrites to nuclear envelope invaginations and beyond: A cell-wide web of cytoplasmic nanocourse coordinates calcium signalling in primary cortical neurons

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It has been proposed that cellular calcium signalling is coordinated through an intracellular network of cytoplasmic nanocourses, namely the cell-wide web, demarcated by sarco/endoplasmic reticulum (S/ER) nanojunctions that span from outer nuclear membrane to the plasma membrane[1]. At the centre of the cell-wide web are cytoplasmic nanocourses demarcated by nuclear envelope invaginations (NEIs). Although their function remains open to question, evidence indicates that the nuclear envelope lumen holds a calcium store that is released into cytoplasmic nanocourses demarcated by NEIs rather than into the nucleoplasm, and that modulating calcium flux across the outer nuclear membrane correlates with changes in gene expression in pulmonary arterial myocytes[1]. Nuclear invaginations are evolutionarily conserved and are found in other mammalian cells[2], including neurons[3]. Our aim was, therefore, to determine whether the cell-wide web coordinates calcium signalling in primary mouse cortical neurons isolated from mouse embryos (E17.5) after 7 days of culture. Fixed neurons were labelled by ER-kit (Abcam), Lamin A and Lamin B1 antibodies following immunolabelling methods adapted from those used previously[1]. 3D reconstruction of deconvolved confocal images strongly suggested that both NEIs and the cell-wide web were present in primary mouse cortical neurons. There were ~55 (given mean ± SEM) NEIs per cell, of which 32.4 ± 3.0 (n = 5) were both lamin A and lamin B1 positive, 21.8 ± 4.9 (n = 5) were lamin A positive and lamin B1 negative, and 1.8 ± 0.7 (n = 5) were lamin B positive and lamin A negative. Lamin B positive NEIs were found to co-localise with the histone mark H3K9me2, consistent with their proposed role in gene expression regulation[1]. Live-cell confocal imaging was then carried out on cortical neurons loaded with the calcium indicator Fluo-4 (Life Technologies), ER-tracker (Thermo Fisher) and Draq5 (Thermo Fisher). This revealed a cell-wide web of Fluo-4 positive cytoplasmic nanocourses that colocalised with ER-Tracker. Within different cytoplasmic nanocourses asynchronous calcium signals were observed in the absence of applied stimuli. More strikingly still, pre-incubation (1 min) of Bicuculine (50 mM) and 4-Aminopyridine (250 mM), which have been shown to induce action potential firing by synaptic release of glutamate[4], evoked calcium transients in primary cortical neurons that arose at dendrites (Fmax/F0 = 2.20 ± 0.15, n = 5) and propagated through cytoplasmic nanocourse into the soma and NEIs (Fmax/F0= 2.46 ± 0.19, n = 5) but not the nucleoplasm, and finally through the axon. Notably the calcium signal within cytoplasmic nanocourse demarcated by NEIs declined more slowly (time to 50% of max = 4.27s ± 0.61s, n = 5) than in extraperinuclear nancourses across the wider cell (time to 50% of max = 2.53s ± 0.40s, n = 5). We conclude that in cortical neurons calcium signalling is coordinated through the cell-wide web at NEIs and beyond.

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PCA089

Laryngeal effects of stimulation of the dorsolateral Periaqueductal Grey Matter in spontaneously breathing anaesthetized rats.

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Background

The stimulation of the Periaqueductal Gray matter (PAG) produces an increase in sympathetic tone includiing an increase in blood pressure, heart rate and respiratory frequency[1]. PAG and nucleus retroambiguus (nRA) are necessary to produce vocalization[2]. The nRA is the perfect target to turn passive into active expiration modifying the activity of laryngeal motoneurons located in the nucleus ambiguous (nA)[3].We have shown that rostral and ventral pontine structures are involved in changes of laryngeal caliber[4]. A high expression of FOXP2 protein (transcription factor closely related to vocalization) at mesencephalic (PAG) and pontine regions (Parabrachial complex and A5 Region) involved in cardiorespiratory control has been described[5].

Objectives

The aim of this study was to characterize the possible role of the dlPAG in modulating laryngeal activity and their effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=27), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/ kg, i.v., as necessary).

Neuromorphological study (n=6)

The pattern of staining for c-Fos and FOXP2 protein immunoreactivity (c-Fos-ir) were examinated throughout the rostrocaudal extent of the nRa/nA region during electrical stimulation of the dlPAG.

Neuropharmacological study (n=21)

A double tracheal cannulation was used to obtain an “isolated glottis in situ” and to record respiratory airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, ±0,1 psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70ml/min with a thermal mass digital air flow meter

controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Thus, at constant air flow, changes in pressure indicate changes in laryngeal resistance. Bilateral parietostomy allowed access to the dlPAG. Electrical stimulations (n=7) of this region using concentric bipolar electrodes (1ms pulses, 20-40μA, 100Hz for 5s) were performed. Microinjections of PBS-Evans Blue (250nl, pH 7.4±0.1, 5-s duration) (n=7) or glutamate (0,25M, 250nl) (n=7) were performed. Respiratory flow, pleural pressure, blood pressure and heart rate were also recorded.

Only data from animals in which the histology showed that the microelectrodes were positioned within the dlPAG and the A5 region were used for statistical procedures.

Results

Activation of the dlPAG elicited a selective increase in c-Fos-ir with an ipsilateral predominance in nRA/nA somatas ($p<0.01$) and confirm the expression of FOXP2 bilaterally in both nuclei. dlPAG PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. dlPAG electrical and chemical (glutamate) stimulations evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,001$), together with a pressor ($p<0,001$) and tachycardic response ($p<0,001$).

Conclusions

Our study contributes with new data on the role of the mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Ethical approval

All experimental protocols were performed in accordance with the recommendations of the European Union directive (2010/63/EU) for animal care and experimental procedures. The experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía.

Keywords

Subglottic Pressure, Laryngeal Motoneurons, dlPAG, Nucleus Ambiguus

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PCA090

The effect of the indoor environment quality (IEQ) on cognition and health

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Background.

The indoor environment quality (IEQ) encompasses temperature, humidity, light, sound and air quality. Previous studies have shown that there was an increase in detrimental effect on cognitive performance and memory at high relative humidity (RH) levels >70% than low RH levels of <30% (Tian et al., 2021; Wu et al., 2020). However, these studies only examined short term exposure (<3 hours) to high humidity levels, outdoor environmental exposure, or field studies. Moreover, increase in thermal discomfort and humid sensitivity has been observed with higher humidity levels when temperatures were >30ºC (Jing et al., 2013).

The effects of heat on cognitive performance depend on multiple personal factors − such as the level of motivation, expertise, sex, hydration status, and heat acclimation (HA) (Gaoua, 2010; Schmit et al., 2017). Many studies thus far have investigated the effects of higher temperatures (>35ºC) on cognition have been conducted in hotter and more humid geolocations, but little have focused on the effects of higher humidity in more temperate landscapes (<35ºC) temperate geolocations.

Aims.

Before the effects of heat and humidity is investigated in the field, it is important to study the acceptance and comfort of hot and humid environment first in a simulated lab study, where we can also measure the most relevant physiological outcomes in a controlled manner. Thus, the main aim of this study is to determine the effect of a humid and warm climate on cognition, physiological responses and environmental perception when compared to a more temperate condition. The primary objective of this study is to investigate the isolated effect of a warm and humid indoor environment for the duration of 8 hours on cognitive function compared with a lower humidity level at the same ambient temperature.

Methods.

Study population: 25 healthy participants - aged between 20 and 40 years with a between BMI >18.5 and <26 kg/m2 will be included.

Study design: Each participant will be exposed to the four conditions in randomized order with at minimum of 1-2 day washout period between sessions. The four conditions differ only in the exposure level of indoor relative humidity (HIGH/LOW) and temperature (NEUTRAL/WARM). The difference between the conditions is the level of humidity in the chamber, which is either 30% of RH (LOW) or 70% of RH (HIGH) and temperature, which is either 25ºC (NEUTRAL) or 32ºC (WARM). Throughout the test day the participant will be conducting cognition tests,

stepping activities, and questionnaires. The main study parameters and endpoints are cognitive performance (cognitive tasks and subjective workload and motivation). While the secondary parameters are physiological measures including core temperature, skin temperature, skin blood flow, local sweat rate, heart rate, blood pressure, urine hydration, and salivary cortisol); along with perceptual evaluations of the environment (thermal, air quality and wetness sensation, comfort, acceptance, preference, pleasure).

Results.

Data collection is ongoing at the moment.

Conclusions.

Conclusions will be made when full results can be determined.

Keywords. Temperature, Humidity, Cognition, Metabolism, Performance

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PCA091

Relief of experimentally-induced breathlessness in healthy individuals, using repetitive Transcranial Magnetic Stimulation of Dorsolateral Prefrontal Cortex

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Background: Use of non-invasive brain stimulation to unravel the brain mechanisms of breathlessness is a novel application that may reveal new targets for relief of intractable breathlessness thereby addressing an urgent clinical need. Similarities in brain mechanisms of pain and breathlessness are evident; both are multidimensional with physical and emotional domains. Brighina *et al.* (2011) showed that capsaicin-induced pain was reduced by repetitive Transcranial Magnetic Stimulation (rTMS) over the left Dorsolateral Prefrontal Cortex (L-DLPFC). We hypothesized that L-DLPFC rTMS will also relieve 'Air Hunger' (AH), an unpleasant component of breathlessness.

Methods: Healthy volunteers underwent breathing tests of AH (hypercapnia with constrained ventilation) before and after 5Hz rTMS over L-DLPFC, before and after 5Hz rTMS over R-DLPFC, and before and after SHAM stimulation, on three separate days in random order. Subjects rated AH on a 100mm Visual Analogue Scale (VAS) during, and completed the D-12 multidimensional dyspnoea questionnaire after, each AH test.

Results: Preliminary data from 10 healthy individuals (aged 21-53yrs; 3 female) showed significant reduction in steady-state AH after L-DLPFC rTMS (mean±sd of -17±15mmVAS; p=0.006), and after R-DLPFC rTMS (-18±22 mmVAS; p=0.04) with smaller non-significant reduction after SHAM (-9 mmVAS). Treatment effects (rTMS minus SHAM responses) for left and right DLPFC rTMS averaged -7.9 and -9.6mmVAS which are below the minimal clinically important difference of 10mmVAS. However, the treatment effect met the minimal clinically important difference for VAS ratings of AH (Ries *et al.*, 2005) in 50% of individuals for both L and R rTMS (Figure 1). The data is currently underpowered and skewed by 2 individuals with disproportionately high SHAM responses. The physical domain of D-12 scores fell by -13, -6 and -2% full-scale for the R-DLPFC, L-DLPFC and SHAM stimulations, approaching significance only for R-DLPFC (p=0.06).

Conclusion: Unlike for pain, 5Hz rTMS of both L-DLPFC and R-DLPFC relieved AH. Attenuation of the physical domain of breathlessness appeared to be linked more to the rTMS of the R-DLPFC. Furthermore, low frequency stimulation of the R-DLPFC is known to relieve anxiety and depression. This suggests that the modulation of dyspnoea from the rTMS of the R-DLPFC and L-DLPFC may operate via separate mechanisms. Reconciling these findings with those of brain imaging studies may help unravel the cerebral network for breathlessness and reveal new targets for relief of clinical dyspnoea.

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PCA092

Blood-Brain and Blood-CSF barriers in a Genetic Model of Hydrocephalus

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Hydrocephalus is a result of cerebrospinal fluid (CSF) accumulating in the brain's ventricles caused by imbalances in CSF secretion, flow, and/or absorption. Treatments for hydrocephalus include invasive procedures such as surgical shunt placement, which commonly fail and are revised throughout a patient's life[1]. Taking a cell and molecular approach to characterize changes occurring during hydrocephalic development may lead to novel targets for pharmacological treatment.

Cell types associated with the blood-brain, brain-CSF, and blood-CSF barriers may be altered in hydrocephalus. This study focuses on choroid plexus epithelial (CPe) cells, ependymal cells, and astrocytes. The choroid plexus is a contiguous layer of tightly regulated epithelial cells surrounding a fenestrated capillary network that is responsible for the production of CSF. The ependymal cells are ciliated neuroglial cells that line the ventricles of the brain and play roles in CSF maintenance and waste clearance. Astrocytes are glial cells that perform various functions in the brain, one of them being blood-brain barrier regulation[2]. These cells serve in brain fluid/electrolyte regulation, an important component of barrier integrity. These cells contain aquaporins (AQP), AQP1 in the CP, and AQP4 in ependymal cells and astrocytes, that are important in regulating CSF and brain interstitial fluid. Aquaporins are implicated in various diseases associated with brain fluid regulation[3].

In Wistar rats, a missense point mutation in the Transmembrane 67 (TMEM67) protein, causes a ciliopathy resulting in hydrocephalus[4]. Using this model, changes in barrier integrity and aquaporins were evaluated using immunohistochemistry, real-time quantitative polymerase chain reaction (RTqPCR), and western blot. Animals were euthanized using 1ml/kg body weight intraperitoneal injection of Euthasol (pentobarbital sodium 390mg/ml, phenytoin sodium 50mg/ml) or carbon dioxide gas according to the IUPUI IACUC protocol, then decapitated. Brains were harvested and processed for their respective experiments. Ventriculomegaly appears in TMEM67 homozygous animals before postnatal day (P)10 by quantitative magnetic resonance imaging[5] and visualized with Nissl (n=3). Barrier integrity was investigated by examining glial activation and tight junction expression and appearance. Increased glial fibrillary acidic protein, a marker of astrocytes, appeared in hydrocephalic animals by immunohistochemistry as early as P10 (n=3). Fluorescent intensity of the tight junction proteins expressed in CPe; claudin-1 and 2, and adherens junction protein; E-cadherin, increased in P15 hydrocephalic animals compared to wildtype (n=3). Interestingly, P10 wildtype animals appeared to have more claudin-1 labeling than hydrocephalic animals (n=3). Expression will be confirmed using western blot (n=3).

Aquaporin localization was examined in hydrocephalic animals at P15, 10, and 5 (n=3). Increased fluorescent intensity of AQP4 in the subventricular zone of hydrocephalic and AQP1 apical localization in the CP was observed in hydrocephalic animals at P15 and P10. RTqPCR showed increased AQP1 in CPe[5] (*p=0.0101) and no change in AQP4 mRNA in the

periventricular cortex (p=0.4359) of P15 hydrocephalic animals compared to wildtype. Post-transcriptional changes will be evaluated using western blot (n=3).

These results provide further characterization of the role of the brain barriers and aquaporins in the pathophysiology of hydrocephalus and elucidate how brain fluid regulation may be altered in hydrocephalus. They also produce targets for pharmacological development.

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PCA093

Investigation of the role of PKCδ in an in vitro model of neuropathic pain

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Background. Neuropathic pain (NP) is caused by lesion or disease of somatosensory system. It impacts quality of life of 8% of UK population and only 33% of these patients have effective NP management with existing medications[1]. Protein kinase C delta (PKCδ) is calcium-independent novel PKC isozyme and is involved in numerous cellular functions, including neurotoxicity, neurodegeneration and apoptosis. PKCδ expression correlates with augmented sensitivity and intensity of NP[2,3]. Rottlerin, a compound isolated from *Mallotus Philippensis* plant, was reported to act as PKCδ inhibitor[4].

Hypothesis. Inhibition of PKCδ activity by rottlerin may represent a novel therapeutic strategy for effective NP management.

Methods. *Cell culture.* HD33n1 human induced pluripotent stem cells (hiPSC) were used to generate fully functional sensory-like neurons according to previously developed protocol[5]. To verify the data obtained on hiPSC-derived sensory-like neurons, we used trigeminal ganglia (TG) neurons isolated from 10-week-old Sprague-Dawley rats (n=3); Schedule 1 for tissue harvesting.

Electrophysiology. Patch-clamp conventional whole-cell electrophysiology technique was used to investigate electrical properties of neurons. Electrophysiological parameters were recorded with Axopatch 200A amplifier, Digidata 1440A digitizer and pCLAMP 10.2 software (Molecular Devices) at room temperature. Bath solution was prepared with 144.8mM NaCl, 2.5mM KCl, 0.5mM $MgCl_2$, 1.2mM $CaCl_2$, 10mM glucose, and 5mM HEPES, pH 7.4 with 1M NaOH. Pipette solution contained 140mM KCl, 6mM NaCl, 4mM Na_2-ATP, 4.2mM Na-GTP, 3mM $MgCl_2$, 1mM $CaCl_2$, 5mM HEPES, pH 7.2 with 1M KOH.

qPCR. RNA was extracted from HD33n1 hiPSCs-derived sensory-like neurons treated with rottlerin in the presence and absence of “pain cocktail” (10 μM adenosine triphosphate, 1μM noradrenaline, 1μM bradykinin and 1μM substance P). The Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit was used for cDNA synthesis following standard procedure. Changes in relative gene expression of *PKCD* in the presence and absence of “pain cocktail” in control and rottlerin treated samples were calculated using the $2^{-\Delta\Delta CT}$ method, and the data were normalised to *GAPDH*.

Statistical analysis. All data are expressed as mean ± S.E.M. Statistical comparisons were performed using Student’s t-tests; differences were considered significant at $p< 0.05$.

Results. Acute application of rottlerin hyperpolarised HD33n1 hiPSCs-derived sensory-like neurons and rat TG neurons in concentration-dependent manner. 10μM rottlerin significantly hyperpolarised HD33n1 hiPSC-derived sensory-like neurons from -45.6±0.1mV (n=3) to -54.2±1.8mV (n=3); $p<0.01$. Similarly, acute application of 10μM rottlerin significantly hyperpolarised rat TG neurons from -57.7±4.0mV (n=5) to -66.4±5.1mV (n=5); $p<0.05$.

Synergetic application of all components of the “pain cocktail” was ineffective to cause depolarisation in rat TG neurons in the presence of rottlerin, thus suggesting its anti-excitatory effect.

Chronic administration of HD33n1 hiPSC-derived sensory-like neurons with 10μM rottlerin decreased PKCδ expression, whereas administration with “pain cocktail” increased it. Combined chronic administration of rottlerin and “pain cocktail” reduced PKCδ expression compare to “pain cocktail” alone.

Summary. These data show for the first-time the ability of PKCδ inhibitor rottlerin to attenuate excitability of HD33n1 hiPSC-derived sensory-like and rat TG neuronal *in vitro* NP model. This may suggest a novel strategy to improve pharmacological management of NP as well as associated co-morbidities.

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PCA094

Ultrastructural and molecular features of rodent brain Calyx-of-Held-like synaptogenesis: neuroendocrine contributions to the molecular logic

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The hindbrain parabrachial complex (PBc) is a sensory relay in the CNS. The forebrain extended amygdala (EA) orchestrates adaptive responses to emotional events. While PBc-EA connection has been extensively studied, cell type specificity and types of synapses remained unclear. In order to address these questions we used a systematic anatomical analysis (immunohistochemistry IHC and double in situ hybridization, DISH) of neuropeptides PACAP, CGRP and neurotensin-NT distribution in rat N=20 and mice N=20 brains, we observed perisomatic ring-like structures containing the three peptides as well as VGLUT1/VGLUT2 in the EA. The origin of those structures was studied using in vivo juxtacellular labeling and tracing study using Adcyap1-Cre for Cre-dependent expression of fluorescent marker fused with artificial channelrhodopsin. PACAP/CGRP/NT/ VGLUT1/VGLUT2 expression was a molecular signature of the pre-synaptic neurons from the posterior-ventral division of PBc. PKCdelta-GABAergic neurons in the EA, co-expressing Adcyap1r1 and Vipr1 mRNA were identified as the post-synaptic cells. This signature, except co-expressing PKCdelta is characteristic of the Calyx-of-Held, a rare perisomatic large glutamatergic synapse in brainstem' medial nucleus of the trapezoid body (MNTB), which receive axons from globular bushy neurons in the ventral cochlear nucleus. Using transmission electron microscopy (TEM) and focused ion beam scanning electron microscopy (FIBSEM), in combination with immunohistochemistry, we demonstrated that the ring-structures in EA are highly similar to the Calyx-of-Held observed in the MNTB. Taken together, our results suggest a common molecular basis for calyceal synaptogenesis both in hindbrain and forebrain. The PBc-->EA Calyx-of-Held synapse may represent a previously unappreciated morphological substrate for high fidelity sensory alert to the forebrain center for adaptive response. This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experiments were approved by the NIMH Institutional Animal Care and Use Committee (ACUC, LCMR-08) and the Research and Ethics Committee of the Faculty of Medicine, Universidad Nacional Autónoma de México (CIEFM 062/2016).

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Effects of stimulation of Cuneiform nucleus and the dorsomedial Hypothalamic nucleus and Perifornical area on the mechanisms involved in the control of laryngeal activity and subglottic pressure in spontaneously breathing anaesthetized rats

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Background

The dorsomedial Hypothalamic nucleus and Perifornical area (DMH-PeF) and the mesencephalic Cuneiform nucleus (CnF) have been involved in sympathetic activity due their connectivity with several nuclei involved in cardiorespiratory control, e.g. dorsolateral Periaqueductal Gray Matter (dlPAG), the Parabrachial/Kölliker-Fuse complex (PBc/KF), the Solitary Tract Nucleus (NTS) and the Rostral Ventrolateral Medulla (RVLM) (1). In previous studies we have demonstrated a functional interaction between hypothalamic and mesencephalic structures (DMH-PeF, dlPAG) with several pontine regions (PBc, A5) (2, 3). We have also shown that rostral and ventral pontine structures are involved in the changes of laryngeal caliber (4).

Objectives

The aim of this study was to characterize the relations between hypothalamic and mesencephalic regions involved in cardiorespiratory control and their possible role in modulating laryngeal activity and their possible effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=42), SPF, Sprague-Dawley (250-300g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/kg, i.v., as necessary). A double tracheal cannulation was used to obtain an “isolated glottis in situ” and to record respiratory airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, ± 0,1psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Thus, at constant air flow, changes in pressure indicate changes in laryngeal resistance.

Bilateral parietostomy allowed access to the DMH-PeF and CnF. Electrical (n=14) and chemical (n=14) stimulations of these regions using concentric bipolar electrodes (1ms pulses, 20-40µA, 100Hz for 5s) or glutamate (0,25M, 250nl) was performed. Microinjections (n=14) of PBS-Evans Blue (250nl, pH 7.4±0.1, 5-s duration) served as control purpose. Respiratory flow, pleural pressure, blood pressure and heart rate were also recorded.

Only data from animals in which the histology showed that the microelectrodes were positioned within the CnFand the DMH-PeF region were used for statistical procedures.

Results

DMH-PeF and CnF PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. However, electrical stimulations in both regions evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,001$), together with a pressor ($p<0,001$) and tachycardic response ($p<0,001$).

Glutamate microinjections within the DMH-PeF and CnF evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,01$ and $p<0,001$ respectively) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,001$ in both cases), together with a pressor ($p<0,001$ and $p<0,01$ respectively) and tachycardic response ($p<0,001$in both cases).

Conclusions

The results of our study contribute with new data on the role of the hypothalamic-mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Ethical approval

All experimental protocols were performed in accordance with the recommendations of the European Union directive (2010/63/EU) for animal care and experimental procedures. The experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía.

Keywords

Subglottic Pressure, Laryngeal Motoneurons, DMH-PeF, CnF, Nucleus Ambiguus

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