OC01

Atrial Compartmentalisation Proteomics: Analysis of Endo-lysosomal proteins using a modified density gradient approach

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Atrial Fibrillation (AF) is the most common cardiac arrhythmia. The incidence and prevalence are expected to increase in the next 30 years by 63% and 66% respectively[1]. As a multigenic disorder, an understanding of the molecular cascades and the cross-communication between total ion exchange and cellular stress in AF is essential for designing effective AF therapies. Mis-regulation of regulatory proteins in the endocytic pathway, including endo-lysosomes and lysosomes, has been reported in AF[2]. The discovery of the cardiac lysosome Ca2+ signaling[3] has opened a new door to unravel the underlying molecular mechanisms of acidic organelles in AF. Organelle isolation using tissue biopsies facilitates studies investigating the roles of regulatory proteins within these pathways. We developed an endo-lysosome isolation method from tissue biopsies using Percoll and sucrose differential density gradient centrifugation. We present what we believe to be the first comprehensive dataset of *C. porcellus* endo-lysosomal focused organelle proteomics using label free MS/MS peptide analysis (QExec LumosTM mass spectrometer) on isolated endo-lysosomes from *C. porcellus* atrial tissue. Experiments performed in accordance with Home Office Guidance on the Animals (Scientific Procedures) Act (1986).

Mass spectrometry data from biological replicates (n = 3) were processed and analysed using Max-quant and Perseus software (v1.5.2.4) to identify proteins. Robust criteria was used for filtration and imputation, and significantly upregulated/downregulated proteins in endo-lysosome (EL) and tissue lysate (TL) fractions were identified by volcano plot. The highest abundant EL proteins were separated using cell component filtration in Gene Ontology (GO) analysis, the molecular function was identified using the PANTHER pathway, and functional network interactions were mapped using Cytoscape analysis (v3.7.2). The presence of the EL marker proteins Lysosome associated membrane protein 2 (LAMP2), beta-Galactosidase (GLB), beta-hexosaminidase (HEXB) and the absence of mitochondrial marker Cytochrome oxidase IV (COX IV) and sarcoplasmic reticulum marker Phospholamban were confirmed by Western blot and enzyme assays.

Our EL preparations demonstrate the enrichment of lysosomal markers GLB, HEXB, Ras-related protein Rab-7a and LAMP2, and identify multiple protein hits relevant to diseases that have been linked to dysfunctional lysosomal enzymes and membrane-bound proteins. These include; lysosomal α-glucosidase (GAA), a key lysosomal enzyme involved in the degradation of glycogen in lysosomes[4]; Cathepsin A, which serves a protective function by regulating stability and activity of beta-galactosidase, neuraminidase enzymes and plays a role in galactosialidosis[5]; Clusterin, identified as a potential biomarker for mucopolysaccharidosis; and Decorin, a protein that, when dysregulated, contributes to cardiac fibrosis or fibrotic stiffness. Furthermore, we identified glycogen phosphorylase, brain form (PYGB) in the EL fraction, a lysosomal enzyme that regulates glycogen mobilization, and plays a prominent role as the only marker protein elevated in the early-stage of

asymptomatic patients with lysosomal storage disease Fabry disease. In addition, we identified a major complement of lysosomal cathepsins in our proteomic data that have been linked with cardiovascular diseases, such as cathepsins B, C, D and Z. The successful identification of such disease related proteins highlights the potential usage of these techniques in understanding the role of lysosomal proteins in complex cardiac diseases such as AF.

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OC02

Cerebrovascular responses during voluntary breath holding are larger than rebreathing in humans

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Both voluntary breath-holding and rebreathing of expired air elicit changes in respiratory gas chemostimuli (CO_2 and O_2) at the metabolic rate. These chemostimuli elicit increases in cerebral blood flow (CBF), proportional to the magnitude of concomitant increases in CO_2 and reductions in O_2. These chemostimuli also activate the sympathetic nervous system, which increases systemic blood pressure. Although blood pressure responses appear small during rebreathing, evidence from obstructive sleep apnea (OSA) patients suggests higher mean blood pressure during sleep in those with worse OSA. We aimed to assess how superimposed changes in blood gases and increases in blood pressure affect the CBF responses during breath holding vs. rebreathing.

We recruited 23 healthy participants (12 females) and instrumented them with a finometer (for beat-by-beat mean arterial blood pressure; MAP), transcranial Doppler ultrasound (middle and posterior cerebral artery velocity; MCAv, PCAv) and a pneumotachometer with gas sampling via a dual gas analyzer to assess the pressure of end-tidal (P_{ET})CO_2 and O_2. Participants carried out two protocols in randomized order: (a) a maximal, voluntary end-inspiratory breath hold (BH) and (b) a rebreathing (RB) test. A breath-by-breath stimulus index (SI) was calculated as $P_{ET}CO_2/P_{ET}O_2$ during rebreathing, whereas the end-tidal gas values used to calculate SI were interpolated during breath holding from initial and break point values. During both BH and RB, cerebrovascular reactivity (CVR) was calculated as the MCAv or PCAv/SI. MAP reactivity (MAPR) was calculated as the slope of the MAP/SI response.

Cerebrovascular conductance (CVC; MCA or PCA/MAP) reactivity (CVCR) was calculated as the slope of the MCA_{CVC} or PCA_{CVC}/SI responses.

We found that (a) CVR was larger during BH vs. RB (MCA: 146.9±69.9 vs. 67.7±29.9 cm/s/SI, P<0.0001, n=20; PCA: 78.1±30.7 vs. 45.6±21.0 cm/s/SI, P=0.0004, n=17), (b) MAPR during BH was significantly higher than during RB (116.8±67.8 vs. 35.9±26.0 mmHg/SI, P=0.0001, n=20). and (c) CVCR during BH vs. RB (MCA: 0.69±0.62 vs. 0.45±0.22 cm/s/mmHg/SI, P=0.64, n=20; PCA: 0.33±0.17 vs. 0.33±21 cm/s/SI, cm/s/mmHg/SI, P=0.9, n=17).

Our data demonstrate that breath holding elicited ~3-fold increases in MAP, translating to a larger anterior and posterior CVR compared to rebreathing. These findings suggest that the sympathetic responses during voluntary breath holding were larger than those during rebreathing across similar chemostimuli, potentially due to the differential sympathetic effects of struggling against a closed glottis during breath holding. This is the first evidence that voluntary apnea has larger effects on brain blood flow beyond that elicited by blood gas stimuli alone. Our data may have implications for understanding stroke risk in clinical populations with obstructive sleep apnea, whereby patients experience intermittent breath holds throughout the night, with associated spikes in arterial blood pressure.

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OC03

A dual potassium channelopathy underlies small vessel disease of the brain in a mouse model of Alzheimer's disease

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The microcirculation of the brain is increasingly viewed as a potential target for disease modifying drugs in the treatment of the patient with Alzheimer's Dementia (AD). However, despite established changes to cerebrovascular autoregulation in patients with AD, underlying pathogenic mechanisms in isolated brain resistance arteries have not been investigated. Here we present the first detailed examination of the principal vasodilatory pathways within small cerebral arteries in an AD mouse model. For our study we used the APP23 mouse which has a seven-fold increase in amyloid precursor protein leading to neuritic plaques and also cerebrovascular accumulation of amyoid-β, similarly to patients with AD. Using a range of physiological techniques; mainly pressure myography, patch clamp electrophysiology and high-speed spinning disc confocal microscopy, we show that a global vascular dysfunction within this model is underpinned by two distinct channelopathies. Firstly we showed an attenuation of large conductance Ca^{2+}–activated K^+ (BK) channel function as a decreased contractility to the BK channel blocker, paxilline (p = <0.001, unpaired t test, n = 8-12 arteries per group). Further investigation determined that the BK channels from the APP23 vascular smooth muscle cells produced significantly less spontaneous transient outward currents (p = <0.01, two way ANOVA, n =

10-14 cells per group) and that this was correlated to a significantly reduced frequency of calcium sparks ($p = <0.05$, unpaired t test, n = 5-7 arteries per group). Thus, the increase in myogenic tone from these animals ($p = <0.01$, unpaired t test, 25-29 arteries per group) was a result of a lack of calcium spark activation of the BK channel. This observation was accompanied by a reduction in the vasodilatory capacity of the endothelial inward rectifier K^+ channel, Kir2.1 ($p = <0.05$, unpaired t test, 10-11 arteries per group), indicating a deficit in neurovascular coupling in these animals. If present in human AD, the combination of these pathologies would account for the clinical cerebrovascular presentation seen in patients: reduced blood flow and a failure of functional hyperaemia. The data directs future research to approaches that will reverse this dual vascular channelopathy, with the ultimate aim of restoring healthy cerebral blood flow and improving clinical outcomes.

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OC04

Cardiotoxicity of particulate matter air pollution in mouse models of health and disease.

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An estimated 4.2 million premature deaths worldwide are attributed to air pollution, primarily through increased cardiovascular (CV) morbidity and mortality. Studies have found particulate matter (PM), a subgroup of pollutants, including polyaromatic hydrocarbons (PAHs) are associated most strongly with CV disease (CVD), due to the ability of these smaller particles to enter the systemic circulation. Studies in fish have shown PAHs, such as phenanthrene (Phe), can directly alter CV electrophysiology, including prolonged action potential duration (APD) and QT interval, reduced heart rate (HR), reduced contractility and changes in underlying ionic currents. This level of detailed mechanistic investigation is lacking in mammalian models.

Using the mouse model, the current study found exposure to even a single pollutant, Phe, can alter cardiac electrophysiology. Acute exposure (15 minutes) to Phe led to a significant reduction in HR and prolongation of the RR interval both *ex-vivo* (25uM Phe, 16% reduction in HR, P=0.022, n=5) using the isolated heart preparation and *in-vivo* (50ug/kg Phe, 10% reduction in HR, P=0.0043, n=7) using electrocardiographs recorded from anaesthetised mice, suggesting Phe has some direct effects on the heart, which are unaffected by peripheral factors. Wild-type mice (10-weeks) were exposed to a lower dose of Phe (30ug/kg, n=8) or vehicle only (DMSO, n=6) for a 14-day period, followed by *in-*

vivo electrocardiograph recording and tissue collection. PQRST intervals were calculated for all animals; a significant prolongation of the QTc and P-wave duration was observed in Phe treated animals ($P<0.05$), suggesting the pro-arrhythmic effects of Phe previously seen in fish, may also occur in mammals. Furthermore, the weight of hearts treated with Phe was significantly higher than animals treated with vehicle only ($P=0.004$), this could indicate a hypertrophic response, however further molecular analysis is needed to confirm this.

This study is the first to show direct effects of Phe on the CV system (CVS) of mice, both in the absence and presence of peripheral factors. This study is also the first to investigate prolonged exposure to Phe in mice, showing actions on both cardiac electrophysiology and potentially structure. More detailed electrophysiological, and long-term exposure studies combined with RNA and protein analysis of tissue are needed to investigate the full effects of Phe and reveal novel mechanisms of action. Ambient PM is now ranked 5th in the global mortality risk factor list and as the global levels of pollution continue to rise, studies like ours will help manage and mitigate this avoidable cause of disease and death.

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OC05

Kinetics of synaptic vesicle pool depletion and adaptation in zebrafish lateral line hair cells

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Background

In auditory, vestibular and lateral line systems, ribbon synapses allow hair cells to drive high rates of sustained synaptic transmission (Goutman and Glowatzki 2007; Pichler and Lagnado 2019) and to maintain the exquisite temporal precision of sensory information. The kinetic properties of vesicle release and replenishment at these synapses have mainly been studied by measuring changes in membrane capacitance during hair cell depolarization using patch clamp, which bypasses the normal process of mechanotransduction. The zebrafish is becoming an important model for the investigation of hair cell development and function (Ricci et al. 2013; Olt et al. 2014; Pichler and Lagnado 2019), with the advantage to investigate both pre- and postsynaptic responses and the activity of afferent neurons in an intact organism *in vivo*. In this study, we investigated the release properties of hair cell ribbon synapses and sensory adaptation *in vivo* using a combination of extracellular recording and fluorescence imaging while displacing the mechanoelectrical transducer apparatus.

Methods

Work was licensed by the UK Home Office and approved by the University of Sheffield Ethical Review Committee. Zebrafish larvae from the following strains were used: *Tg(brn3c:Gal4), Tg(UAS:iGluSnFR)*, *Tg(UAS:GCaMP7a)* and *Tg(nbt:GCaMP3).* Zebrafish larvae (<5.2 days post fertilisation) were anaesthetised in MS-222 (0.01%) prior to injection of α-bungarotoxin (125 μM) into the heart to suppress movement. Recordings from the ganglion cell body were performed using loose-patch voltage-clamp as previously described (Trapani et al. 2011). Mechanical displacement of the cupula was achieved by using a piezoelectric fluid jet. iGluSnFR and Ca^{2+} signals were recorded using a two-photon laser-scanning microscope based on a mode-locked laser system operating at 925 nm.

Results

Sustained displacement of the neuromast cupula elicited a transient increase in hair cell synaptic release at the stimulus onset, which quickly adapted to a lower, sustained level. The release time course was consistent with the recruitment of two pools of synaptic vesicles with different release kinetics. The time constant of the fast component, which we identify as the readily releasable pool (RRP), was 19 ms (18 neuromasts, 10 zebrafish). Short, repeated pressure stimuli lead to progressive depletion of the RRP. Recovery from depletion showed a biphasic time course, with a fast time constant of 129 ms and a slow time constant of 2.8 s (33 neuromasts, 16 zebrafish).

The relatively fast adaptation of synaptic responses (21 hair cells, 15 neuromasts, 7 zebrafish) was likely due to vesicle depletion, as the hair cell calcium responses (39 hair cells, 18 neuromasts, 4 zebrafish) did not show any significant decline throughout the stimulation. Both the calcium responses in the afferent terminals (37 afferent terminals, 17 neuromasts, 5 zebrafish) and the firing rate in the afferent ganglion neurons (32 neurons, 29 zebrafish) showed adapting responses comparable to fast adaptation at the hair cell ribbon synapse.

Conclusions

We conclude that the hair cell synaptic release properties allow the lateral line to maintain its sensitivity in the presence of sustained stimuli, such as those produced by a constant flow of water.

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OC06

Inhibition of mitochondria fission in astrocytes in the Dorsal Vagal Complex of the brain to target the metabolic profile of brown adipose tissue in high fat diet-fed rats.

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Background and aim: The prevalence of obesity worldwide is increasing sharply, resulting in growing costs for healthcare systems worldwide. The brown adipose tissue (BAT) is being probed as a potential target to treat obesity. BAT activation is driven by the central nervous system (CNS) and circulating glucose and fatty acids. The nucleus of the tractus solitarius (NTS) in the brainstem is an important centre that receives information regarding the nutritional status from the visceral vagal afferents and relays them to the forebrain. Astrocytes are the most abundant cells in the brain that work as energy sensor and provide the right environment for the neurones to respond to altered metabolic status. Their role in regulating systemic metabolism is of growing interest, and evidence shows their involvement in BAT thermogenesis (1).

Previous studies have shown that targeting all cells or specifically astrocytes with a dominant-negative form of Drp1 (Drp1-K38A) to inhibit mitochondria fragmentation in rats NTS decreased food intake, restored insulin sensitivity in the NTS and prevented weight gain in high-fat diet (HFD)- fed-obese rats (2).

Our main aim is to investigate whether targeting NTS with Drp1-K38A can affect the metabolic profile of BAT and increase adrenergic discharge to the organ and glucose uptake, which associate with enhanced metabolic demand.

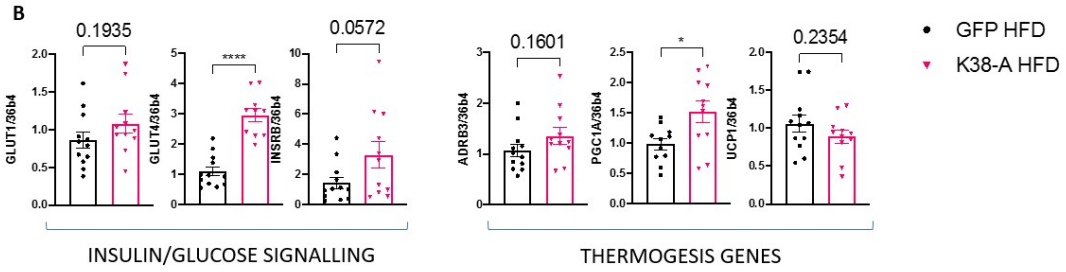
Materials and methods: The experimental protocol was approved by the ethical committee for the use of animals in research of the Faculty of Biological Sciences,University of Leeds and by the Home Office. 1) Rats (male, n=24) were subjected to stereotactic surgery on day 0 and received an injection of either GFAP:Drp1-K38A or GFAP:GFP in the NTS on day 1 and fed HFD diet for 15 days. On day 16 animals were sacrificed by pentobarbital overdose (60 mg/kg) and BAT dissected. 2) Rats (male, n=3) were fed with HFD for 28 days, submitted to stereotactic surgery on day 29 and injected with an adenovirus expressing CMV:Drp1K138A or CMV:GFP in the NTS on day 30. Rats where then maintained on HFD for additional 15 days and submitted to PET scan.

Results: Noradrenaline precursor Tyrosine Hydroxylase (TH) levels in BAT of HFD-fed animals that received GFAP:Drp1K38-A in the NTS are increased 144% when compared to GFAP:GFP controls ($p<0.001$). The mRNA levels of insulin-dependent glucose transporter 4 (*GLUT4*) and thermogenic gene peroxisome proliferator-activated receptor gamma (*PGC1A*) are significantly upregulated in

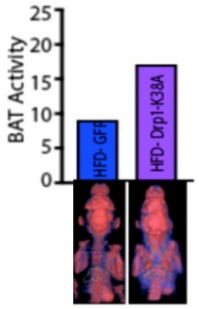
animals that received GFAP:Drp1K38-A in the NTS when compared to controls ($p<0.0001$ and $p<0.05$) respectively. On a protein level PGC1A is upregulated ($p<0.05$). Our Pilot study revealed a potential increase in glucose uptake in BAT of rat fed long-term HFD (50 days) that received CMV:Drp1K38-A when compared to control.

Conclusions: Our results suggest that inhibition of mitochondria fission in NTS astrocytes restored TH reservoirs in BAT-essential for beta adrenergic-driven thermogenesis, and regulator of GLUT4 mRNA in BAT(3). Consistently with the PET scan pilot, these results may suggest that glucose uptake in BAT is favoured in animals that received either CMV:Drp1K38-A or GFAP:Drp1K38-A when compared to controls; more studies are required to test this hypothesis.

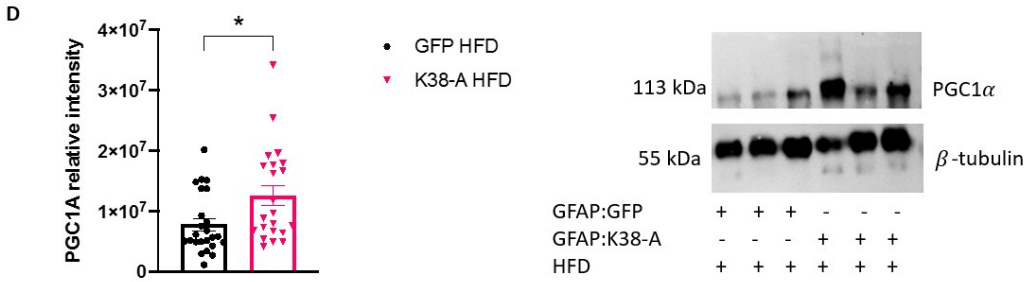
C) representative western blot showing UCP1 levels in GFAP:Drp1-K38A-expressing and GFAP:GFP-expressing HFD-fed rats. All data are expressed as mean ±SEM n=12 for both GFP and Drp1-K38A from two independent quantifications. Data was normalised to total protein amount, and Statistical test: unpaired T-test [*p < 0.05, **p <0.01, *** p<0.001]



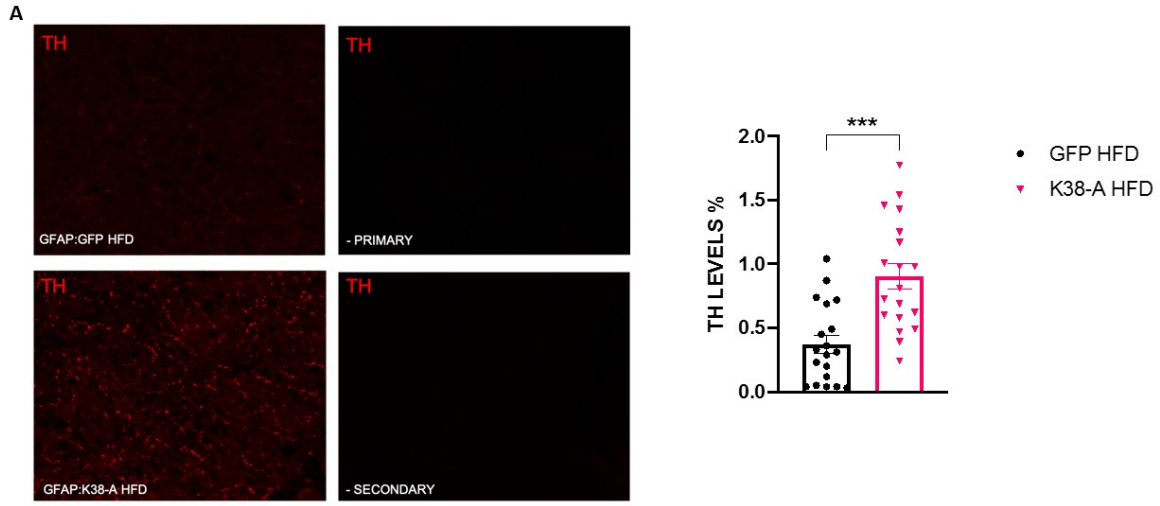
Bi



B) mRNA levels in BAT of rats expressing GFAP:Drp1-K38A-or GFAP:GFP in the NTS (n=12) and fed HFD for 15 days.
Bi) Pilot PET scan showing an increase of glucose uptake in BAT in a long term HFD (50 days) model of rat expressing either CMV:Drp1-K38A or CMV:GFP in the NTS (n=1).



D) representative western blot showing PGC1A levels in GFAP:Drp1-K38A-expressing and GFAP:GFP-expressing HFD-fed rats. All data are expressed as mean ±SEM n=12 for both GFP and Drp1-K38A from two independent quantifications. Data was normalised to total protein amount, and Statistical test: unpaired T-test [*p < 0.05, **p <0.01, *** p<0.001]



A) representative confocal image showing tyrosine hydroxylase pools in BAT of GFAP:Drp1-K38A-expressing and GFAP:GFP-expressing HFD-fed rats. All data are expressed as mean ±SEM n=4 with 4 technical replicates per animal for both GFP and Drp1-K38A from four independent quantifications. Statistical test: unpaired T-test [*p < 0.05, **p <0.01, *** p<0.001]

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OC07

Role of CPT1C in motor function, energy state, mood, synaptic plasticity and cognition

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CPT1C is an enzyme present throughout the nervous system, which is specifically expressed in neurons, showing remarkable amounts in the hypothalamus, hippocampus, cortex, cerebellum and amygdala. Unlike other CPT1 isoforms, which are involved in the transport of fatty acids inside the mitochondria for their subsequent b-oxidation, CPT1C is located in the endoplasmic reticulum (ER), where it regulates ceramide metabolism. Previous studies reported that CPT1C plays an important role in energy homeostasis and control of body weight. Although the molecular mechanisms underlying these functions remain partially unclear, CPT1C involvement in dendritic spine maturation, ER-mediated axonal transport and AMPA receptor synthesis and trafficking have been described. Due to the wide expression of this protein together with the diverse cellular mechanisms attributed, CPT1C might be involved in additional functions in different brain regions that remain unexplored. Here we carried out a systematic characterization of the role of CPT1C at different —molecular, synaptic and behavioral— levels of complexity. First, CPT1C expression pattern in different brain areas was studied by immunohistochemistry in CPT1C Knock-out (KO) mice and wild-type littermate mice. Then, the role of CPT1C in locomotor activity, energy state, anxiety- and depression-like behavior was explored. In addition, CPT1C involvement in different types of learning was investigated studying motor learning, hippocampal-dependent spatial and habituation memory, and associative instrumental learning *in vivo*, in behaving mice. To correlate hippocampal-dependent memory processes with synaptic plasticity changes, neural activity and dendritic spine maturation in the hippocampus were evaluated *in vitro* in these animals.

Our data showed energy deficits and impaired locomotor activity in CPT1C KO mice. These animals also exhibited deficits in motor and instrumental learning, as well as in spatial and habituation memory. The latter effects could be attributed to an inefficient hippocampal dendritic spine maturation and long-term plasticity impairments observed at the CA3-CA1 synapse. No changes in mood state were found. Together, our results show that CPT1C is needed for learning and memory processes taking place in brain areas that underlie motor, associative, and non-associative learning, as well as confirm the role of CPT1C in hippocampal synaptic plasticity and energy homeostasis.

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OC08

STERNOHYOID MUSCLE WEAKNESS IN PRE-SYMPTOMATIC ALS MICE

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration and loss of motor neurons, resulting in severe muscle weakness and paralysis. The respiratory system is implicated in ALS, with patients experiencing sleep-disordered breathing and progressive respiratory muscle weakness, ultimately leading to respiratory muscle paralysis and failure. The $SOD1^{G93A}$ mouse model of ALS displays motor neuron degeneration and a phenotype consistent with human ALS. It remains to be determined whether muscle dysfunction in ALS is a consequence of motor unit degeneration subsequent to motor neuron loss, or whether there is a primary muscle disorder in ALS that contributes to muscle dysfunction, thereby disrupting neuromuscular junction physiology and contributing to muscle dysfunction. These views are broadly considered as "dying forward" (nerve to muscle) or "dying back" (muscle to nerve) phenomena. It is suggested that muscle impairment may exist early, before the onset of overt motor impairments in ALS mice and perhaps humans. Therefore, we hypothesize that upper airway muscle dysfunction presents early in ALS and precedes cranial motor neuron dysfunction. We aimed to examine upper airway and diaphragm muscle function in young (48-49 days) pre-symptomatic ALS mice.

We examined *ex vivo* muscle contractile function of sternohyoid (a representative pharyngeal dilator) and diaphragm muscles for wild-type (n=12) and ALS (n=12) mice. Muscle preparations were attached to a dual-mode lever transducer system and studied in a water-jacketed muscle bath containing Krebs solution at 35°C aerated with $95\%O_2/5\%CO_2$. Isometric contractions were performed to examine force-generating capacity. Isotonic contractions were performed at 0% load to examine maximum shortening (Smax) and shortening velocity (Vmax) and at 50% load to examine maximum work (Wmax) and power (Pmax) output. Values are expressed as mean ± S.D. Data were statistically compared using unpaired Student's *t* test, Mann Whitney test or two-way ANOVA with Bonferroni *post hoc* test. $P<0.05$ was considered statistically significant.

Sternohyoid muscle tetanic force was significantly reduced in ALS mice compared to wild-type. For the force-frequency relationship, specific force was significantly reduced at 100-150 Hz. Smax, Vmax, Wmax and Pmax were all significantly reduced in ALS sternohyoid muscle compared to wild-type. Conversely, for diaphragm muscle, there was no significant difference in force production in ALS mice compared to wild-type. Smax, Vmax, Wmax and Pmax were also equivalent for ALS and wild-type diaphragm muscle preparations.

These data demonstrate sternohyoid muscle weakness (evidenced by reduced specific force and power output) in young pre-symptomatic ALS mice. Diaphragm muscle function was unaffected in pre-symptomatic ALS mice. Our study indicates that upper airway muscle dysfunction may be a prodromal signature of ALS, with potential implications for the control of upper airway patency in ALS and other neurodegenerative diseases. Current studies seek to examine brainstem and spinal cord motor neuron pools to compare motor and muscle changes in young ALS mice.

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OC09

The effect of local cooling strategies on the perceptual and inflammatory response to passive heating in healthy young males

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Introduction Passive elevation of body temperature can induce an acute inflammatory response that has been proposed to be beneficial for metabolic health. However, the heat strain associated with whole-body heating can be perceived as uncomfortable. While implementing local cooling strategies may partly alleviate thermal discomfort during passive heating, it is not known how such protocol adaptations impact on the acute inflammatory response. The aim of this series of studies was to investigate the effect of local cooling strategies on the acute inflammatory response to passive heating. **Methods** *Study 1* Using a water-perfused suit, ten recreationally active males underwent three 90 min conditions: heating of the legs (50°C water) with upper-body cooling (2°C water and ice packs; LBH), whole-body heating (50°C water; WBH) and exposure to a thermoneutral temperature (36°C water; CON). Blood samples were collected before and up to 3h post-session to assess inflammatory markers. Rectal temperature, basic affect and thermal comfort were recorded at regular intervals. *Study 2* Nine recreationally active male participants were immersed up to the waist for three 60 min water immersion conditions: 42°C hot water immersion (HWI), 42°C HWI with simultaneous upper-body cooling using a fan (FAN), and 36°C thermoneutral water immersion (NEU). Blood samples to determine interleukin (IL)-6 plasma concentration were collected pre- and post-water immersion; rectal temperature, basic affect and thermal comfort were assessed throughout the intervention. **Results** *Study 1* The IL-6 incremental area under the curve (iAUC) was higher for LBH (1096±851 pg/mL*270min) and WBH (833±476 pg/mL*270min) compared with CON (565±325 pg/mL*270min; $p<0.047$). Rectal temperature was elevated to a larger extent in WBH (38.6±0.4°C) compared with the other trials ($p<0.001$), and was higher at the end of LBH (37.1±0.3°C) compared with CON (36.7±0.2°C, p=0.001). Basic affect and thermal comfort were more negative during WBH compared with LBH and CON ($p<0.010$). *Study 2* Plasma IL-6 concentration was higher for HWI and FAN when compared with CON ($p<0.001$) and did not differ between HWI and FAN ($p=0.221$; pre to post, HWI: 1.0±0.6 to 1.5±0.7 pg/mL, FAN: 0.7±0.5 to 1.1±0.5 pg/mL, CON: 0.5±0.2 to 0.5±0.2 pg/mL). Rectal temperature was elevated to a larger extent in HWI (38.7±0.6°C) compared with FAN and NEU ($p<0.001$), and was higher in FAN (38.1±0.4°C) compared with NEU (37.1±0.3°C, p<0.001). At the end of immersion, basic affect was lowest for HWI (HWI: -1.8±2.0, FAN: 0.2±1.6, CON 1.0±2.1, $p<0.020$); thermal comfort for HWI was changed from pre into the uncomfortable range (3.0±1.0, $p<0.001$),

whereas FAN (0.7±0.7, *p*=0.160) and CON (-0.2±0.7, *p*=0.283) remained in the comfortable range throughout. **Conclusions** Both aggressive upper-body cooling through a water-perfused suit and ice packs (study 1) as well as modest cooling using a fan (study 2) resulted in more positive perceptual responses compared with whole-body heating. Moreover, although LBH attenuated the response in additional cardiometabolic markers, both LBH and FAN did not attenuate the acute IL-6 response. The use of a fan may thus be considered as a practical and low-cost tool to enhance the tolerability of passive heating.

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OC10

Low intensity resistance exercise with blood flow restriction induces local and systemic hypoalgesia via endogenous opioid-mediated mechanisms of pain modulation

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Resistance exercise can acutely decrease pain sensitivity (hypoalgesia) both local and remote to the exercising muscle. This effect is maximised with higher intensity exercise (1) and is partly driven by endogenously produced opioid and endocannabinoid substances, which have antinociceptive effects. Resistance exercise is used to treat individuals with chronic pain, however many of these individuals are contraindicated to higher intensity exercise. It has been hypothesised that performing low intensity exercise with blood flow restriction (BFR) in the working limb could increase the analgesic effect of exercise to a similar level as high intensity exercise (2). However, the magnitude of this effect and possible mechanisms are not known. To investigate this, healthy active individuals (n=12) performed 4 trials of unilateral leg press exercise in a randomised, crossover design. The trials included: 1) low intensity exercise (30% 1RM, LIRE); 2) high intensity exercise (70% 1RM, HIRE); 3) BFR exercise (30% 1RM) with a low pressure (BFR-L) and 4) BFR exercise (30% 1RM) with a high pressure (BFR-H). Sensitivity to a pressure stimulus (pressure-pain threshold, [PPT]) measured in kilograms of force (kgf) was assessed before and 5min post-exercise using algometry, whereby an increase in PPT value represents hypoalgesia. This was performed in the exercising quadriceps muscle and three remote non-exercising muscles. Venous blood samples were taken before and 10min post-exercise. Plasma concentration of beta-endorphin and 2-arachidonoylglycerol (marker of opioid and endocannabinoid systems, respectively) were determined using enzyme linked-immunosorbence assays. Values are mean±SD and analysed with two-way repeated measures ANOVA for each outcome. Mediation analysis was performed to determine the impact of any change in plasma beta-endorphin and 2-arachidonoylglycerol concentrations on any change in PPT. There were no differences in baseline values between repeated trials for any outcome measured. In the exercising limb, at 5min post-exercise the PPT was 2.43±1.60 kgf higher, 3.72±2.24 kgf higher and 1.68±1.53 kgf higher following BFR-L, BFR-H and HIRE, respectively, compared to LIRE (all $p<0.05$). Following BFR-H, PPT was 1.29±1.18 kgf higher and 2.04±1.75 kgf higher compared to BFR-L and HIRE

(both p<0.05). In the non-exercising remote muscles, a greater change in PPT occurred with BFR-L (11-17%), BFR-H (11-21%) and HIRE (10-18%) compared to LIRE (2-4%) (all p<0.05). At 5min post-exercise, plasma beta-endorphin concentration was greater following BFR-L (108.56±20.18 pg/ml), BFR-H (109.54±18.83 pg/ml) and HIRE (92.87±16.82 pg/ml) compared to LIRE (90.62±17.13 pg/ml) (all p<0.05). The increase in beta-endorphin was greater in BFR-L and BFR-H compared to HIRE (both p<0.05). There were no changes in plasma 2-arachidonoylglycerol concentration. The mediation model suggested that plasma beta-endorphin mediates 42% of the total effect of the exercise intervention on change in PPT. Thus, this data shows that addition of BFR to LIRE can increase the local and systemic analgesic effect of exercise (3). Importantly, the magnitude of this effect is either greater (local exercising muscle) or similar (remote non-exercising muscle) to HIRE. Moreover, analgesia with BFR exercise is partly driven by endogenous production of opioids. BFR exercise may be effective for treating individuals with chronic pain who cannot tolerate higher intensity exercise.

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OC11

Different ventilatory response to hypoxia and hypercapnia in prematurely born adults

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INTRODUCTION

Pre-term birth, typically defined as birth occurring prior to the 36th week of gestation, has been reported to significantly hamper functional and anatomical development of the respiratory system

(1). Prematurely born individuals present reduced cardiopulmonary control, as well as impaired alveologenesis and pulmonary vasculogenesis, leading to inefficient gas diffusion capacity. Either ventilation-to-perfusion mismatch and/or right heart dysfunction have been suggested as potential mechanisms responsible for alteration in respiratory function (2). Recent work from our group has also demonstrated higher oxidative stress in prematurely born adults (3), which may negatively affect both peripheral and central chemosensitivity to hypoxia and hypercapnia, respectively. The aim of this study was to investigate the resting ventilatory responses to normoxic hypercapnia and normobaric hypoxia in prematurely born, but otherwise healthy, adults.

METHODS

Seventeen pre-term adults (Mean±SD; age, 21±2 years; height, 177±7 cm; body mass, 68.9±7.5 kg; gestational age, 29±3 weeks; gestational weight, 1234±315 g) and fourteen age-matched full-term controls (age, 22±2 years; height, 180±5 cm; body mass, 73.5±5.6 kg; gestational age, 39±2 weeks; birth weight, 3672±499 g) underwent two 5-min resting normoxic hypercapnic periods (3% CO_2 and 6% CO_2, respectively), interposed by 5-min ambient air exposure (*visit 1*). On a second occasion, the participants underwent, again at rest, 4-min of normoxic breathing, before being exposed to 4-min of normobaric hypoxia (F_IO_2=0.11) (*visit 2*). Minute ventilation ($\dot{V}_E$), breathing frequency, tidal volume, and end-tidal partial pressure of CO_2 ($P_{ET}CO_2$), were continuously recorded during both sessions.

RESULTS

On both occasions, pre-term adults showed lower $P_{ET}CO_2$ during rest in normocapnic normoxia than their full-term counterparts (*visit 1*: 36±4 vs. 39±2 mmHg, p=0.02; *visit 2*: 36±3 vs. 38±2 mmHg, p=0.04), while no difference was observed in $\dot{V}_E$. During 3% normoxic hypercapnia, $P_{ET}CO_2$ increased to a greater extent over the first minute in pre-term, relative to full-term, adults (+11±3% vs. +16±5%, p=0.05). No difference in $\dot{V}_E$ was observed between groups while breathing 3% CO_2. During 6% hypercapnic exposure, $P_{ET}CO_2$ increased similarly in both groups, while $\dot{V}_E$ was higher in pre-term compared to full-term adults (30.2±7.5 vs. 23.7±4.5 L/min, p<0.0001). The relative increases in $P_{ET}CO_2$ and $\dot{V}_E$ during the first minute breathing 6% CO_2 were higher in preterm (+31±8% and +64±35%, respectively) compared to full-term (+21±5% and +35±21%, p=0.002 and p<0.04, respectively). During normobaric hypoxia, $P_{ET}CO_2$ and $\dot{V}_E$ increased from normoxia to a similar extent in both groups, thus maintaining significantly lower $P_{ET}CO_2$ in pre-term compared to full-term adults. Interestingly, exposure to normobaric hypoxia resulted in significantly higher breathing frequency among the prematurely born group, relative to their full-term counterparts.

CONCLUSIONS

These findings support previous evidence on prematurity-induced alterations in pulmonary function which persist into adulthood (1). Pre-term individuals presented a hypocapnic status at rest which may be related to ventilation-to-perfusion mismatch and/or cardiac dysfunction. Moreover, this was the first study showing evidence that pre-term birth is associated with increased chemosensitivity to hypercapnia, but not to hypoxia, compared to full-term birth. These novel findings might suggest that prematurity results in exaggerated central chemosensitivity despite unchanged peripheral chemosensitivity.

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OC12

Understanding the role of objective physiological tests in assessment of survivors of severe COVID-19 pneumonia

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The COVID-19 pandemic has affected millions of people worldwide. Acute COVID-19 infection can cause persistent and burdensome symptoms, impacting physiological function and quality of life (D'Cruz *et al.*, 2021). Ideally, holistic post-COVID assessment includes physiological tests, yet infection control policies favour remote assessment of symptoms where possible. The availability of tests involving aerosol generating procedures, such as spirometry, is restricted. This study aimed to investigate the relationship between patient-reported symptoms and physiological outcomes in survivors of severe COVID-19 pneumonia, to investigate the role of in-person physiological assessment. This was a prospective single-centre observational study in the outpatient department of an urban South London teaching hospital. Survivors of severe COVID-19 pneumonia were invited for face-to-face assessment at 4-6 weeks post hospital discharge. Relationships between subjective self-reported symptoms (outcome measures listed in Table 1), 4 metre gait speed (metres/second (4MGS)) (Hirabayashi *et al.*, 2020), and 1 minute sit to stand (repetitions/min (1STSreps) and SpO_2% desaturation) (Briand *et al.*, 2018) were assessed using correlation and multiple regression analysis. 168 patients attended clinic between June and November 2020. Patient characteristics and results of correlation analysis are shown in Table 1. Age (Beta = -0.284, $p<0.001$) and current mMRC dyspnoea score (Beta= -0.352, $p=0.001$) were independent predictors of 4MGS (overall model fit $R^2=0.35$, $p<0.001$). Body mass index was the only independent predictor of 1STSreps (Beta=0.214, $p=0.007$, overall model fit $R^2=0.33$, $p<0.001$). NRS pain (Beta=0.298, $p=0.004$) and cough (Beta=-0.179, $p=0.041$) were the only independent predictors of SpO_2% nadir during 1STS (overall model fit $R^2=0.13$, $p=0.039$). Respiratory symptoms were not strong predictors of 4MGS and 1STS performance, emphasising the importance of objective physiological tests in post-COVID

assessments. The challenge of providing holistic care to survivors of COVID-19 has highlighted the importance of clinical respiratory physiology to the multidisciplinary team.

		Correlation analysis					
		4MGS		1STSreps		SpO_2% nadir	
	Results	r	p-value	r	p-value	r	p-value
Gender (% male)	63.7 (55.9-71.0)						
Age (years)	60 ± 13	-0.210	0.008*	0.015	0.86	-0.151	0.064
BMI (kg/m^2)	31.1 ± 6.1	-0.136	0.095	0.162	0.139	-0.004	0.966
Pre-COVID mMRC dyspnoea score	0 (0-1)	-0.315	<0.001*	-0.304	<0.001*	0.025	0.763
Current mMRC dyspnoea score	1 (0-2)	-0.405	<0.001*	-0.440	<0.001*	0.137	0.098
NRS breathlessness	2 (0-5)	-0.218	0.006*	-0.402	<0.001*	0.143	0.085
NRS fatigue	3 (0-5)	-0.304	<0.001*	-0.400	<0.001*	0.209	0.011*
NRS cough	0 (0-2)	-0.092	0.252	-0.131	0.120	-0.57	0.493
NRS pain	0 (0-5)	-0.233	0.003*	-0.294	<0.001*	0.255	0.002*
NRS sleep difficulty	2 (0-5)	-0.245	0.002*	-0.349	<0.001*	0.226	0.006*
4MGS < 0.8 m/s (%)	42.5 (34.7-50.6)						
1STSreps <LLN (%)	56.8 (48.4-65.0)						
SpO_2% desaturation >/= 4% from baseline (%)	33.8 (26.3-41.9)						

Table 1. Patient characteristics and results of correlation analysis. *Data are presented as mean ± standard deviation if normally distributed, or as median (interquartile range) or frequency (proportion %; 95% confidence interval). SpO_2% nadir = lowest SpO_2% recorded during 1 minute sit to stand test; 1STSreps = repetitions per minute during 1 minute sit to stand test; 4MGS = 4 metre gait speed; BMI = body mass index; mMRC = modified Medical Research Council; NRS = 0 – 10 numerical rating scale; LLN = lower limit of normal; r = Spearman correlation coefficient. *indicates statistical significance at 0.05 level.*

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OC13

The influence of hyperglycaemia on the circadian clock in the TallyHO/Jng mouse model of type II diabetes

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Circadian rhythms refer to endogenous biological oscillations with a 24-hour periodicity which are directly under the control of the core clock located within the hypothalamus. The disruption of said circadian rhythms via rotational or night-shift work has been linked to the development of metabolic diseases such as obesity and type 2 diabetes (T2D). The specific contributions of the core clock to this dysregulation have been elucidated following the development of tissue-specific knockout mice. For example, both skeletal muscle and hepatic-specific CLOCK and BMAL1 knockout mice display impairments in glucose control [1 2]. Despite this, evidence of circadian disruption in more physiological models of T2D is limited. In particular, although the use of diet-induced models is common, these models tend not to develop hyperglycaemia and overt T2D. Therefore, we have utilised the TallyHO/Jng mouse (JAX stock #005314) as a model of T2D. Interestingly, the development of the hyperglycaemic phenotype within this strain is not 100% penetrant which allows us the segregate the mice into two divergent strains of normoglycaemic (Tally-Low) and hyperglycaemic (Tally-High) TallyHO/Jng mice. The SWR/J mouse (JAX stock #000689) strain was utilised as the recommended healthy control. Therefore, our aim was to characterise the development of the diabetic phenotype in Tally-Low and Tally-High mice in comparison to SWR/J mice. In addition, we aimed to determine how hyperglycaemia influences the expression of circadian clock genes across the circadian cycle. Body weight and blood glucose was monitored on a weekly basis from 8 to 16 weeks of age (n=8/group). Plasma insulin was determined at 8 and 16 weeks of age (n=8/group). At 16 weeks of age, SWR/J, Tally-Low and Tally-High (n=6/group) mice were anaesthetized (50 mg pentobarbital/kg BW) and sacrificed via cervical dislocation at ZT3 (9am) and ZT15 (9 pm) and tissue samples collected. Skeletal muscle circadian clock related gene expression was assessed via RT-qPCR and protein levels via immunoblotting. Data were analysed via two-way ANOVA with Bonferroni correction and data presented as mean ± standard deviation. All procedures were approved by the Danish Animal Experiments Inspectorate and complied with European Directive 2010/63/EU for animal experiments. Tally-High mice displayed overt hyperglycaemia by 8-weeks of age (19.9 ± 5.2 mmol) in comparison to Tally-Low (8.9 ± 2.2 mmol) and SWR/J (6.7 ± 1.1 mmol) mice ($P < 0.05$). At 8-weeks of age, both Tally-Low (3.5 ± 1.2 ng/ml) and Tally-High (3.1 ± 1.0 ng/ml) display hyperinsulinemia in comparison to SWR/J mice (0.7 ± 0.3 ng/ml) ($P < 0.05$). At 16-weeks of age, skeletal muscle *Bmal1* expression displayed diurnal rhythmicity between ZT3 and ZT15 in both SWR/J and Tally-Low mice ($P < 0.05$) which was not apparent in Tally-High mice ($P > 0.05$). In conclusion, the divergent phenotype development in the TallyHO/Jng mouse represents an interesting model to study the effects of hyperglycaemia. The exacerbated hyperglycaemia in the Tally-High mice resulted in disrupted diurnal rhythmicity in *Bmal1* expression in skeletal muscle. These data suggest that disruptions to the clock machinery in peripheral tissues may contribute to the divergent phenotype development in TallyHO/Jng mice.

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OC14

Accentuated early postprandial appetite and appetite-related hormone responses in people with SCI versus able-bodied controls

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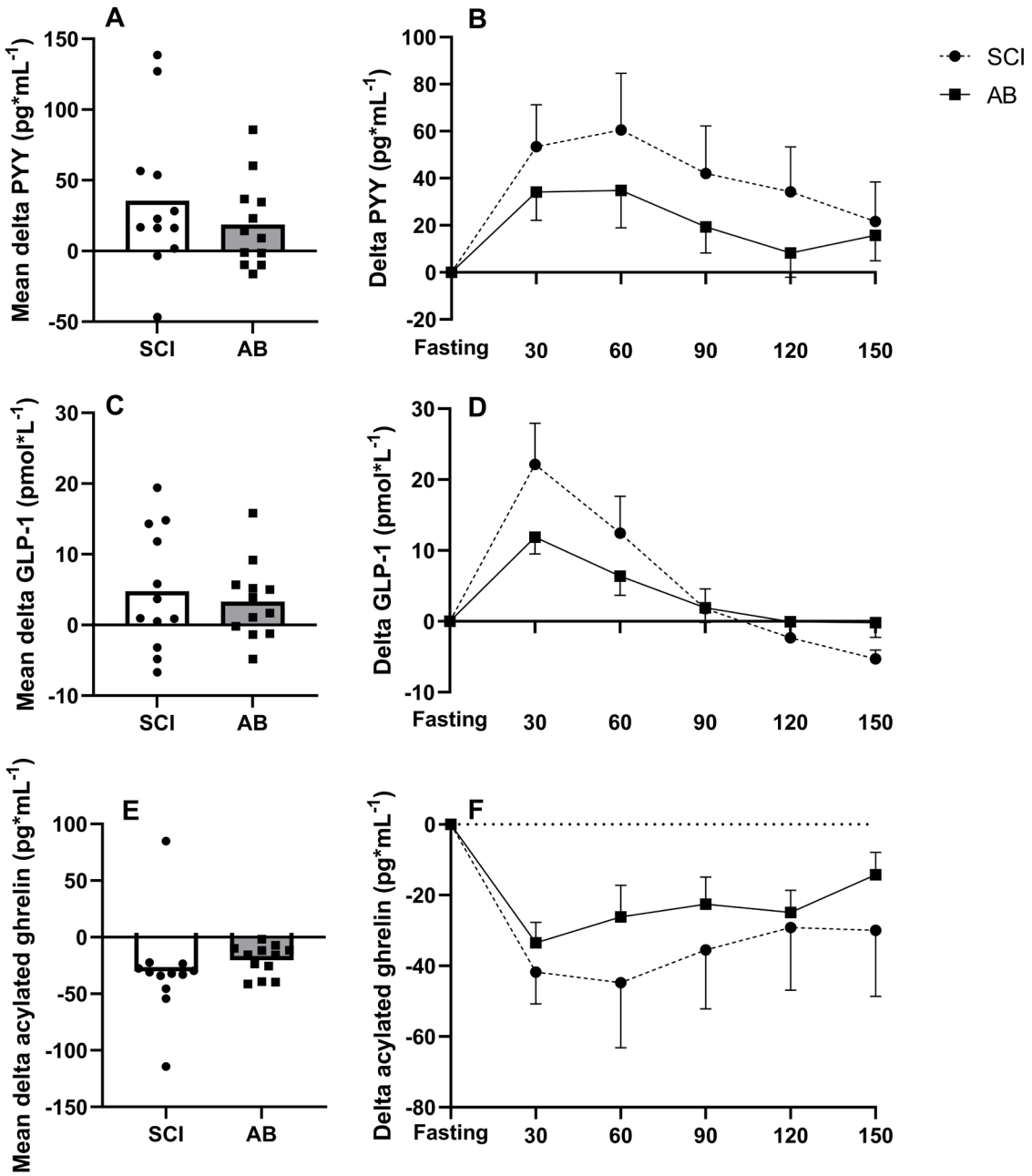
Introduction: In persons with spinal cord injury (SCI), reduced fat-free mass and movement-related energy expenditure contribute to the increased obesity risk found in this population. Impairments in gastrointestinal function in this population are well established (Holmes & Blanke, 2019), but the regulation of appetite-related hormones, and their influence on energy intake regulation remains unknown. Additionally, low levels of total daily energy expenditure have been linked to reduced appetite sensitivity and weight gain (Long et al., 2002), potentially further impacting on energy intake regulation in persons with SCI. This study compared postprandial responses of appetite-related hormones, appetite perceptions and appetite sensitivity in persons with SCI and AB controls.

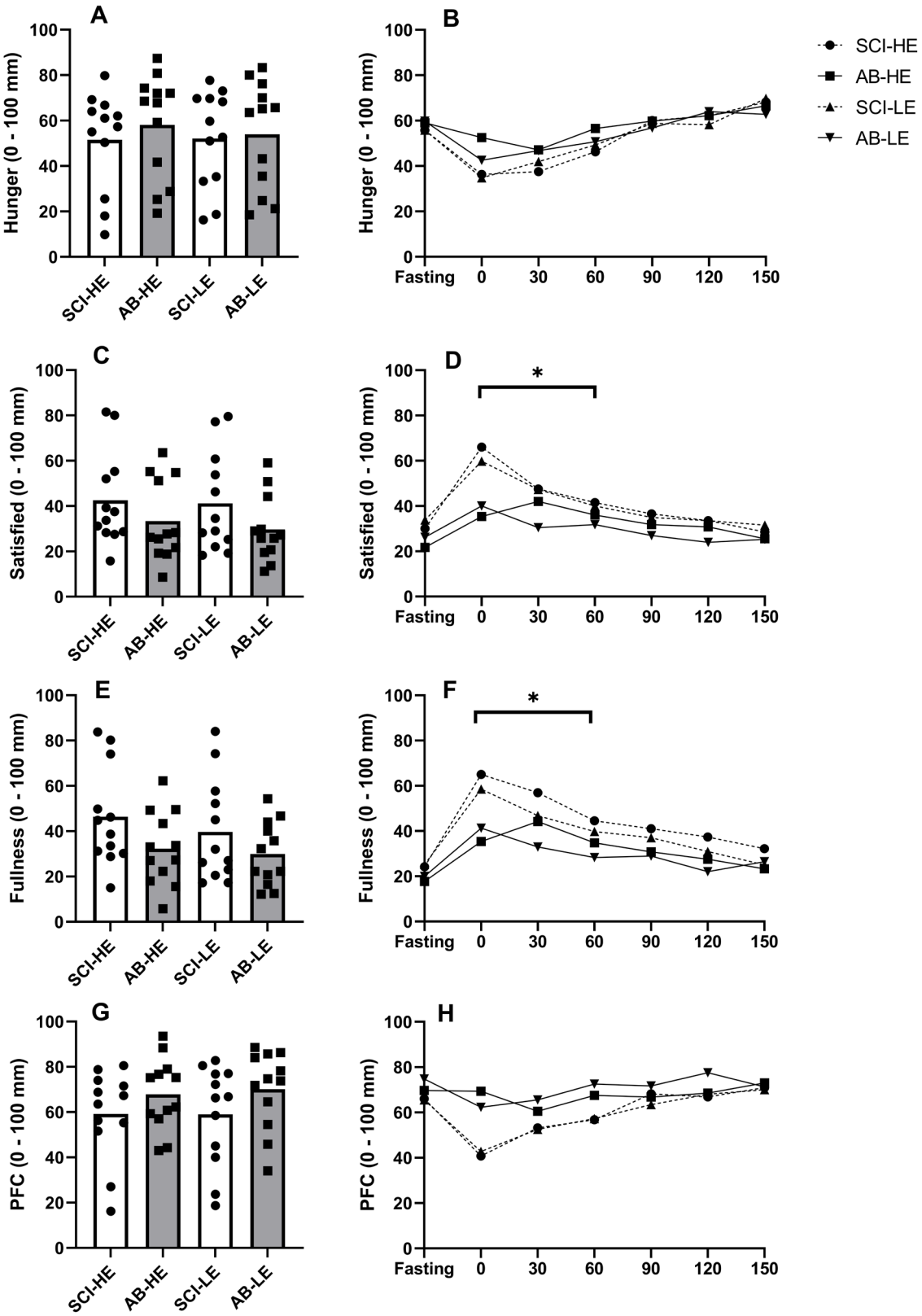
Methods: In a counterbalanced order, 12 men with high-level SCI (≥T4 vertebrae) and 12 body mass and age-matched AB controls completed two trials, consuming a covert high-energy (HE; 2513 kJ) and low-energy (LE; 1008 kJ) preload on separate occasions. Subjective appetite perceptions (hunger, fullness, satisfaction and prospective future consumption [PFC]) were assessed at 30 min intervals following preload consumption (up to 150 min) and appetite sensitivity was subsequently determined from *ad libitum* meals. Appetite-related hormone (total PYY, GLP-1 and acylated ghrelin) responses were measured in the HE trial. Test-specific effect sizes (Cohen's *d*) were calculated in adjunct to each statistical test (a large, moderate, small and trivial effect size was defined as ≥ 0.8, 0.5 < 0.8, and 0.2 < 0.5, and < 0.2, respectively) (Cohen, 1988).

Results: Within the early postprandial phase (0-60 min), the SCI group displayed accentuated meal-responses for PYY (d = 0.72; p = 0.365), GLP-1 (d = 1.00; p = 0.134) and acylated ghrelin (d = 0.87; p = 0.112), though no group difference were seen from 60-150 min ($d \leq 0.59$; $p \geq 0.340$) (Figure 1). Moreover, subjective ratings of fullness (d = 1.09) and satisfaction (d = 0.91) were higher ($P \leq 0.028$) during the first hour (Figure 2). *Ad libitum* energy intake was also lower in the SCI group (1086 vs. 1713 kJ, respectively, d = 1.00; P = 0.020) but no effect of trial (preload) was found (d = 0.003; p = 0.974) (Figure 3).

Discussion: These findings suggest that postprandial satiety may be augmented in people with SCI. Such responses may be related to colonic dysfunction, which is believed to reduce motility in the

upper gastrointestinal tract (Fynne et al., 2012). As PYY and GLP-1, and AG play a role in the inhibition and stimulation of gastrointestinal motility, respectively, our findings suggest that the accentuated postprandial gut hormone secretion may be mediated via colonic dysfunction. Additionally, reduced lean mass, commonly found in persons with SCI, may be related to the augmented satiety response (Gorgey & Dudley, 2007). In conclusion, this study shows that individuals with SCI exhibit accentuated appetite-related hormone and perceived satiety responses in the early postprandial phase, compared to age and weight-matched AB controls. These findings may reflect wider gut dysmotility in people with SCI but imply that weakened satiety may not be associated with higher rates of obesity.




A
Hunger (0 - 100 mm)
SCI-HE
AB-HE
SCI-LE
AB-LE
B
Hunger (0 - 100 mm)
Fasting
0
30
60
90
120
150
SCI-HE
AB-HE
SCI-LE
AB-LE
C
Satisfied (0 - 100 mm)
D
Satisfied (0 - 100 mm)
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E
Fullness (0 - 100 mm)
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G
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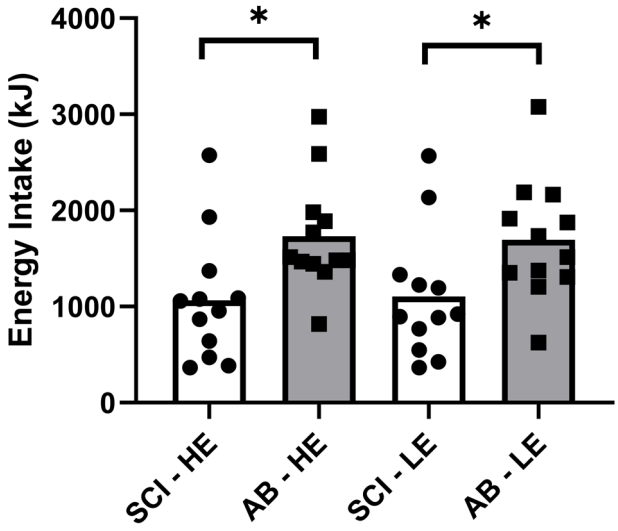

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OC15

Neuropeptide Y neurons within the nucleus of the solitary tract regulate feeding

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Neuropeptide Y (NPY) is one of the most potent orexigenic neuropeptides in mammals. Although NPY is widely expressed within the brain, the preponderance of research investigating NPY's effects on appetite have focussed on the hypothalamus. In the present work, we examined the distribution, neurochemical phenotype and function of NPY cells within the key homeostatic brain region, the nucleus of the solitary tract (NTS) using NPY Humanized Renilla Green Fluorescent protein (NPY-GFP; n=38) and NPY-Cre mice (n=17). All animal studies were conducted in accordance with The Animals (Scientific Procedures) Act 1986. Using in situ hybridisation histochemistry (ISSH) to label endogenous *Npy* mRNA and immunohistochemistry to label GFP, we observed that nearly all NPY-GFP cells co-expressed *Npy* mRNA (n=6). These findings validate the NPY-GFP line. Next, we assessed the distribution and neurochemical phenotype of NPY neurons within the NTS. The majority of NPY neurons are localised between -7.20 and -7.56 from bregma (n=29). As expected, the majority (63.62±4.92%) of NPY-GFP cells co-expressed paired-like homeobox 2b (Phox2b), the transcription factor essential for the development of the autonomic nervous system (n=4), and none co-expressed the neurotransmitter acetylcholine (using choline acetyltransferase), which is localised outside the NTS (n=3). Similar to the arcuate nucleus of the hypothalamus, NPY-GFP co-expression was observed with the neurotransmitter GABA (using glutamate decarboxylase67, GAD67; 22.20±2.78%; n=3). Perhaps counter-intuitively, a larger subset (54.66±7.50%; n=7) of NPY-GFP cells showed co-localisation with the anorectic protein nesfatin, although nesfatin's function within the NTS has not been well described. Roughly a quarter (29.20±7.75%; n=7) showed co-expression for the catecholamines (using tyrosine hydroxylase) which are also classically anorectic. No NPY-GFP cells were co-expressed with the enzyme for nitric oxide, nitric oxide synthase (nNOS) (n=4). To examine the function of NTS NPY neurons, NPY-Cre mice were stereotaxically injected with the Cre-dependant designer receptor exclusively activated by designer drug (DREADD-Gq-mCherry or DREADD-Gi-mCherry) into the NTS (n=8/9 per group) under gas (2-3% isoflurane in O_2) anaesthesia. We observed that activating NPY NTS neurons (DREADD-Gq-mCherry) with designer drug clozapine-N-oxide (CNO, i.p., 1 mg/kg/10 ml) significantly increased food intake whereas inhibiting NPY NTS neurons (DREADD-Gi-mCherry) significantly decreased food intake compared with saline treatment (two-tailed paired t-test or Wilcoxon sign-rank test, $p<0.05$). These data provide the first detailed characterisation of NPY neurons within the NTS and reveal that this population of neurons regulate appetite in mice.

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OC16

The obese rat liver displays overlapping lipid signatures to the hypoxic liver, yet retains metabolic flexibility to respond to acute hypoxic stress.

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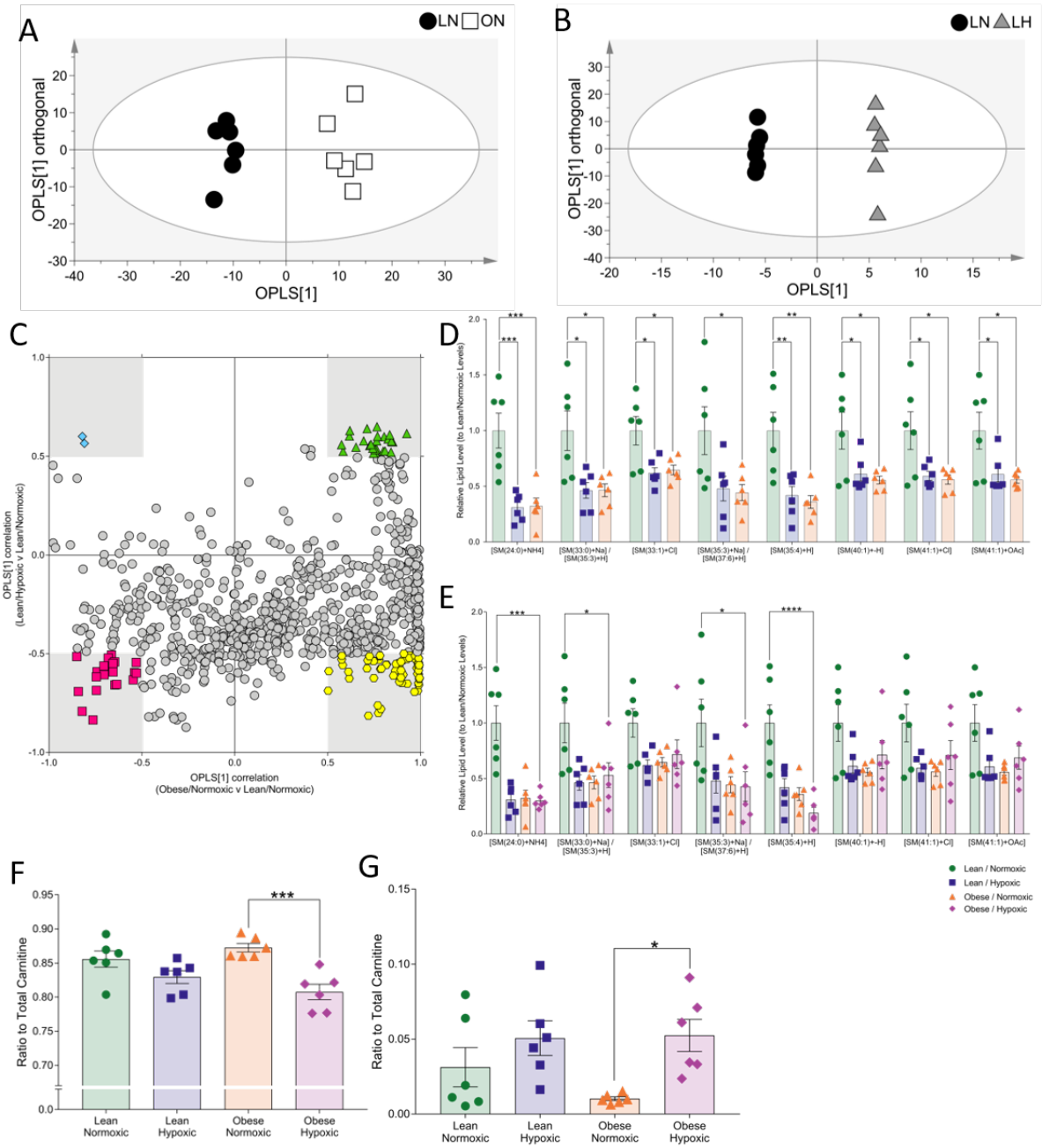
Hypoxia is associated with the aetiology of obesity. For instance, expanded adipose tissue experiences a lower oxygen tension and shows altered metabolism as a result [1]. However, in addition to adipose, the liver enlarges during obesity in association with ectopic fat storage [2]. Moreover, systemic inflammation leads to localised hypoxia as activated immune cells increase oxygen consumption and therefore deplete tissue oxygen tension [3]. We therefore sought to investigate the metabolic overlap between hypoxia and obesity in rat liver to determine whether hypoxia signalling plays a role in the development of obesity-related hepatic conditions, such as non-alcoholic liver disease.

All experiments were carried out in accordance with the UK Home Office, Animals (Scientific Procedures) Act (1986) and were reviewed by the University of Cambridge Animal Welfare and Ethical Research Committee. Following 7-day baseline measurements in controlled environmental conditions (22°C, 55% humidity, 12h photoperiod), female Zucker obese (ZUC-*Lepr*$^{fa/fa}$) and lean (ZUC-*Lepr*$^{fa/+}$) rats were either exposed to two days of normobaric hypoxia (10.3 ± 0.2% O_2; *hypoxia* groups) or maintained in the same environmental conditions (*normoxia* groups; n = 6 per group). At the end of the two-day exposure, the left lateral lobe of the liver was harvested and snap-frozen for metabolic analysis.

Liquid chromatography-mass spectrometry (LC-MS) was utilised to carry out open-profile lipidomics on the liver. Using orthogonal partial least squares-discriminate analysis, the obese/normoxic lipidome was shown to differ significantly to that of the lean/normoxic lipidome ($R^2X_{(cum)}$ = 81.4%, $Q^2_{(cum)}$ = 84.9%, $p<0.0001$). Likewise, the lean/hypoxic lipidome differed to the lean/normoxic lipidome ($R^2X_{(cum)}$ = 85.1%, $Q^2_{(cum)}$ = 81.3%). These models were used to generate a shared and unique structures plot, with species within the ±0.5 region on either axis being of interest as shared or unique to obesity and/or hypoxia. Of these species of interest, a series of sphingomyelins (chain length 24:0 – 41:1) were found at lower levels in both the hypoxic and obese liver ($p<0.05$, one-way ANOVA with Benjamini-Hochberg false discovery rate correction, Tukey's *post hoc* test for multiple comparisons). These species remained at lower levels (compared to lean/normoxic controls) when the insults were combined in the obese/hypoxic liver.

Using LC-MS, the acyl-carnitine complement of the liver was also assayed. The obese/hypoxic liver had higher levels of long-chain acyl-carnitines (≥13 carbons) compared with the obese/normoxic liver, alongside lower levels of short-chain acyl-carnitines (<6 carbons). This suggests that during hypoxia, fatty acid oxidation decreases in the obese liver [4], showing that the obese liver remains metabolically flexible to respond to hypoxic conditions by promoting less oxygen-expensive pathways.

Taken together, these results highlight that there are metabolic signatures common to the obese and hypoxic liver. However, the obese liver can still respond metabolically to hypoxic stimuli despite potential lipotoxicity and insulin resistance concurrent with obesity development [5].



There are overlapping metabolic signatures in the obese and hypoxic rat liver. Orthogonal partial least squares-discriminant analysis (OPLS-DA) demonstrates the lean/normoxic liver has a lipidome distinct to that of the obese/normoxic lipidome (**A**) and the lean/hypoxic lipidome (**B**). Comparison of these models allows the construction of a shared and unique structures plot (**C**) with species highlighted in green triangles higher in both the obese and hypoxic liver, pink squares lower in both hypoxia and obesity, yellow hexagons higher in obesity only and blue diamonds higher in hypoxia only. Univariate analysis of these species, highlighted a group of sphingomyelins (**D**) as being found at lower levels in both the obese and hypoxic liver; these species remained at lower levels that lean/normoxic controls when the two insults were combined (**E**). However, the obese/hypoxic liver is able to alter its metabolism in response to the hypoxic insult, as it has lower levels of short-chain acyl-carnitines (**F**) and higher levels of long-chain acyl-carnitines (**G**) compared with obese/normoxic rat livers. N = 6/group. Bars show mean ± SEM. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05; one-way (if comparing across 3 groups), or two-way (if comparing across genotype and oxygen) ANOVA with Benjamini-Hochberg false discovery rate correction, Tukey's *post hoc* for multiple comparisons.

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OC17

Menstrual cycle phase but not oral contraceptive use modulates monocyte TNFα release following exposure to combined lipopolysaccharide and hyperthermia

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Exercise and hyperthermia can increase intestinal permeability and cause an influx of lipopolysaccharide (LPS) into the intravascular space. LPS initiates a robust pro-inflammatory cascade which can contribute to heat-related illnesses. Fluctuations in oestrogen throughout the menstrual cycle have been shown to modulate intestinal barrier function, with indirect evidence linking high oestrogen concentrations in the luteal phase with an improved intestinal barrier function. Improved barrier function may impact pro-inflammatory responses across the menstrual cycle in response to hyperthermia-mediated LPS influx. There is little research on whether pro-inflammatory responses to LPS and/or hyperthermia differ in eumenorrheic women compared to women who take an oral contraceptive, or whether responses fluctuate across monthly hormone/pill cycles. This study aimed to quantify monocyte release of pro-inflammatory cytokine tumour necrosis factor-alpha (TNFα) following LPS-stimulation with or without hyperthermia in whole blood obtained from oral contraceptive users and eumenorrheic women throughout the pill/menstrual cycle. Volunteers using

oral contraceptives (OC, n=7) were tested during the pill-free phase (PF; day 6±1), and twice during the pill phase (P1; day 14±2, and P2; 21±2 including the pill-free week). Eumenorrheic volunteers (MC, n=7) were tested during the early follicular (EF; day 6±1), ovulation (OV; day 16±2), and mid-luteal (ML; 7±1 days' following positive ovulation test) phases of the menstrual cycle. Participants rested for 20-minutes before a 20 mL venous blood sample was drawn into a syringe and transferred into sodium-citrate tubes. Whole blood (1 mL) was stimulated with 100μg·mL^{-1} of LPS and one aliquot was incubated at 37°C (body temperature), and another at 40°C (simulated hyperthermia) for 6 hours. Plasma was diluted 1:40 in 1% bovine serum albuminate in phosphate-buffered saline, and TNFα concentration determined via ELISA (R&D Duoset, within-plate coefficient of variation (CV) 4%, between-plate CV 14%). Absolute TNFα concentrations (ng·mL^{-1}) were analysed by repeated-measures ANOVA, with temperature (37°C and 40°C) and time (OC: PF, P1, P2; MC, EF, OV, ML] phase) within-subject factors and group (OC and MC) a between-subject factor. Effect sizes are expressed at partial eta^2 (η_p^2). TNFα concentrations were 28% greater after incubation at 40°C compared to 37°C (main effect for temperature, p<0.001, η_p^2=0.610). The temperature-mediated response was similar in both groups (temperature x group interaction, p=0.784, η_p^2=0.006), regardless of menstrual cycle phase or pill phase (main effect of time, p=0.212, η_p^2=0.121). The TNFα response was similar between P1 vs. PF (+4%), and P1 vs. P2 (+7%) in the OC group regardless of temperature. TNFα concentrations were increased between ML vs. EF (+39%) and between ML vs. OV (+55%) regardless of temperature, however, the time x group interaction did not meet the conventional threshold for statistical significance (p=0.068, η_p^2=0.200). TNFα responses were similar between the two groups when stimulated with LPS with/or without hyperthermia. A larger pro-inflammatory response occurred during the ML phase of the MC across both temperatures, despite evidence linking oestrogen with a reduction in pro-inflammatory responses. In conclusion, our data suggest that monocyte responses to immunogenic stimuli fluctuate throughout a hormone cycle in eumenorrheic women, but are more stable in those taking oral contraceptives.

OC18

Distinct roles for branched-chain amino acids and branched-chain keto acids in mediating cardiac insulin resistance in the healthy and failing heart

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Perturbed branched-chain amino acids (BCAA) oxidation positively correlates with the severity of cardiac insulin resistance in heart failure [1, 2]. We previously showed that cardiac specific deletion of the BCAA oxidative enzyme mitochondrial branched-chain aminotransferase (BCATm), which increases cardiac BCAA levels and decreases branched-chain keto acids (BCKA) levels, increases insulin-stimulated cardiac glucose oxidation rates [3]. This enhanced cardiac insulin sensitivity is associated with an increase in the phosphorylation of protein kinase B (Akt) and activation of pyruvate dehydrogenase (PDH), the rate-limiting enzyme of glucose oxidation. However, whether it

is the accumulation of BCAA or BCKA that is critical in mediating cardiac insulin resistance is unknown. How perturbed BCAA oxidation may mediate cardiac insulin resistance in heart failure is also unknown.

To address these questions, we first examined the effects of selectively enhancing cardiac BCKA levels on cardiac insulin-stimulated glucose oxidation. We perfused isolated working mice hearts (male and female C57BL/6N, n=8-10) with high levels of BCKA (**α**-keto-isocaproate 80 mM, **α**-keto-**β**-methylvalorate 100 mM, **α**-keto-isovalorate 70 mM), levels that can be seen in diabetes and obesity [4]. High levels of BCKA completely blunted insulin-stimulated glucose oxidation rates and increased fatty acid oxidation rates. We also found that BCKA abolished insulin-stimulated mitochondrial Akt, an effect that was associated with PDH deactivation.

We next determined the potential protective effect of reducing cardiac BCKA levels in the failing heart. We randomized $WTCre^{+/+}$ and cardiac-specific $BCATm^{-/-}$ mice (male, 25-30g, n=6-8) to undergo either sham surgery or transverse aortic constriction surgery to induce heart failure. Five weeks post-surgery there was a marked increase in insulin-stimulated glucose oxidation rates in the BCATm-/- failing hearts compared to the $WTCre^{+/+}$ failing hearts (1.48 ± 0.2 vs 0.78 ± 0.02 µmol.g dry $wt^{-1}.min^{-1}$ respectively, $p<0.05$), with no significant effect on glycolysis rates. Enhanced cardiac insulin sensitivity was associated with a significant decrease in fatty acid oxidation rates in the $BCATm^{-/-}$ failing hearts compared to the $WTCre^{+/+}$ failing hearts (0.31 ± 0.06 vs 0.67 ± 0.2 µmol.g dry $wt^{-1}.min^{-1}$, respectively, $p<0.05$). This decrease in fatty acid oxidation in the $BCATm^{-/-}$ failing hearts was associated with a significant decrease in myocardial oxygen consumption rates. As a result, cardiac efficiency (cardiac work/myocardial oxygen consumption) was significantly increased in the $BCATm^{-/-}$ failing hearts compared to the $WTCre^{+/+}$ failing hearts. We conclude that the accumulation of BCKA, and not BCAA, is a major contributor to cardiac insulin resistance via abrogating mitochondrial translocation of Akt. Targeting BCKA may represent a potential therapeutic approach to improve cardiac insulin-stimulated glucose oxidation in the setting of heart failure, obesity and diabetes.

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OC19

The Effects of Oral Contraceptives on Exercise Performance in Women: A Systematic Review and Meta-analysis

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Background: Oral contraceptive pills (OCPs) are double agents, which downregulate endogenous concentrations of oestradiol and progesterone whilst simultaneously providing daily supplementation of exogenous oestrogen and progestin during the OCP-taking days. This altered hormonal milieu differs significantly from that of eumenorrheic women and might impact exercise performance, due to changes in ovarian hormone-mediated physiological processes.

Objective: To explore the effects of OCPs on exercise performance in women and to provide evidence-based performance recommendations to users.

Methods: This review complied with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. A between-group analysis was performed, wherein performance of OCP users was compared with naturally menstruating women, and a within-group analysis was conducted, wherein performance during OCP consumption was compared with OCP withdrawal. For the between-group analysis, women were phase matched in two ways: (1) OCP withdrawal versus the early follicular phase of the menstrual cycle and (2) OCP consumption versus all phases of the menstrual cycle except for the early follicular phase. Study quality was assessed using a modified Downs and Black Checklist and a strategy based on the recommendations of the Grading of Recommendations Assessment Development and Evaluation working group. All meta-analyses were conducted within a Bayesian framework to facilitate probabilistic interpretations.

Results: 42 studies and 590 participants were included. Most studies (83%) were graded as moderate, low or very low quality, with 17% achieving high quality. For the between-group meta-analysis comparing OCP users with naturally menstruating women, posterior estimates of the pooled effect were used to calculate the probability of at least a small effect ($d \geq 0.2$). Across the two between-group comparison methods, the probability of a small effect on performance favouring habitual OCP users was effectually zero ($p < 0.001$). In contrast, the probability of a small effect on performance favouring naturally menstruating women was moderate under comparison method (1) ($d \geq 0.2$; $p = 0.40$) and small under comparison method (2) ($d \geq 0.2$; $p = 0.19$). Relatively large between-study variance was identified for both between-group comparisons (!0.5 = 0.16 [95% credible interval (CrI) 0.01–0.44] and !0.5 = 0.22 [95% CrI 0.06–0.45]). For the within-group analysis comparing OCP consumption with withdrawal, posterior estimates of the pooled effect size identified almost zero probability of a small effect on performance in either direction ($d \geq 0.2$; $p \leq 0.001$).

Conclusions: OCP use might result in slightly inferior exercise performance on average when compared to naturally menstruating women, although any group-level effect is most likely to be trivial. Practically, as effects tended to be trivial and variable across studies, the current evidence does not warrant general guidance on OCP use compared with non-use. Therefore, when exercise performance is a priority, an individualised approach might be more appropriate. The analysis also indicated that exercise performance was consistent across the OCP cycle.

OC20

The atypical antipsychotic drugs aripiprazole and clozapine have direct effects to increase beta-cell proliferation and protect against apoptosis

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Motivation: Atypical antipsychotic drugs (AAPs) are prescribed to control various psychiatric conditions, such as schizophrenia, bipolar disorder, or they are used as an add-on therapy for major depressive disorder. The prevalence of diabetes is 3-fold higher in people suffering from mental illnesses [1] and some antipsychotic drugs have been linked to increased risk of hyperglycaemia and type 2 diabetes (T2D) [2]. However, AAPs vary in side effects related to T2D and the mechanisms of action of individual AAPs at islet beta-cells are not fully understood. Therefore, this project aimed to investigate the direct effects of therapeutic concentrations of two widely used AAPs, aripiprazole and clozapine, on mouse beta-cell function. Methods: Beta-cell proliferation was measured by quantifying BrdU incorporation into MIN6 beta-cell DNA after 48h exposure to 1µM aripiprazole or 2µM clozapine using a BrdU ELISA, and viability was measured by Trypan blue staining. ATP generation and apoptosis of MIN6 cells and mouse islets were quantified following a 48h incubation in the absence or presence of AAPs using CellTiter-Glo 3D and Caspase-Glo assays, respectively. Chronic effects of AAPs on glucose-stimulated insulin secretion from MIN6 cells and mouse islets were determined by static incubation experiments and secreted insulin was measured by radioimmunoassay. Results: Aripiprazole and clozapine significantly increased MIN6 beta-cell proliferation (OD 450nm, control: 0.59±0.02; +1µM aripiprazole: 0.65±0.01; +2µM clozapine: 0.67±0.02, n=8, $P<0.05$) and metabolic activity (by 15.22±1.38% and 14.49±2.04%, respectively), without any effect on Trypan blue dye uptake (% viability after 48h, control: 95.5±2.6; +1µM aripiprazole: 99.0±1.0; +2µM clozapine: 97.7±2.3, n=3, $P>0.1$). These AAPs had a protective effect against palmitate-induced apoptosis of MIN6 cells and mouse islets (MIN6 cells: luminescence units, control: 653,970±28,403; +1µM aripiprazole: 541,061±13,281; +2µM clozapine: 555,632±16,741, n=8, $P<0.001$; mouse islets: control: 62,424± 7,921; +1µM aripiprazole: 40,799±4,841; +2µM clozapine: 41,627±3,857, n=8, $P<0.05$). However, they did not significantly affect glucose-stimulated insulin secretion from MIN6 cells (ng/20,000 cells/h, control: 2.18±0.15; +1µM aripiprazole: 2.40±0.32, n=7, $P>0.1$; control: 0.92±0.09; +2µM clozapine: 1.18±0.26, n=7, $P>0.1$) or mouse islets (ng/islet/h, control: 2.77±0.37; +1µM aripiprazole: 3.97±0.67, n=7, $P>0.1$; control: 1.70±0.74; +2µM

clozapine: 2.15±0.79, n=7, P>0.1). Conclusion: Therapeutically relevant concentrations of aripiprazole and clozapine are well-tolerated by beta-cells, with no impairment of viability. They induce beta-cell proliferation and reduce apoptosis, which is of benefit to restore functional beta-cell mass. Our data support these AAPs being recommended for treating psychiatric conditions in patients with glucose dysregulation.

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OC21

A comprehensive analysis of gene expression in the kidney of the one-humped Arabian camel (*Camelus dromedarius*) reveals a role for cholesterol in water conservation during dehydration

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[1]University of Bristol, Bristol, United Kingdom [2]United Arab Emirates University, Al-ain, The United Arab Emirates [3]Alfaisal University, Riyadh, Saudi Arabia [4]Vetmeduni, Vienna, Austria [5]College of Natural and Health Sciences, Abu Dhabi, The United Arab Emirates

The one-humped Arabian camel is the most important livestock animal in arid and semi-arid regions and continues to provide basic necessities to millions of people in areas where food production is problematic. In the current context of global warming, there is renewed interest in the adaptive mechanisms that enable camelids to survive in arid conditions. Extensive evidence shows the impressive set of adaptations that allows dromedaries to thrive in such environment and recent investigations described genomic signatures that revealed evolutionary adaptations to desert environments (1-2). We now present a comprehensive catalogue of the transcriptomes of the male dromedary kidney.

We performed RNAseq in samples from 15 adult dromedaries (5 controls, 5 dehydrated and 5 rehydrated). Using DESeq2, we identified 985 DEG in the kidney (cortex and medulla) of dehydrated camels. Gene Ontology (GO) analyses suggested an enrichment of the cholesterol biosynthetic pathway and an overrepresentation of categories related to “ion transmembrane transport”. The RNAseq data confirmed that 13 key genes involved in the cholesterol biosynthesis pathway were significantly ($p_{adj} < 0.05$) downregulated in the kidney medulla during dehydration. These data were validated by RT-qPCR (log_2FC ranged 0.34 – 0.87). Further, we recently reconstructed the transcriptional regulatory network of the camel kidney during chronic dehydration. We identified 49 differentially expressed transcription factors and 10 enriched regulons. Among those, *SREBF1* and *SREBF2* were significantly downregulated in dehydration compared to control ($p_{adj} < 0.05$). They play

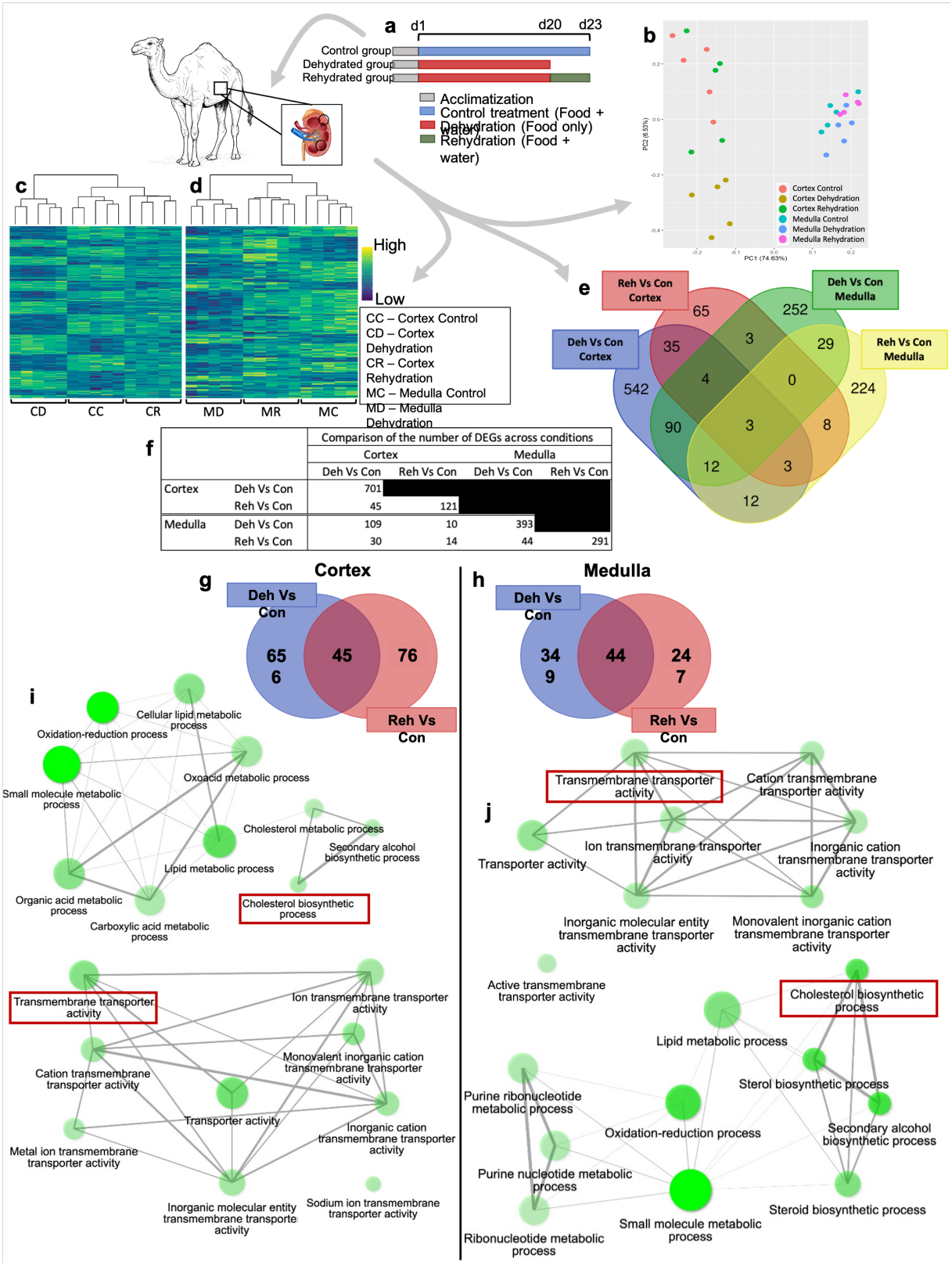
a key role in the regulation of cholesterol synthesis and GO analyses of their significantly changed targets confirmed an enrichment of that pathway.

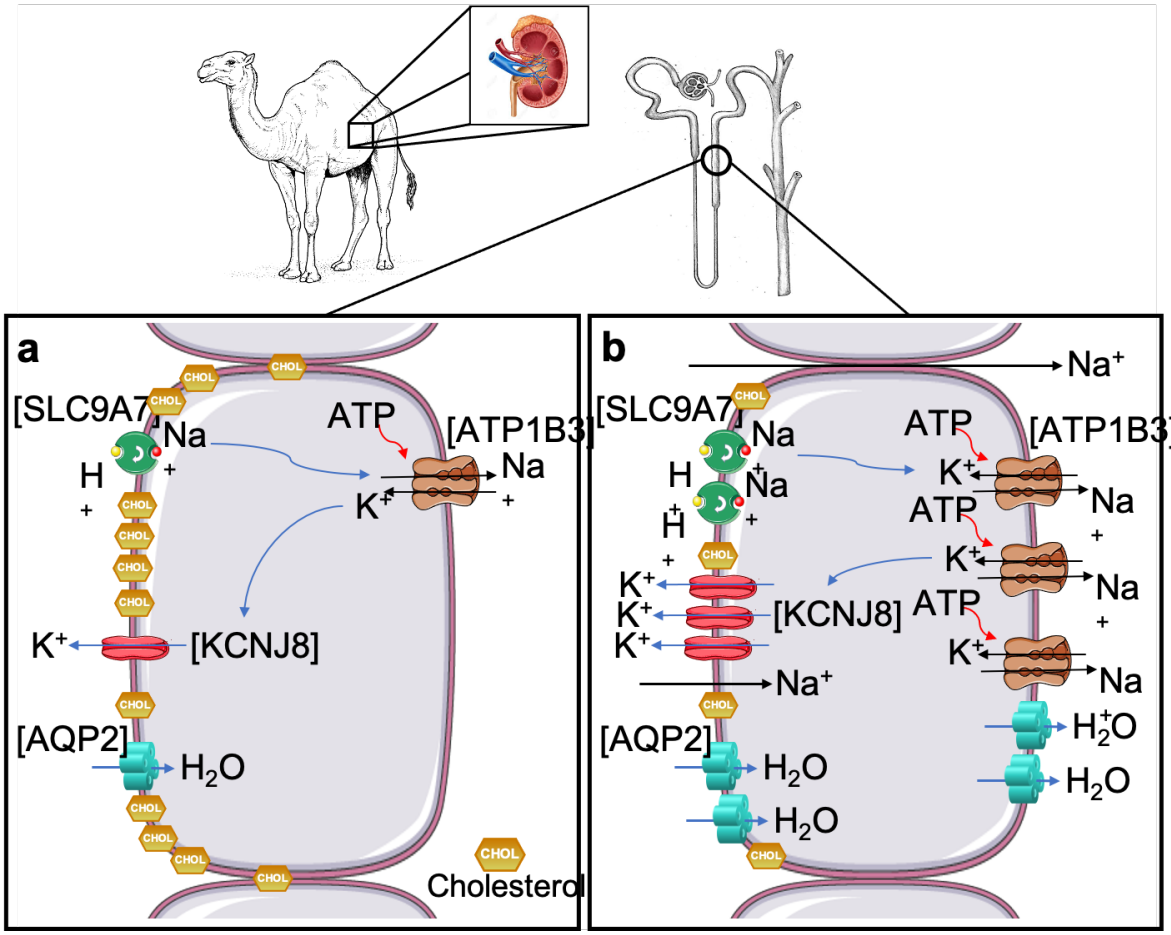
Based on our hypothesis of a role for cholesterol during dehydration, we identified DEGs with known roles in the countercurrent multiplication process which are affected by changes in the level of cholesterol. Cholesterol changes the state of K^+ inward rectifiers (*KCNJ8*), by directly binding onto it, to "silent", it also inhibits Na^+ leakage and indirectly decreases Na^+-K^+-ATPase activity. Moreover, cholesterol depletion results in the accumulation of AQP2 in the plasma membrane. *KCNJ8* (p_{adj} < 0.01) and *ATP1B3* (non-significant) showed a ~0.5-2 fold increase in gene expression during dehydration in the medulla, whilst *SLC9A7* expression level trended towards a smaller increase (non-significant). Relative expression of *AQP2* showed a 2-fold increase (p_{adj} < 0.001).

Our datasets suggest that the suppression of cholesterol biosynthesis may facilitate water retention in the kidney of the dromedary by indirectly enhancing the osmotic gradient along the medullary interstitium that is required for the subsequent AQP-2-mediated water reabsorption.

Ethical statement and statistics

Samples were shipped to the University of Bristol under the auspices of a DEFRA Import Licence (TARP/2016/063). This study was approved by the Animal Ethics Committee of the United Arab Emirates University (approval ID: AE/15/38) and the University of Bristol Animal Welfare and Ethical Review Board. Benjamini-Hochberg was used to calculate significance levels in differential gene expression analysis and One-way ANOVA to determined differences between RT-qPCR experimental groups (n=5). Alternatively, we used Kruskal-Wallis test combined with Benjamini-Hochberg method for groups which followed non-normal distribution.


a
d1
d20
d23
Control group
Dehydrated group
Rehydrated group
Acclimatization
Control treatment (Food + water)
Dehydration (Food only)
Rehydration (Food + water)
b
Cortex Control
Cortex Dehydration
Cortex Rehydration
Medulla Control
Medulla Dehydration
Medulla Rehydration
PC1 (74.63%)
PC2 (8.53%)
c
d
High
Low
CC – Cortex Control
CD – Cortex Dehydration
CR – Cortex Rehydration
MC – Medulla Control
MD – Medulla Dehydration
CD
CC
CR
MD
MR
MC
e
Reh Vs Con Cortex
Deh Vs Con Medulla
Deh Vs Con Cortex
Reh Vs Con Medulla
65
252
35
3
29
542
4
0
224
90
3
8
12
3
12
f
Comparison of the number of DEGs across conditions
Cortex
Medulla
Deh Vs Con
Reh Vs Con
Deh Vs Con
Reh Vs Con
Cortex
Deh Vs Con
701
Reh Vs Con
45
121
Medulla
Deh Vs Con
109
10
393
Reh Vs Con
30
14
44
291
g
Cortex
Deh Vs Con
65
6
45
76
Reh Vs Con
h
Medulla
Deh Vs Con
34
9
44
24
7
Reh Vs Con
i
Cellular lipid metabolic process
Oxidation-reduction process
Oxoacid metabolic process
Small molecule metabolic process
Cholesterol metabolic process
Secondary alcohol biosynthetic process
Lipid metabolic process
Organic acid metabolic process
Cholesterol biosynthetic process
Carboxylic acid metabolic process
Transmembrane transporter activity
Ion transmembrane transporter activity
Monovalent inorganic cation transmembrane transporter activity
Cation transmembrane transporter activity
Transporter activity
Inorganic cation transmembrane transporter activity
Metal ion transmembrane transporter activity
Inorganic molecular entity transmembrane transporte activity
Sodium ion transmembrane transporter activity
j
Transmembrane transporter activity
Cation transmembrane transporter activity
Transporter activity
Ion transmembrane transporter activity
Inorganic cation transmembrane transporter activity
Inorganic molecular entity transmembrane transporter activity
Monovalent inorganic cation transmembrane transporter activity
Active transmembrane transporter activity
Cholesterol biosynthetic process
Lipid metabolic process
Sterol biosynthetic process
Purine ribonucleotide metabolic process
Oxidation-reduction process
Secondary alcohol biosynthetic process
Purine nucleotide metabolic process
Steroid biosynthetic process
Small molecule metabolic process
Ribonucleotide metabolic process



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OC22

Nutraceutical targeting of the bile acid receptor, farnesoid X receptor, for intestinal disease.

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Introduction: Bile acids, classically known for their roles in dietary fat absorption are now recognised as hormones critical to regulating intestinal and metabolic function, including mucosal immune responses, epithelial proliferation/apoptosis, and transepithelial transport and barrier function. Downregulation of the nuclear bile acid receptor, farnesoid X receptor (FXR), in intestinal epithelium occurs in inflammatory bowel disease, colorectal cancer, obesity and diabetes, whereas FXR activation prevents disease progression in pre-clinical models. Thus, strategies to upregulate epithelial FXR expression have been proposed to treat such conditions. Previous studies suggest that a particular class of plant-derived phytochemicals, denoted KFS1, modulates FXR activity. Plant sources have been reported to contain these phytochemicals, such as KFS1, which are proposed to have FXR modulatory activity.

Aim: The aim of the current study was to investigate the potential for developing plant extracts, rich in phytochemicals, such as KFS1, as a new novel nutraceutical-based approach to treat and prevent intestinal and metabolic diseases.

Methods: T_{84} colonic epithelial cells and primary cultures of murine epithelial colonic enteroids were employed to examine the effects of a common-dietary phytochemcial, KFS1, and a KFS1-rich plant extract on FXR expression and FXR signaling *in vitro*. *Ex vivo* studies were carried out on human colonic tissue obtained during endoscopy with ethical approval from Beaumont Hospital. *In vivo* studies in C57BL/6 mice were carried out and proximal colonic tissue were examined with ethical approval from the HPRA. Expression of FXR and FGF19/15, an established marker of FXR activation, was assessed by qRT-PCR, ELISA and immunoblotting. Data are expressed as mean ± SEM for a series of *n* experiments.

Results: KFS1 treatment (5 µM; 24hrs) increased FXR mRNA and protein expression in T_{84} cells by 4.2 ± 0.4 ($p<0.05$; n = 4) and 1.7 ± 0.1 fold ($p<0.05$; n = 7), respectively. KFS1 also enhanced FGF-19 protein expression, an established marker of FXR activity, in response to the FXR agonist, GW4064 (5 µM), by 4.1 ± 0.5 fold ($p<0.001$; n = 7). Similarly, KFS1 significantly upregulated FXR expression by 2.1 ± 0.4 fold in human biopsies ($p<0.05$; n = 6). Oral KFS1 administration to mice had a tendency to upregulate colonic epithelial FXR signalling *in vivo* and increased FXR expression and GW4064-induced FGF15 mRNA by 2.3 ± 0.2 ($p<0.01$; n = 4) and 2.2 ± 0.1 fold ($p<0.01$; n = 4) in murine epithelial colonic enteroids. Finally, a methanolic KFS1-rich plant extract, denoted QE1, verified by

LC/MS to contain KFS1, increased FXR protein expression in T_{84} cells by 1.8 ± 0.2 fold ($p<0.05$; n = 5) and enhanced GW4064-induced FGF19 protein release by 1.9 ± 0.1 fold ($p<0.01$; n = 9).

Conclusion: In conclusion, our data demonstrate that KFS1-containing plant extracts modulate expression and activity of the nuclear bile acid receptor, FXR, in the intestinal epithelium. Given the critical role of FXR in maintenance of gut health and metabolic function, such extracts have significant therapeutic and commercial potential to be developed as a novel first-in-class "FXR-targeted nutraceutical" for treatment and prevention of common intestinal and metabolic disorders.

Acknowledgements :- The RCSI StAR PhD Programme & Science Foundation Ireland (SFI)

OC23

Modulation of cerebrospinal fluid production by the Transient Receptor Potential Vanilloid 4 channel: implications for hydrocephalus and other disorders of brain fluid volume.

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Cerebrospinal fluid (CSF) secretion into the brain ventricular system is an active process via the choroid plexus (CP) - a specialized, convoluted epithelial sheet surrounding a fenestrated capillary bed which is fed from the cerebral arterioles. The final CSF composition differs from a simple plasma filtrate implicating the CP epithelial cells (CPe) as an important regulatory site controlling CSF composition and volume. Solute and water transport across the blood-CSF barrier are tightly controlled by ion and water transport proteins. Transient Receptor Potential Vanilloid 4 (TRPV4) is a polymodal, non-selective cation channel which functions as a regulatory hub protein in epithelia, including the CP. Previous studies from our group demonstrated d that TRPV4-selective antagonists were effective at reducing ventriculomegaly in a genetic rat model of severe postnatal hydrocephalus, a condition resulting from excess CSF (Hochstetler and Smith et al., 2020). In CPe, it was found that activation of TRPV4 caused the influx of both Ca^{2+} and Na^{+} ions, and entry of both of these ions was selectively blocked by a TRPV4-selective antagonist. Systemic treatment with TRPV4 antagonists reduced TRPV4 mRNA expression in the CP in hydrocephalic rats, but not in normal rats, indicating a role for TRPV4 as part of a regulatory pathway in CSF production under pathological conditions. To study the mechanism of CSF secretion by the CP, we utilized two continuous, high resistance CP cell lines: the porcine choroid plexus – Reims (PCP-R) and the human choroid plexus papilloma (HIBCPP) cells. The current experiments utilize Ussing chamber electrophysiological techniques, western blotting, RT-PCR, and fluorescent immunolabeling. Immunolabeling and mRNA expression demonstrate a robust abundance for TRPV4 and other well-characterized membrane proteins in both cell lines. In the lines, TRPV4 activation stimulates a large change in short circuit current (I_{SC}), a measure of net electrogenic ion flux. This change in I_{SC} is coupled with a substantial change in transepithelial membrane conductance, a measure of cellular permeability. The transepithelial membrane transport changes due to TRPV4 activation for human and porcine cells are similar in overall effect, but differ in baseline, duration, and magnitude, indicating the possibility of species-specific CP transport mechanisms. TRPV4 activation can be inhibited by pre- incubation with

TRPV4 antagonists, and the changes in ion flux and conductance can be reversed by addition of TRPV4 antagonists after initiation of the response, these studies substantiate the specificity of the mediator-induced changes and provide an in vitro model for testing potential drug actions. Furthermore, in both the porcine and human cells, the TRPV4 response is dependent upon K^+ secretion, Cl^- absorption, and Na^+ secretion. These data confirm a role for TRPV4 in regulating ionic gradients, thereby modulating both electrogenic and electroneutral transporters, and controlling CSF production. The promising results of these studies, coupled with published data from other investigators suggesting a role for TRPV4 in blood-brain-barrier permeability and astrocyte volume regulation, shows the importance of TRPV4 as a regulatory hub protein in maintaining brain fluid volume.

Reference 1 :- Hochstetler AE, Smith HM, Preston DC, Reed MM, Territo PR, Shim JW, Fulkerson D, Blazer-Yost BL. TRPV4 antagonists ameliorate ventriculomegaly in a rat model of hydrocephalus. JCI Insight. 2020 Sep 17;5(18):e137646. doi: 10.1172/jci.insight.137646. PMID: 32938829; PMCID: PMC7526552.

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OC24

Acidic pH Affects the Expression of ASIC1 and is Associated with Hypomethylation of ASIC1 Promoter Region in Pancreatic Ductal Adenocarcinoma

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The development and progression of pancreatic ductal adenocarcinoma (PDAC) are linked to the physiology and microenvironment of the exocrine pancreas. The temporally highly variable extracellular pH landscape in pancreatic ducts challenges epithelial cells in maintaining a constant intracellular pH [1]. We hypothesize that changes in pH affect cellular processes leading to an altered expression of ion channels. This in turn helps driving transformation from usual to malignant cell behaviour. We investigate if the already known dysregulation of the acid sensing ion channel 1 (ASIC1) [2] is regulated by DNA methylation.

To investigate if a prolonged acidic pH treatment has an effect on DNA methylation and thereby the expression of ASIC1, PDAC cell lines Panc-1 and MiaPaca-2 were incubated in medium with a pH of 7.4 or 6.5 over 30 days. A ductal-like control cell line hTERT-HPNE was grown in pH 7.4. Under these conditions, mRNA expression of ASIC1 was obtained by qPCR, and DNA methylation levels were

obtained by sodium bisulfite-treated genomic DNA sequencing. Human PDAC tissue samples from non-tumour and tumour tissue were used to acquire the ASIC1 expression by classical immunohistochemical analysis (IHC).

Our preliminary IHC results showed that ASIC1 tends to be upregulated in tumour tissue compared to normal tissue (n=10, intensity score in tumour =2, *vs.* 0 in non-tumour tissue). ASIC1 was significantly upregulated in Panc-1 and MiaPaca-2 cell lines, compared to the control hTERT-HPNE (n=3, Welch Correction test, $p< 0.0001$ and $p<0.01$, respectively). The acidic pH treatment (pH 6.5) resulted in further ASIC1 upregulation, when compared to cells treated with pH 7.4, both in Panc-1 and MiaPaca-2 cells (n=3, Welch Correction test, $p< 0.001$ and $p< 0.01$, respectively). To test our hypothesis that ASIC1 gene expression is regulated by DNA methylation, we performed bisulfite sequencing interpreted by a semi-quantitative analysis, where the ratio between the peak of thymines and cytosines was measured. Different CpG islands of the ASIC1 promoter region were to some extent methylated in DNA from MiaPaca-2 cells treated with pH 7.4 (n=2), sum of methylation in 26 CpG islands was Σ95% and Σ28%, respectively. In contrast, CpG islands of the ASIC1 promoter region from MiaPaca-2 treated with pH 6.5, had lower DNA methylation levels (n=2), sum of methylation in 26 CpG islands was Σ1.35% and Σ1.18%, respectively.

Collectively, these results show that ASIC1 is upregulated in human tissue, and is significantly upregulated in PDAC cell lines Panc-1 and Miapaca-2. Prolonged exposure to acidic pH, results in an upregulation of ASIC1 in both cell lines. This upregulation might be governed by DNA methylation of specific ASIC1 CpG sites, suggesting that the ASIC1 gene promoter region is hypomethylated in cells exposed to prolonged acidic treatments. Further studies should assess ASIC1 expression and DNA methylation status in human tissue. Furthermore, a quantitative analysis and method to show that DNA methylation levels are reversible can reveal that the upregulation of ASIC1 in PDAC is governed by DNA methylation and affected by the temporally highly variable pH landscape in pancreatic ducts.

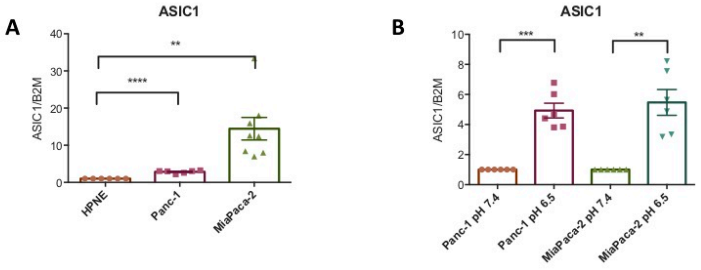

Figure 1. ASIC1 mRNA expression in PDAC cell lines is affected by pH. 1A) ASIC1 mRNA expression is upregulated in PDAC cell lines (Panc-1 and MiaPaca-2), compared to a normal pancreatic duct cell line (hTERT-HPNE). 1B) ASIC1 mRNA expression is significantly higher in PDAC cells grown in pH 6.5 compared to cells grown in pH 7.4. Data are shown as means with SEM error bars. ** indicate P < 0.01, *** indicate P< 0.001, **** indicate P< 0.0001, Unpaired t-test with Welch's correction. Data are representative of 3 independent experiments.

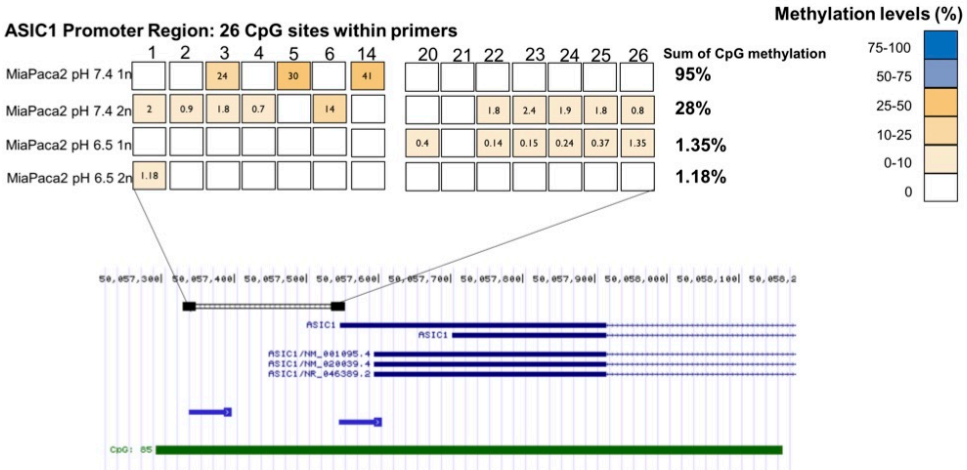

Figure 2. Methylation of ASIC1 Promoter Region. Promoter sites in ASIC1 gene within the specifically designed primers (black dotted box). MiaPaca2 cancer cells grown in pH 7.4 are more methylated than MiaPaca2 cancer cells grown in pH 6.5. Sum of the methylation in MiaPaCa2 cells grown in pH 7.4 (Σ95% and Σ28%, respectively) and the sum of MiaPaCa2 cancer cells grown in pH 6.5 (Σ1.35% and Σ1.18%, respectively) are shown. (CpG sites which are not showed, are not methylated). n=2.

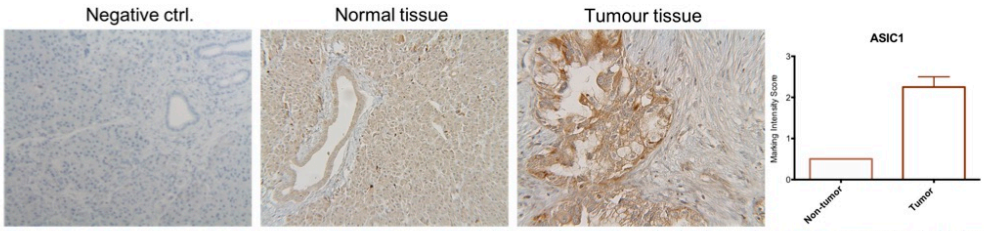

Figure 3. ASIC1 is upregulated in human PDAC tissue. IHC staining with ASIC1 antibody shows that ASIC1 expression is higher in tumour tissue compared to non-tumour tissue. The histogram shows the marking intensity score of ASIC1 in tumour vs. non tumour tissue (n=10). Negative control represent stain of tissue without primary antibody.

Reference 1 :- Pedersen, S.F., et al., *Alternating pH landscapes shape epithelial cancer initiation and progression: Focus on pancreatic cancer.* Bioessays, 2017. **39**(6).

Reference 2 :- Zhu, S., et al., *ASIC1 and ASIC3 contribute to acidity-induced EMT of pancreatic cancer through activating Ca(2+)/RhoA pathway.* Cell Death Dis, 2017. **8**(5): p. e2806.

Acknowledgements :-

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OC25

Students' Perspectives of Physiology E-Learning during the COVID-19 Pandemic in Nigerian Universities

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During the COVID-19 pandemic, several institutions and medical schools globally transited to online learning, as evident in few Nigerian Universities (1). This study explores the perspectives of medical students regarding Physiology e-learning amid the COVID-19 lockdown. An online descriptive, cross-sectional study was conducted between July and September, 2020. Current undergraduate medical students aged 17 years or older across five private-owned Universities who transited to virtual learning constituted the study population. Using Google forms, a web-based questionnaire was administered to participants via WhatsApp messenger. The questionnaire was developed using validated questions from previously published studies. Overall, 200 participants responded (response rate = 65%). Of the 190 valid responses, 48 (25.3%) were males, and 157 (82.6%) were in the age bracket of 17-20 years. Of the 190 participants who engaged in learning Physiology online during the lockdown, 78 (41.1%) were pursuing the Bachelor of Medicine and Bachelor of Surgery (MBBS) program, 69 (36.3%) Bachelor of Pharmacy (B.Pharm), while 16 (8.4%) were studying Bachelor of Science in Physiology (Majors; B.Sc.). More than half (n = 122, 64.2%) of the study participants agree to actively participate in online classes, 68 (35.8%) had a suitable home environment for online learning, 32 (16.8%) agree that the quality of online teaching equals classroom teaching, while 36 (19%) students are satisfied with learning Physiology online. Furthermore, 135 (71.1%) participants prefer mornings as their learning periods compared to afternoons, 149 (78.4%) supplement the online lectures with further private study, while only 46 (24.2%) students agree that online lectures only will sufficiently equip them with required Physiology knowledge. The evaluation of the experiences and perspectives of students learning Physiology online during the COVID-19 pandemic in Nigeria is imperative due to the novelty of the learning mode. Thus, there is a need to encourage

the consideration of adopting and integrating blended (virtual) learning in Physiology education in Nigeria.

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OC26

Interactive quizzing in online classes: The suitability of Kahoot! for face-to-face and online delivery in health sciences and medical education

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Recent events have fast-tracked the transition of university teaching from face-to-face to online, remote delivery. The disruption to the delivery of standard classes, as a result of the COVID-19 pandemic, affected students across all levels of education.[1] In order to continue teaching and learning, the delivery of content was replaced with live online modalities. This placed a significant challenge on tertiary educators to find learning activities that are transferrable between face-to-face and online teaching whilst promoting equal engagement.[2] One such method, interactive quizzing, is a tool commonly used in face-to-face classes that could enhance engagement and enjoyability.[3] The primary outcome of this study was to evaluate the suitability of using the interactive quizzing platform, Kahoot!, as a teaching tool that can be effective between face-to-face and online classes. In order to achieve this outcome, this study aimed to compare learner perceptions of the interactive quizzing platform Kahoot! when used in either a face-to-face or online setting within a health sciences and medical course. A total of 174 first-year health sciences and medical students from an Australian university enrolled in this study. Two study groups were formed based on whether the participants were currently enrolled in a face-to-face (n = 72) or online (n = 102) provision of their subject. Participants attended a one-hour physiology lecture, either in a face-to-face class or online during live sessions, then completed a 10-item Kahoot! interactive quiz based on the session content. Following the provision of the quiz, participants completed a four-question Likert scale survey related to their experiences and provided written responses to three open-ended questions regarding their perceptions of using the interactive quizzing platform. Overall, participants in both the face-to-face and online learning groups highly rated their learning experience using interactive quizzing. There were no significant differences (Student's two-tailed *t*-test) between experiences from using Kahoot! during a face-to-face or online session. In particular, responses from the face-to-face and online participants on the Likert scale (*1 = strongly disagree*, *5 = strongly agree*) for the statement "*I enjoyed using Kahoot!*" were 4.71 ± 0.54 and 4.81 ± 0.68, respectively ($p = 0.3$). Three overall themes emerged from qualitative analysis of student perceptions that were comparable between the two groups. These themes were (1) interactive quizzing is enjoyable, (2) interactive quizzing is engaging, and (3) interactive quizzing helps my learning. Participants utilising interactive quizzing in an online setting reported increased engagement whilst learning due to the fun, attractive environment and

interactive nature of the platform, despite being isolated in a passive learning environment. This study identifies Kahoot! as a teaching tool that is equally effective across both face-to-face and online teaching sessions, and thus presents interactive quizzing as a variable instrument for assessment and revision that can be utilised in health sciences and medical courses. As many tertiary institutions are currently split between online, face-to-face or mixed-mode curricula, it is increasingly important to highlight technology that can be rapidly and easily transferred between various modes of delivery.

Reference 1 :- UNESCO. Universities tackle the impact of COVID-19 on disadvantaged students. May 2020. Accessed January 20, 2021. https://en.unesco.org/news/universities-tackle-impact-covid-19-disadvantaged-students

Reference 2 :- Moro C, Phelps C, Stromberga Z. Utilizing serious games for physiology and anatomy learning and revision. *Adv Physiol Educ.* 2020;44(3),505-507. https://doi.org/10.1152/advan.00074.2020

Reference 3 :- Felszeghy S, Pasonen-Seppänen S, Koskela A, et al. Using online game-based platforms to improve student performance and engagement in histology teaching. *BMC Med Educ*. 2019;19(1):273-273.

OC27

Using 3D holographic technology (HoloLens) for asthma education in health sciences and medicine.

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Medical and health sciences education requires constant updating and refining to best prepare students for the expectations of working in the modern healthcare professional environment. Learning about a range of diseases is a core component within these courses, and a fundamental part of many curricula and assessment-focussed outcomes. One common example is asthma, a widespread and prevalent respiratory condition estimated to affect up to 339 million people globally [1]. However, learning about the management of asthma requires students to integrate knowledge of physiology, anatomy, pathology, immunology, pharmacology and more. It also necessitates a need for an understanding of the lungs and its associated structures in 3D space. This can be difficult when studying from a textbook or lecture notes alone. In recent years, there has been a shift towards technology-enhanced learning to deliver content in an engaging manner [2]. Emerging technology, such as the Microsoft HoloLens, is of great interest as it can provide 3D representations of the human body, while also encouraging interactivity with any presented organs or systems [3]. Though never employed for the specific use of teaching asthma, the HoloLens shows potential as a way to effectively explain the mechanisms underlying asthma, and its associated multidisciplinary concepts. The aim of this honours research project will be to assess whether a textbook-style written delivery, or a three-dimensional (3D) augmented reality HoloLens resource, is more effective for learning. This will be a randomised-control trial utilising pre- and post-testing with

first year health sciences and medical students. Lessons will be set up with an instructional module explaining the epidemiology, anatomy, physiology, pathophysiology, immunology and pharmacology of the respiratory system and asthma. The control group are to be provided with a printed textbook-style version of the lesson, with 2-dimensional diagrams, while the HoloLens intervention group viewing the models in 3D, with the text read out as an audio transcript. Though data collection will commence shortly, it is hypothesised that learning through augmented reality using the HoloLens device will provide a better overall learning experience and improve test performance for health sciences and medical students.

Reference 1 :- Global Asthma Network, *The Global Asthma Report 2018*. 2018: Aukland, New Zealand. p. 6-7.

Reference 2 :- Moro, C., Birt, J., Stromberga, Z., Phelps, C., Clark, J., Glasziou, P., & Scott, A. M. (2021). Virtual and augmented reality enhancements to medical and science student physiology and anatomy test performance: A systematic review and meta-analysis. *Anatomical Sciences Education.*. https://doi.org/https://doi.org/10.1002/ase.2049

Reference 3 :- Moro, C., Phelps, C., Redmond, P., & Stromberga, Z. (2021). HoloLens and mobile augmented reality in medical and health science education: A randomised controlled trial. *British Journal of Educational Technology*, e13049. https://doi.org/https://doi.org/10.1111/bjet.13049

PC01

Preventing cardiotoxicity: Lifestyle changes or pharmacological interventions? Preliminary results in a doxorubicin animal model

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Breast cancer (BC) is the most common cancer among women. Advances in BC therapy have improved the survival and quality of life of BC patients. However, these improvements are accompanied by cardiac dysfunction that may occur in a subset of patients and compromise chemotherapy continuation. Cardiac dysfunction might be due to an increase in sympathetic (SNS) tone promoted by chemotherapy, contributing to left ventricular ejection fraction decrease. Different preventive strategies, such physical exercise, and β-blockers, have been proposed to maintain the physiological homeostasis. At present, the methods used are insufficient and do not have enough sensitivity so it is not possible to accurately predict which patients will be affected. Thereby it is not clear what is the most effective preventive approach to maintain the physiological homeostasis in these patients.

Using animal experiments is possible to develop and functionally characterize a doxorubicin (DOX) treatment model to deeply evaluate it effect in cardiac function and to compare the efficacy of two different approaches to prevent negative outcomes: one pharmacological, using atenolol (ATN), a β1

selective antagonist without antioxidant properties, and other non-pharmacological, using a treadmill training in an animal model of DOX.

Healthy female Wistar rats, aged >12 weeks, were divided into 4 groups: Doxorubicin (n=13) (DOX; ip. cumulative dose 8 mg/kg, 1 time/week, for 4 weeks), DOX with physical exercise (n=8) (DOX + EX; treadmill, 22 cm/seg for 30 minutes, 5 times/week), DOX with Atenolol (n=10) (DOX + ATN: DOX, ip. cumulative dose 8 mg/kg, 1 time/week and 4 mg/ml; ATN, oral administration, 5 times/week, for 4 weeks) and controls (n=7) (ip. with saline solution). Echocardiography measurements were recorded at baseline and 1 week after of treatment and blood pressure (BP), cardiac (HR) and respiratory (RF) frequency, baro- and chemoreflexes were evaluated under anaesthesia.

Our results reveal that DOX treatment triggered a significant decrease in BP and HR, caused hypopnea, decreased baro- and chemoreflexes, without evidence of sympatho excitation. These changes can be explained by the decline in cardiac function, respiratory muscle weakness, autonomic dysfunction and vascular changes induces by DOX. During treatment with DOX, the physical activity protocol countered some of the adverse effects caused by DOX. It normalized BP, HR and RF to physiological values, and decreases the loss in baroreflex gain. Chemoreflex sensitivity, sympathetic and parasympathetic activities remained similar. Systolic and diastolic pressures and cardiac output increased after exercise. This might be due to an increase in stroke volume due to longer diastoles as showed by echocardiography despite total peripheral resistance was not evaluated. ATN treatment, similar to physical activity effect, also increased baroreflex gain and RF to normal values, causing also a clear tendency to maintain BP values.

Although these results are still preliminary and complementary data is still needed, with these results we can conclude that lifestyle changes, such as physical exercise, can improve cardiovascular health and is more effective than atenolol in counteracting the adverse effects of DOX treatment.

PC02

To what extent does ethnicity affect five minute heart rate variability recordings in healthy humans?

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Over the last few decades, heart rate variability (HRV) has been used to estimate the balance between the sympathetic and the parasympathetic nervous system in healthy and diseased humans. There exists very little data on the effect of different ethnic groups on short term heart rate variability. The aim of our study was to investigate the extent to which ethnicity affects 5 minute heart rate variability recordings in healthy humans. A secondary aim was to assess other factors that could influence HRV in these subjects, such as resting blood pressure, levels of exercise, alcohol or smoking intake.

Previous data collected in our lab was compiled and analysed. In total, HRV data from 264 healthy subjects was analysed. Most subjects were staff and students of Plymouth University (PU). Previous studies were given ethical approval by the Research Ethics committee, PU. All participating subjects gave informed and signed consent. An ECG lead was attached to each limb. LabChart software and a PowerLab were used for data acquisition. Subjects were supine and underwent one standard five minute HRV measurement. Data was also normalised by taking into account the heart rate [1]. Short term HRV was assessed using time domain (HR, SDNN), frequency domain (LFnu and HFnu alongside normalised LFnu and HFnu) and Poincaré analysis (SD1 and SD2). Different ethnic groups were investigated (Arab, Asian, Black, South Asian and White). Mixed subjects were excluded. Analysis was across all five ethnicities, and then both Asian and South Asian subjects were combined, to allow for analysis to be made across four ethnicities. The results were analysed by one way ANOVA. $P < 0.05$ was considered as significant.

The subjects' mean (± S.D.) age was 25.8 ± 10.0 years and mean BMI was 24.9 ± 5.2 kg m^{-2}. Ethnic details of the 264 healthy subjects are shown in Table 1 below.

Ethnic group	Subject numbers
Arab	74
Asians	25
Black	23
Mixed	4
South Asians	31
White	107

Table 1. Subject numbers of each ethnic group in the study (n=264).

HR (p=0.286) and SDNN (p=0.333) were not different across all five ethnicities. With four ethnic groups, HR (p=298) and SDNN (p=0.206) again did not show any differences. LFnu was significantly different across all five groups (p=0.001), while HFnu was not (p=0.348). Pairwise comparison showed Blacks (35.83 Hz) had a lower LFnu than Whites (52.55 Hz; p=0.002). With four ethnic groups, a similar significant difference was noted in LFnu, with Blacks having a lower value than Whites. Normalised LFnu was also significantly higher in Whites than Blacks, across both four and five ethnic group analysis.

LFnu is an indicator for sympathetic response while HFnu is an indicator of parasympathetic response. Therefore, it seems our White participants had a greater sympathetic (fight or flight) response, relative to an attenuated response in Black participants. Further research is needed to

elucidate possible reasons for these differences in frequency domain HRV variables across ethnic groups.

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PC03

Cerebral Blood Flow Pulsatility Index is Unchanged during Superimposed Lower-Body Negative Pressure in Head-Up Tilt in Anterior and Posterior Cerebral Circulations

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Lower body negative pressure (LBNP) chambers can be utilized to experimentally-elicit reduction in blood pressure, cerebral blood flow (CBF), and associated presyncope symptoms. There is high variability in tolerance to LBNP between individuals, however, the underlying physiological responses affecting tolerance remain unclear. Pulsatility in CBF may affect LBNP tolerance, as more pulsatile flow may protect the delivery of oxygen to cerebral tissues during hypotension. We aimed to assess the pulsatility index (PI) in anterior and posterior cerebral circulation during LBNP superimposed head-up tilt (HUT), where gravitational-induced blood volume redistribution augments volume unloading during LBNP. We recruited and included 12 healthy male participants and instrumented them in a custom-built integrated 45° HUT-LBNP chamber. All experiments and protocols were reviewed and approved by the Mount Royal University Human Research Ethics Board (Ethics ID 2011-91Sa) and conformed to the Declaration of Helsinki and the Canadian Government Tri-Council Policy Statement on research ethics (TCPS2). All participants provided written informed consent prior to participation in this study. Participants were instrumented for heart rate (HR; ECG), end-tidal CO_2 ($P_{ET}CO_2$; mouthpiece and gas analyzer), beat-by-beat mean arterial pressure (MAP; finometer), and middle and posterior cerebral artery velocity (MCAv, PCAv; transcranial Doppler ultrasound). All measures were recorded during baseline (BL) and during -50 mmHg LBNP exposure up to a maximum of 10-minutes (600s). Presyncope was defined as a 30% reduction in systolic blood pressure (i.e., investigator stop) or onset subjective symptoms (i.e., participant stop). We quantified all variables as a 30-sec mean bin during BL and the final 30-sec of LBNP prior to presyncope using a paired t-test. MAP, MCAv, and PCAv PI were calculated as systolic-diastolic/mean. LBNP tolerance time was 480.9±134.3 sec. HR increased from 72.3±10.3 (BL) to 103.0±18.2 bpm ($P<0.01$) during LBNP, suggesting a baroreflex response, and $P_{ET}CO_2$ was reduced from 32.0±2.9 (BL) to 28.7±2.9 Torr ($P<0.01$) during LBNP, suggesting mild hyperventilation. MAP decreased from 84.8±11.3 (BL) to 76.2±14.7 mmHg ($P<0.01$) during LBNP, with associated reductions in mean MCAv from 52.1±15.0 to 44.3±14.5 cm/s ($P<0.01$), and mean PCAv from 35.5±12.6 to 29.0±9.9 cm/s ($P<0.01$) during LBNP. MAP PI was reduced from 0.76±0.2 to 0.59±0.02 ($P<0.01$) during LBNP. However, MCAv and PCAv PI were unchanged during ($P=0.6$ and $P=0.9$, respectively).

Our results suggest that although MAP and MAP PI were both significantly reduced during LBNP at pre-syncope, with associated reductions in mean MCAv and PCAv, MCAv and PCAv PI remained stable with LBNP in HUT LBNP likely due to cerebral autoregulation. These findings suggest that PI in CBF variables at the cardiac frequency are not related to LBNP tolerance in healthy men.

Acknowledgements :- NSERC Discovery Grant

PC04

Elderberry extract increases cell proliferation in endothelial cells potentially via Akt pathway

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Introduction

Previous research has shown that Elderberry extract (EE) can improve endothelial cell viability, which is vital for preventing endothelial dysfunction a subclinical marker for most cardiovascular diseases (1,2). However, the exact mechanisms have not been explored making it difficult for understanding application in vivo. Some research has postulated that AKT is related to cell survival (3).

Objective

Therefore, the main aim of this study was to confirm whether EE can improve endothelial cell viability and to explore the molecular mechanisms.

Methods

Primary Human Umbilical Vein Endothelial cells (HUVEC) were maintained in Media 200 supplemented with 10% foetal bovine serum and low serum growth supplement. The EE was dissolved in PBS. For the MTT assay cells were seeded at 3.5 $x10^3$ per well in a 96 well plate, two concentrations were used including a low 50 µg/ml and high 500 µg/ml with two controls PBS and hydrogen peroxide (H_2O_2). The absorbance of each well was measured/quantified at 560 nM using a Promega GloMax Discovery system. For western blots, cells were treated with different concentrations (50, 125, 250, 325, and 500 µg/ml) for 1-hour and the protein levels of Phospho-AKT and AKT were measured by probing with specific antibodies. Levels of proteins were quantified and normalised using β-actin as an internal control, and the ratio of phosphorylated (activated) protein vs total protein was calculated. All data were analysed using GraphPad and were expressed as means ± standard deviation.

Results

H_2O_2 treated cells displayed a significant increase in cell cytotoxicity compared the control cells ($p < 0.01$). Cells treated with low concertation (50 µg/ml) of EE improved cell viability over a 24-hr period ($p = 0.0055$). The highest concentration showed no significance ($p = 0.3496$). Furthermore, over a 48-

hr period the lowest concentration 50 µg/ml vs control (p = 0.153) showed an effect whereas the highest concertation 500 µg/ml showed no significance in comparison with control (p = 0.1307). No significant differences were observed between EE concentrations (p > 0.05). Western blots were analysed by chemiluminescence detection using Li-Cor imaging system. For western blots time points which showed significant difference 125 µg/ml (p = 0.0098), 325 µg/ml (p = 0.0288), 500 µg/ml (p = 0.0412) although 50µg/ml the lowest concentration showed the largest change ($F_{(7.821,\ 4)}$ = 425.3 vs 100, p = 0.0019). Additionally, no difference was observed between the lowest and higher concertation (p > 0.05).

Findings

The main findings of this study show that a lower concentration of EE can cause proliferation within HUVECs and we also observed the highest AKT activity in the lowest concentration which could suggest there is an association. Further experiments are required with an AKT inhibitor (wortmannin) to confirm the association with AKT (4). Moreover, the PI3K/Akt pathway within endothelial cells has been known to induce eNOS phosphorylation which has many cardioprotective properties (5). Further research is required to understand the full functional properties of EE has within endothelial cells.

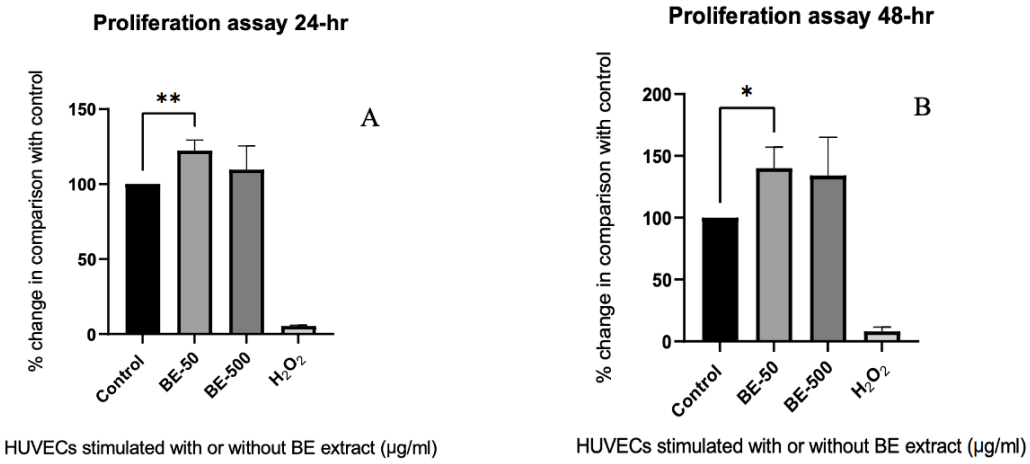

Figure 1. MTT assay to assess cell proliferation, only Elderberry 50 µg/ml (BE-50) showed significant difference in comparison with both controls, PBS and H_2O_2. Elderberry 500 µg/ml (BE-500) only showed significant effect in comparison with H_2O_2. Significant value was set at * = 0.05 and ** = 0.01.

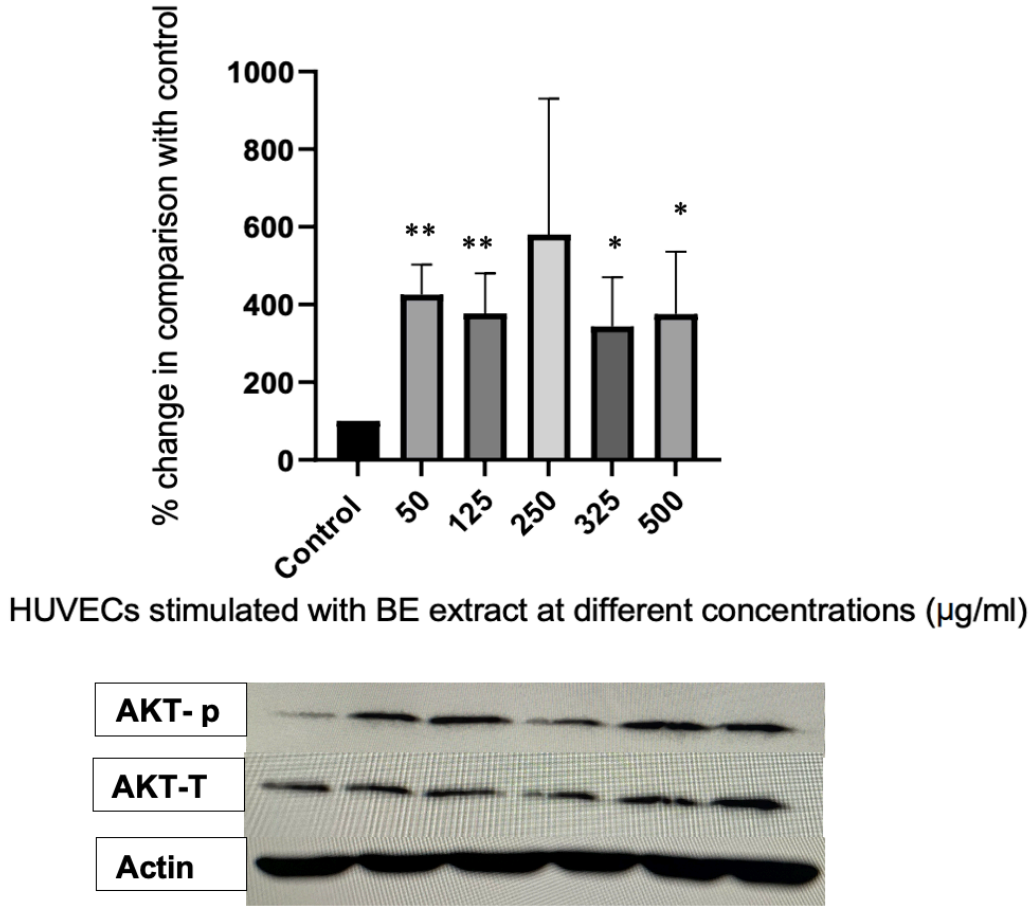

Figure 2. Western blot, concentration course (50, 125, 250, 325, and 500 µg/ml) at 1-hr time point to determine the optimal dosage. Significant value was set at * = 0.05 and ** = 0.01.

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PC05

The effects of emetine based anti-malarial leads on calcium wave dynamics in sheep ventricular myocytes.

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The emergence of anti-malarial drug resistance poses a major threat to world health and necessitates a need to develop new anti-malarial agents. These new anti-malarial agents could arise through the process of drug repositioning. Work by Matthews *et al*., (2017) and Panwar *et al*., (2020) demonstrates the anti-amoebic drugs Emetine dihydrochloride (EDC) and Dehydroemetine (DHE) are effective anti-malarial compounds. However, both are associated with cardiotoxicity including arrhythmogenesis (DHE less so), which may compromise their clinical use. To elucidate the cellular basis of this cardiotoxicity, our previous work showed that only DHE produced sustained negatively inotropic alterations to Ca dynamics, but *spontaneous* Ca waves were fewer than in cells exposed to EDC. However, both transiently (1-2 beats) increased systolic Ca on application; indicative of ryanodine receptor (RyR) potentiation. To investigate this further, we next investigated the effects of EDC and DHE on induced intracellular Ca waves.

All animals were killed in accordance with the Home Office Animal (Scientific Procedures) Act 1986. Sheep ventricular myocytes were loaded with the ratiometric Ca indicator Fura-2 then intracellular Ca waves induced using 0.3 mM ouabain octahydrate and 5 mM extracellular Ca. The effects of EDC (50 nM) or DHE (80 nM) were then measured using epi-fluorescent photometry. To estimate SR Ca threshold for waves, we measured the amplitude of caffeine (10 mM) evoked Ca transients on wave onset.

DHE decreased Ca wave amplitude by 25 ± 6 % (n=20, p = 0.004) and increased wave frequency by 10 ± 4 % (n = 20, p = 0.02). DHE also decreased SR threshold Ca content for waves (Control (n = 9): 0.93 ± 0.15, DHE (n = 9): 0.49 ± 0.12, p = 0.03). EDC decreased Ca wave amplitude by 19.83 ± 3 % (n = 21, p = <0.001) but had no effect on wave frequency or SR Ca threshold.

These data suggest DHE potentiates the RyR; a phenomenon associated with increased risk of arrhythmia. This contradicts our previous observation that DHE produces fewer *spontaneous* Ca waves. It may be that the negatively inotropic effects of DHE offset those on the RyR reducing net arrhythmogenicity. Certain evidence suggests that to an extent, EDC also potentiates the RyR.

However, that we observe no negatively inotropic effects may explain why this drug produces more *spontaneous* Ca waves, providing a substrate for reported arrhythmia risk.

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PC06

Stable Expression of VEGF-A in C2C12 Muscle Cells and Angiogenic Potential

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Rationale: Angiogenesis is where new blood vessels form from pre-existing vessels. With adequate oxygen transport and waste removal essential for tissue homeostasis, restrictions in blood supply can lead to ischaemia. Ischaemia due to defective angiogenesis in muscle can contribute to disease pathology in conditions such as atherosclerosis, peripheral artery disease or genetic conditions such as muscular dystrophy. Therapeutic angiogenesis is the co-ordinated delivery of vascular growth factors with the aim of promoting blood vessel growth to re-establish blood flow. Vascular endothelial growth factor (VEGF) is paramount for the induction of angiogenesis, while also playing a role in the promotion of myogenesis, having been shown to stimulate muscle repair following injury, making it an ideal candidate as an angiogenic (and myogenic) stimulant in muscle [1] . The C2C12 immortal cell line consists of isolated murine skeletal muscle cells. Stable transfection, unlike in transient transfection, is where DNA is introduced into a cell line long term, passing the introduced DNA onto their progeny.

Hypothesis: VEGF-A expressing C2C12 muscle cells exert a pro-angiogenic effect potentiating therapeutic angiogenesis in muscle disease.

Objectives: To establish a muscle cell line stably expressing VEGF-A and to determine its potency in eliciting an angiogenic effect in the chick chorioallantoic membrane (CAM) model of angiogenesis.

Methodology: C2C12 cells were transfected with VEGF-GFP and GFP plasmids respectively, and then underwent antibiotic selection to isolate separate high and low expressing cell lines for each transgene. GFP was included to visually inspect for transfection efficiency. ELISA determined VEGF-A concentration secreted from stable cells into cell media and a CAM filter disc assay was used to investigate the angiogenic effect of the C2C12 conditioned media. A CAM Matrigel assay was employed to explore the angiogenic effects the different cell lines directly. Angiogenesis was quantitated using a standard stereological scoring method applied to images of the CAMs [2] .

Findings: Herein, we successfully have characterised VEGF-A expression and the angiogenic potential of stably transfected C2C12 mouse myoblast cells using the CAM assay. Values are expressed as means ± S.E.M., compared by ANOVA. VEGF-A stable transfected C2C12 cells secreted significantly increased amount of VEGF-A, compared to GFP transfected alone (VEGF-A: 10198 ± 230.3 pg/mL vs GFP: 1081 ± 30.9 pg/mL, $p<0.01$). Conditioned media from cells produced a significant increase in angiogenesis in the CAM filter disc assay (Non-transfected: 15.4 ± 1.5; GFP: 15.5 ± 1.8; VEGF: 23.9 ± 2.1; $p<0.01$) (**fig. 1**). The CAM Matrigel assay determined that stable transfected C2C12 cells secreted VEGF-A which produced an angiogenic response when applied directly to the CAM assay (GFP: 6.3 ± 2.1 vs VEGF: 15.0 ± 1.2; $p<0.01$). In summary, C2C12 cells were successfully stably transfected to secrete elevated levels of VEGF-A and are proven to elicit a pro-angiogenic effect in the CAM model of angiogenesis. These studies qualify the potential use of a genetically modified muscle cell line in therapeutic angiogenesis for the treatment of muscle disease associated with vascular defects.

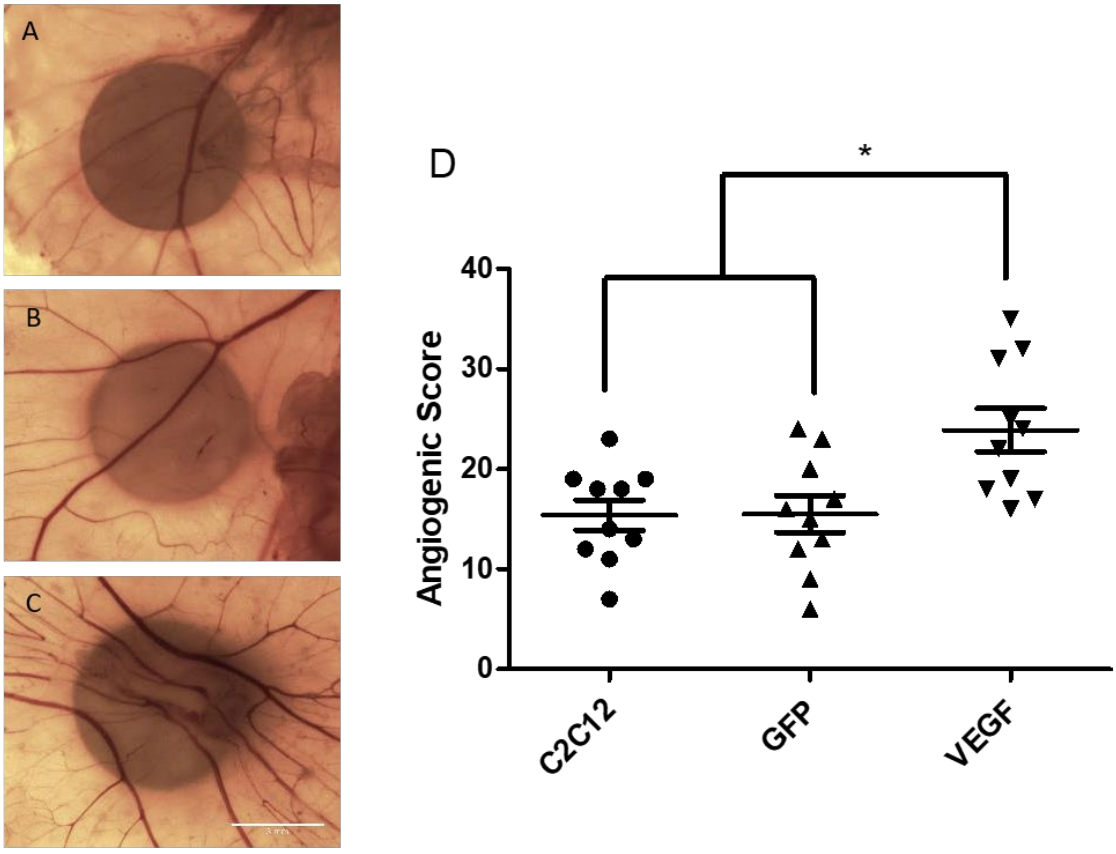

Figure 1. Angiogenic response of CAM following application of filter discs treated with conditioned media. Representative images of filter paper discs treated with 40 µL of conditioned DMEM media taken from (A) non-transfected (B) GFP and (C) VEGF-GFP transfected C2C12 cells following 72 hours incubation. Images taken at 16x magnification. Scale bar=2 mm (D) Angiogenic score of filter discs. Error bars indicate± SEM with n=10. *$p<0.01$.

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PC07

Regional thermal hyperaemia in the human leg: insight into the role of thermosensitive mechanisms in the control of the peripheral circulation

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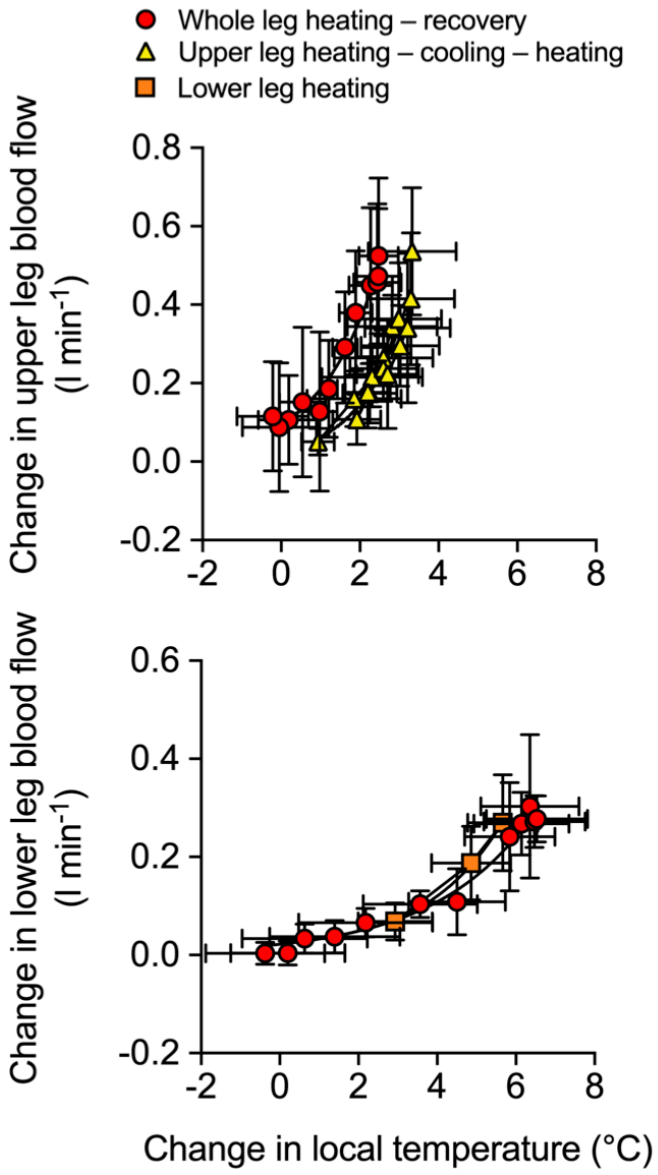
Hyperthermia is thought to increase limb tissue blood flow through the activation of thermosensitive mechanisms within the limb vasculature (1). However, the precise vascular locus in which hyperthermia modulates perfusion, specifically in the different segments of the human leg, remains elusive. We tested the hypothesis that temperature-sensitive mechanisms alter limb haemodynamics by regulating microvascular blood flow. **Methods**: A cohort of healthy males and females (30±12 years; mean ± SD) participated in three protocols: (a) 3 h of whole-leg heating followed by 3 h of recovery (n=9); (b) 1 h of upper-leg heating followed by 30 min of cooling and 1 h bout of upper-leg heating (n=8) and (c) 1 h of lower-leg heating (n=8). Leg haemodynamics of the common (CFA), superficial (SFA) and profunda (PFA) femoral arteries and popliteal artery (POA) of the experimental and control leg were measured at regular intervals (every 10–-30 min depending on the protocol) throughout protocols using a duplex Doppler ultrasound system. Furthermore, temperature and oxygenation profiles of the experimental and control leg were measured continuously throughout the protocols. Two-way repeated measures ANOVA tests were performed to investigate differences in haemodynamics, flow profiles and temperature between and within the experimental and control leg/leg segment over time. Additionally, regression analysis was performed to assess the relationship between various key data. **Results**: Whole-leg heating increased experimental average leg temperature (T_{Leg}) by 4.2±1.2ºC and blood flow in the CFA, SFA, PFA and POA by ≥3-fold, whilst core and control-leg temperatures and haemodynamics remained stable. Upper- and lower-leg blood flow increased by 525±199 and 278±49 ml·min^{-1} (p<0.0001), respectively, in direct response to the rise in local T_{Leg} (R^2=0.95; p<0.0001) and then declined during recovery. Furthermore, upper-leg heating increased upper T_{Leg} by 3.3±1.1 ºC (p<0.0001) and upper-leg blood flow by 536±162 ml·min^{-1} (p<0.0001), without any changes to lower T_{Leg} tissue oxygenation or blood flow. These are comparable to whole-leg heating responses. Conversely, lower-leg heating increased lower T_{Leg} and blood flow—5.7±0.9ºC and 270±98 ml·min^{-1} (p<0.0001)—without altering upper T_{Leg}, tissue

oxygenation or skin and PFA blood flow. Neither of three protocols resulted in changes to systemic haemodynamics, perfusion pressure or conduit artery diameter across all vessels (p>0.1). Throughout all three protocols, changes in blood flow were directly related to changes in local T_{Leg} (R^2=0.97; p<0.0001) (Fig. 1). **Conclusion**: Findings demonstrate that whole-leg hyperthermia induces profound and sustained elevations in upper- and lower-limb blood flow and that segmental hyperthermia matches the regional thermal hyperaemia, without causing thermal or haemodynamic alterations in the non-heated limb segment. These observations support the notion that heat-activated haemovascular mechanisms in the microcirculation regulate limb tissue perfusion during hyperthermia. Importantly, the markedly enhanced hyperaemia and tissue oxygenation lend support to the therapeutic potential of local hyperthermia for treatment of circulatory diseases.

Figure 1. Close curvilinear relationships between the change in local temperature ($\overline{T}_{Leg}$) and the change in local blood flow during whole-leg, upper-leg, and lower-leg and foot heating. Vertical error bars signify Δ blood flow SD, whilst horizontal error bars signify $\Delta\,\overline{T}_{Leg}$ SD, respectively.



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PC09

MORINGA OLEIFERA CYCLOTIDE FRACTION AMELIORATES DOXORUBICIN INDUCED CARDIAC DYSFUNCTION IN ADULT MALE ALBINO WISTER RATS

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Background: Cardiovascular disease (CVD) remains the leading cause of death globally. In many cases, a leading cause of morbidity and mortality in patients receiving chemotherapy has been cardio-toxicity. Anthracyclines are widely used as chemotherapeutic agent and is now part of standard therapeutic regimens for a variety of cancers. Anthracyclines' clinical use however is limited by its induced cardio-toxicity. Although a variety of approaches to protect the heart against Anthracycline-induced cardio-toxicity is currently part of clinical practice but the mortality rate of such patients is still high. The search for plant derived anti-oxidant peptides is on the increase. Such alternatives may be safer than other antioxidant as they are from food sources and have been consumed over the years without side effects.The present study was thus designed to evaluate the cardioprotective effect of Moringa Oleifera cyclotide (MOC) in a rat model of doxorubicin (DOX) induced cardiomyopathy

Method: MOC fraction was obtained by solvent extraction and peptide purification. Male adults albino Wister rats (48) weighing between 180-200 g were randomly divided into six groups (n=8). Animal experimentation lasted for 17 days. Group 1 animals served as control group and were untreated. Group 2-6 animals were induced with cardiomyopathy by Intra-peritoneal injection of DOX (5 mg/Kg) at day 15. Group 4 was pre-treated on day 15 with dexamethasone (3mg/kg) 20 minutes before DOX treatment. Group 3-6 were orally pre-treated with MOC 10, 20, 40 mg/kg respectively for 15 days. Cardiovascular measurements and animal sacrifice were done 48 hours after DOX treatment. plasma and tissue samples were obtained for biochemical, histological and molecular assays.

Result: Rats treated with doxorubicin alone (Group 2) showed high LF/HF ratio, increased blood pressure, cardiac hypertrophy, disseminated congestion of cardiac tissue, raised homocysteine, cardiac troponin I and KIM-1 levels when compared with control. However, MOC pre-treatment especially at 10 mg/Kg ameliorated the adverse DOX induced cardiac dysfunction which was comparable with animals pre-treated with dexamethasone.

Conclusion: MOC exhibits cardio-protective effects against doxorubicin induced cardiac dysfunction in male adult albino Wister rats.

PC10

GENDER DIFFERENCES IN CARDIOTOXICITY AND DYSLIPIDEMIA INDUCED BY CADMIUM EXPOSURE IN WISTAR RATS

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BACKGROUND: Sex and gender differences have been documented to influence cardiovascular diseases with an impact on disease management. Cadmium is a ubiquitous environmental toxicant that has been suggested to be a cardiovascular disease risk factor based on scientific evidence. This study investigated the gender difference in cardiotoxic and dyslipidemic effect associated with cadmium as there are dearth of scientific information in this regards.

METHODS: Ten male and ten female Wistar rats (150-170g body weight) were fed with standard rat feed and water ad libitum under a condition of 12/12 hour light/dark cycle daily. The study was approved by the Bowen University Ethical committee with the number BUTH/REC-103. The rats were acclimatized for 7 days before subjecting them to different treatments and were treated for 21 days as follows (n=5): Control – Male; Cadmium chloride - Male (5mg/kg, p.o); Control – Female; Cadmium chloride – Female (5mg/kg, p.o). After the days of administration, rats were sacrificed by cervical dislocation under light ether. Blood and tissue samples were collected for estimation of tissue injury biomarkers, lipid peroxidation (MDA) and antioxidant, lipid profile and lipoprotein lipase according to standard laboratory procedures. Values are mean ± S.E.M, compared with ANOVA using Graph pad prism5.

RESULTS: The results showed that creatinine kinase myocardial band (CK-Mb) and lactate dehydrogenase increased significantly in both gender as compared with their control (18.29 ± 2.5 vs 43.47 ± 1.2, $p<0.001$; 36.57 ± 8.012 vs 82.71 ± 10.72, $p<0.05$) . However, it increased significantly more in female than in male (43.47±1.2 vs 82.71 ± 10.72, $P<0.05$). Lipid peroxidation (MDA) increased in both gender but significantly in female compared with control (4.875 ± 0.07327 vs 6.176 ± 0.3531, $P<0.05$). Superoxide dismutase (SOD) reduced significantly in both treated groups compared with control (1.368 ± 0.07 vs 0.75 ± 0.06, $P<0.0001$; 1.15 ± 0.05 vs 0.75 ± 0.09, $P<0.05$) but no significant difference between male and female. No significant changes in catalase, glutathione and glutathione peroxidase. Total cholesterol increased in both male and female treated groups (110.6 ± 3.113 vs 138.2 ± 8.298, $P<0.05$; 124.7 ± 11.94 vs 162.4 ± 7.347, $P=0.05$). Although not significant, the value was higher in female than in male. Triglyceride increased significantly only in male compared with control (57.33 ± 1.704 vs 87.60 ± 11.45, $P=0.05$). There was no significant changes in low density lipoprotein across the groups, though the value increased in female by 17.8% compared with control (65.71 ± 7.874 vs 79.96 ± 5.553) and by 20.1% compared with male (63.82 ± 4.9 vs 79.96 ± 5.5). The high density lipoprotein and lipoprotein lipase increased significantly in the treated groups compared with their controls and not between male and female groups.

CONCLUSION: Cadmium induced oxidative damage in the heart by enhancing membrane lipid peroxidation and also altered plasma lipid profile. Furthermore, the findings suggest that female gender is more susceptible than the male gender to cardiac oxidative stress and dyslipidemia associated with cadmium exposure.

PC11

ACUTE SALT LOADING MODULATES HEMODYNYMIC RESPONSE TO POSTURAL CHANGE AND EXERCISE IN MALE STUDENTS OF A NIGERIAN UNIVERSITY

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Background: Dietary salt consumption has been shown to alter fluid and electrolyte balance and plays important role in arterial blood pressure regulation. In this study, we hypothesized that short-term salt loading would impair blood pressure regulation, blood and urinary electrolyte levels, and urinary volume after exercise and postural adjustments in young Nigerian males.

Method: Eight (8) healthy male subjects between the age of twenty to thirty one (20-31) years who were recreationally active and non-smokers were recruited for 5 days. Anthropometric data were taken while blood pressure and heart rate readings were recorded at rest and after postural changes and exercise on the first day before salt load, and on the last day after salt load respectively. Blood and urinary samples were collected on the first day and last day before and after salt loading were analyzed using two analytical methods (flame photometry and indirect ion-selective electrode potentiometry). Data was analyzed using graph pad statistical software (version 5) and statistical significant was set at $p<0.05$.

Results: Data showed that moderate salt ingestion in healthy young males led to insignificant increases in SBP, DBP, MABP, HR, serum and urine electrolytes, and urine volume compared to baseline. Postural changes from supine to standing elicited progressive decreases in SBP, DBP, MABP, but increase in HR after salt loading. Exercise led to significantly increased SBP and MABP following salt loading, while DBP was unchanged. However, HR was increased after exercise compared with before salt load and exercise.

Conclusion: The present results demonstrate that short-term salt ingestion elicits slight increases in hemodynamic and urinary parameters in young healthy adults. Short-term salt loading shows the propensity to increase arterial blood volume and trigger sympathetic response to regulate blood flow following postural change and during exercise.

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PC12

Simulation of acute doxorubicin cardiomyopathy in Wistar rats

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In the conditions of successful anticancer treatment, complications associated with its toxic effect on healthy tissues and organs began to come to the fore. Anthracycline drugs (doxorubicin (DOX), epirubicin, idarubicin) are widely used in oncological practice to treat a wide range of solid tumors and hematological malignancy. This determines the need to search for mechanisms that implement the development of those effects of chemotherapy drugs that have not been previously encountered. To solve these problems, new models are needed that could reflect these effects. The aim of the work was to develop an optimal model of doxorubicin cardiomyopathy with fibrotic lesion and distant development of diastolic dysfunction in Wistar rats. The study was carried out on 35 male Wistar rats, weighing 393 ± 38 g. The experiments were carried out in compliance with the principles of humane treatment of animals, regulated by the requirements of the European Convention on the maintenance, feeding and care of experimental animals. Throughout the study, animals were weighed and echocardiography recorded (left ventricular internal dimension / LVID /, thickness of the anterior and posterior LV walls during diastole, the shortening fraction / FS). The administration of DOX for groups A (15 mg / kg) and C (20.4 mg / kg) was performed 6 times every other day. In group B (25 mg / kg) DOX was administered 10 times every day. The control group was injected with 1 ml of 0.9% sodium chloride solution 6 times every other day. At the time of the end of the study, under the conditions of inhalation anesthesia (isoflurane), cardiac arrest was performed using KCl solution, after which vital organs were taken. The Kruskal-Wallis test was used to assess the significance of differences. ECHO results in group A on day 13 showed a significant decrease in FS (Before - 54.5 ± 5.9%; After - 37.5 ± 5.5%; *** - $p \leq 0.001$) and LVIDs (Before - 3, 1 ± 0.53 mm; After - 4.1 ± 0.37 mm; *** - $p \leq 0.001$). Histological examination of the heart revealed capillary plethora in all groups, which was most pronounced in group B. Also in this group there is a partial loss of myofibrils of cardiomyocytes. In group A, there is moderate venous congestion in the liver, dilated spaces of Disse. The capillaries of the kidney medulla are dilated and full-blooded. In groups B and C, venous plethora, necrosis of hepatocytes, diffuse disturbance of the structure of the hepatic tracts, and foci of hydropic dystrophy are noted in the liver. Group B and C has venous plethora of the

cortical and medulla of the kidneys, moderate tubular atrophy and necrosis of individual epithelial cells. The results obtained indicate that the model of administration with a total dose of DOX from 20 mg / kg is suitable for the assessment of acute cardiotoxicity. It can be assumed that a total dose of 15 mg / kg is suitable for the assessment of chronic cardiotoxicity. In this regard, further research will be carried out.

PC13

Effect of menopause on cerebral blood flow and cerebrovascular function: Systematic review and meta-analysis

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Background : Menopause and its associated decline in oestrogen is linked to chronic conditions like cardiovascular disease and osteoporosis, that may be difficult to disentangle from the effects of ageing. Further, post-menopausal women exhibit increased cerebrovascular disease risk, linked to declines in cerebral blood flow (CBF) and cerebrovascular reactivity (CVR), yet the direct understanding of the impact of the female menopause on cerebrovascular function is unclear. The aim of this systematic review and meta-analysis was to examine the available literature investigating CBF and CVR in pre- compared with post-menopausal women.

Methods : Five databases were searched for cross-sectional studies assessing CBF or CVR in pre-and postmenopausal women. Meta-analysis examined the effect of menopausal status on middle cerebral artery velocity (MCAv), and GRADE assessed evidence certainty.
Results : Nine studies (n=504) included cerebrovascular outcomes. Six studies (n=239) reported negligible differences in MCAv between pre- and post-menopausal women [2.11cm/s (95% CI: -8.94 to 4.73, p=0.54)], but with a “low” certainty of evidence. CBF was lower in post-menopausal women when MCAv was expressed as cerebrovascular conductance. CVR was lower in post- compared with pre-menopausal women in two of three studies, but high-quality evidence is lacking. Across outcomes, study methodology and reporting criteria for menopause were inconsistent.

Conclusions : MCAv was similar in post- compared with pre-menopausal women. Methodological differences in characterizing menopause, and inconsistent reporting of CBF outcomes make current comparisons difficult. Comprehensive assessments of cerebrovascular function of the intra- and extracranial arteries to determine the physiological implications of menopause on cerebral with healthy ageing is warranted.

PC14

Acetate supplementation improves Dyslipidemia and Cardiac aconitase activity in oral contraceptive-treated insulin-resistant Dams

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Cardiometabolic disease is associated with energy dysregulation. Short chain fatty acids have shown potential to improve insulin resistance-associated disturbances. We hypothesize that acetate would enhance key cardiac metabolism enzymes in combined oral contraceptive (COC)-treated dams with insulin resistance.

Female rats (130-150g) were mated to achieve timed pregnancy and delivery. Twenty-four (24) dams were randomly grouped into vehicle *(p.o)*, fructose exposed dam (FDAM), FDAM + COC, FDAM + acetate (ACE) and FDAM + COC + ACE groups (6rats/group). The groups received distilled water (vehicle, *p.o.*), 10% Fructose (w/v) , 10% Fructose plus 1.0 µg ethinylestradiol plus 5.0 µg levonorgestrel (*p.o.*), and 10% Fructose plus COC + ACE [200mg/kg *bw* (*p.o.*)] respectively. Pregnant rats were exposed to fructose drink daily for 19 days. After weaning, dams received COC 3rd and 6th weeks postpartum. After anaesthetisia with pentobarbital sodium (50 mg/kg, *ip*), blood sample was collected via cardiac puncture and cardiac tissue was harvested for biochemical assays. The Use of Laboratory Animals and was approved by the Institutional Ethical Review Board of University of Ilorin. All data were expressed as means ± SEM and significance were accepted at $p<0.05$ and $n=6$.

Data show that postpartum COC administration in fructose-exposed dams worsened insulin resistance, plasma and cardiac lipid deposition, hyperuricemia. IR phenotype in this model is accompanied with decreased high density lipoprotein-cholesterol level, pyruvate dehydrogenase and aconitase activities in the heart. However, supplementation with acetate improved insulin resistance-related disturbances and enhanced cardiac pyruvate dehydrogenase and aconitase activities in COC-treated dams with insulin resistance.

Our findings suggest that cardiac dysfunction caused by postpartum COC treatment in fructose-exposed insulin-resistant dams is associated with dyslipidemia, cardiolipotoxicity and impaired key mitochondrial enzymes in the heart. Acetate supplementation however shows plausible ameliorative potential against postgestational COC-worsened IR-related cardiac dysfunction.

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PC15

ASSESMENT OF OSMOTIC FRAGILITY IN DIFFERENT BLOOD GROUPS AMONG ABU ZARIA STUDENTS

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Several blood group systems have been discovered but amongst the most important is the ABO group. Landstainer demonstrated four groups according to antigens available A, B, AB and O and showed that an individual possess antibodies against those antigens he lacked on the red cell. Several other red cell blood groups and hence antigens have been shown but the rhesus group is another of utmost importance. Cells which have Rhesus antigen on their surface are described as Rhesus positive while those without this rhesus antigen are Rhesus negative. Osmotic fragility is a test to measure red blood cell (RBC) resistance to hemolysis when exposed to a series of increasingly dilute saline solutions. Osmotic fragility tests are based on the measure of red blood cell lysis as a function of osmotic stress. When erythrocytes are placed in hypotonic solutions, they begin to take on water osmotically. This results in swelling of the cell until the critical volume is reached, afterward the membrane at first leaks and then bursts releasing hemoglobin. This study evaluated the comparison of blood groups and osmotic fragilities under different concentrations of saline solution respectively, the blood group that has the highest frequency of haemolyses is considered most osmotically fragile. The purpose of this study is to aid in diagnosis of disease with red cell membrane abnormality by providing which blood group is more fragile osmotically and also to add to the general knowledge in medical physiology. An ethical clearance was sought from Ahmadu Bello University Teaching Hospital Ethical Committee on Human Research. Seventy blood samples from different subjects were used which includes different ethnic groups, age and sex. Using a 5ml syringe, 2ml of blood were collected and the experiment was carried out in the laboratory and both the blood group and osmotic fragility were determined. This study showed the mean value and standard deviation of 0.4694 ± 0.11909, 0.6050 ± 0.11459, 0.4917 ± 0.09003 and 0.7000 ± .14142; and percentage haemolysis of 36%, 20%, 12% and 2% for O, A, B, and AB groups respectively and the groups were compared using analysis of variance (ANOVA) followed by Turkey's *post hoc* test. The result in comparison shows that blood group O is more fragile osmotically followed by B, A then AB is least fragile which was statistically significant at p value of 0.000. Therefore, this result will help providing information in medical practitioners in blood maintenance and diagnosis of patients with abnormal red cell membrane.

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PC16

Effects of diabetes mellitus and obesity on the electrical conduction system of the heart in the diabetic and fatty Zucker rat.

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Introduction: Obesity is a major risk factor for type 2 diabetes mellitus. Cardiovascular disease is the major cause of morbidity in diabetic patients. The electro-mechanical function of the heart is frequently compromised in diabetic heart. Few studies have investigated the effects of obesity and obesity/diabetes (diabesity) on the electrical conduction system of the heart.

Aims & Objectives: *In vivo* biotelemetry techniques were used to evaluate the effects of obesity and diabesity on the electrical conduction system of the heart in the Zucker lean (ZL), Zucker fatty (ZF) and Zucker diabetic fatty (ZDF) rat.

Methods: Experiments were performed in ZL, ZF and ZDF rats (Charles River, UK). Heart biopotential, physical activity and body temperature were monitored using an *in vivo* biotelemetry system (Data Sciences, USA). The transmitter devices were surgically implanted in 30 rats (10 ZL, 10 ZF, 10 ZDF) aged 75 days under general anesthesia. The transmitter devices were inserted into the peritoneal cavity and the electrodes from the devices were arranged in Einthoven Lead II configuration (right foreleg and left hindleg). Data recording was continued for a period of 4 months. Data are presented as mean ± SEM. Statistical analysis was performed using Two way repeated measures ANOVA and Independent and Paired samples t Test as appropriate. Ethical approval for the project was obtained from the Animal Research Ethics Committee, UAE University.

Results: At the end of the biotelemetry experiments ZL, ZF and ZDF rats body weights were (429.0±16.0 g, n=11), (725.3±18.5 g, n=12) and (608.6±45.8 g, n=13), respectively and fasting blood glucose values were (92.3±2.2 mg/dl, n=15), (131.3±3.8 mg/dl, n=16) and (208.3±13.9 mg/dl, n=16), respectively. Heart rates (HRs) were significantly ($p<0.05$) lower in ZDF (265±8 bpm, n=10) compared to ZF (336±9 bpm, n=10) and ZL (336±10 bpm, n=10). Results also showed significant differences in heart rate variability (HRV) between ZDF (22±1 bpm, n=10), ZF (27±1 bpm, n=10) and ZL (31±1 bpm, n=10) rats. Analysis of the electrocardiogram revealed a progressive age-dependent prolongation of PQ and QRS intervals in all 3 groups of rats. At 105, 135, 165 and 195 days (3.5, 5.5 and 6.5 months) of age the QRS interval was significantly prolonged in ZDF compared to ZF rats. Power spectral

analysis revealed no significant (p>0.05) differences in HRV at low frequencies, reduced HRV at high frequencies and increased sympathovagal balance in ZDF compared to ZF and ZL rats.

Conclusions: HR was reduced by aging and additionally reduced by diabesity in the absence of changes in physical activity and body temperature. Reductions in HRV associated with altered sympathovagal drive might partly underlie disturbed HR in the ZDF rat.

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PC17

The Middle Cerebral Artery Blood Velocity Response to Moderate and Heavy Intensity Cycling In Healthy Adults

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The kinetic response of the middle cerebral artery blood velocity (MCAv) response to steady-state moderate-intensity exercise on a recumbent stepper has been modelled previously in healthy adults, using a mono-exponential function with a time delay. However, no data exist regarding the MCAv kinetic response to whole body upright cycling, nor heavy-intensity exercise, which may require a more complex approach due to hyperventilation-induced hypocapnia. Data from incremental exercise suggest that moderate-intensity exercise elicits the greatest increases in MCAv, though this has yet to be explored during constant work rate exercise. The aim of this study was to compare the amplitude and time-based parameters of the MCAv response to moderate and heavy-intensity cycling in adults using an exponential model. Seventeen healthy adults (23.8±2.4 years, 8 males) completed a ramp incremental test to exhaustion on a cycle ergometer to determine maximal oxygen uptake and the gas exchange threshold (GET). Participants then completed three 6-minute transitions at a moderate-intensity (90% GET) and three 6-minute transitions at a heavy-intensity (40%Δ) in a counterbalanced order, all on separate visits. Bilateral MCAv was measured throughout using transcranial Doppler ultrasonography. MCAv mean data were exported as 1-sec averages and time aligned to exercise onset. For each participant, left and right MCAv data from each corresponding repeat transition were obtained and pooled, resulting in six sets of MCAv data per person that were subsequently averaged. The MCAv response to each intensity were analysed using a mono-exponential model with a time delay for each participant. Differences in kinetic parameters between moderate and heavy-intensity exercise were explored using paired t-tests. Baseline MCAv before each trial was similar between intensities (69.4±9.1 vs. 70.8±8.4 $cm \cdot s^{-1}$, P=0.16). At exercise onset, consistent, rapid fluctuations in MCAv were observed within both intensities for ~25 s, before MCAv increased in an exponential-like fashion. The time constant of the MCAv kinetic response was similar between moderate- and heavy-intensity cycling (25.4±9.7 vs. 26.0±7.7 s, P=0.82), as was the time delay (29.3±11.3 vs. 29.1±9.6 s, P=0.95). The amplitude of the MCAv increase from baseline was

significantly greater during heavy (23.9±10.0 $cm \cdot s^{-1}$, 34.1±14.4%) compared to moderate (12.7±4.4 $cm \cdot s^{-1}$, 18.7±7.5%) intensity cycling (both P<0.01). After attaining a peak or steady state, MCAv decreased as the exercise bouts progressed within each intensity. This occurred significantly earlier during heavy intensity exercise (164±43 vs. 248±90 s, P<0.01), and MCAv decreased by a significantly greater magnitude during heavy intensity exercise, compared to moderate (9.5±6.9 vs 2.8±3.8 $cm \cdot s^{-1}$, P<0.01). Nevertheless, MCAv at the end of the bout remained elevated during heavy intensity exercise, compared to moderate (85.2±9.6 vs. 79.3±7.7$cm \cdot s^{-1}$, 21.1±12.7% vs. 14.9±7.8%, P≤0.01). These novel findings indicate that the MCAv kinetic response to moderate and heavy intensity cycling in adults can be modelled using an exponential function, and revealed that increases in MCAv are greater during constant work-rate heavy intensity cycling, compared to moderate, but that the time constant and time delay of the responses are similar between intensities. These novel analysis techniques form an important area to explore cerebrovascular responses and regulation during exercise.

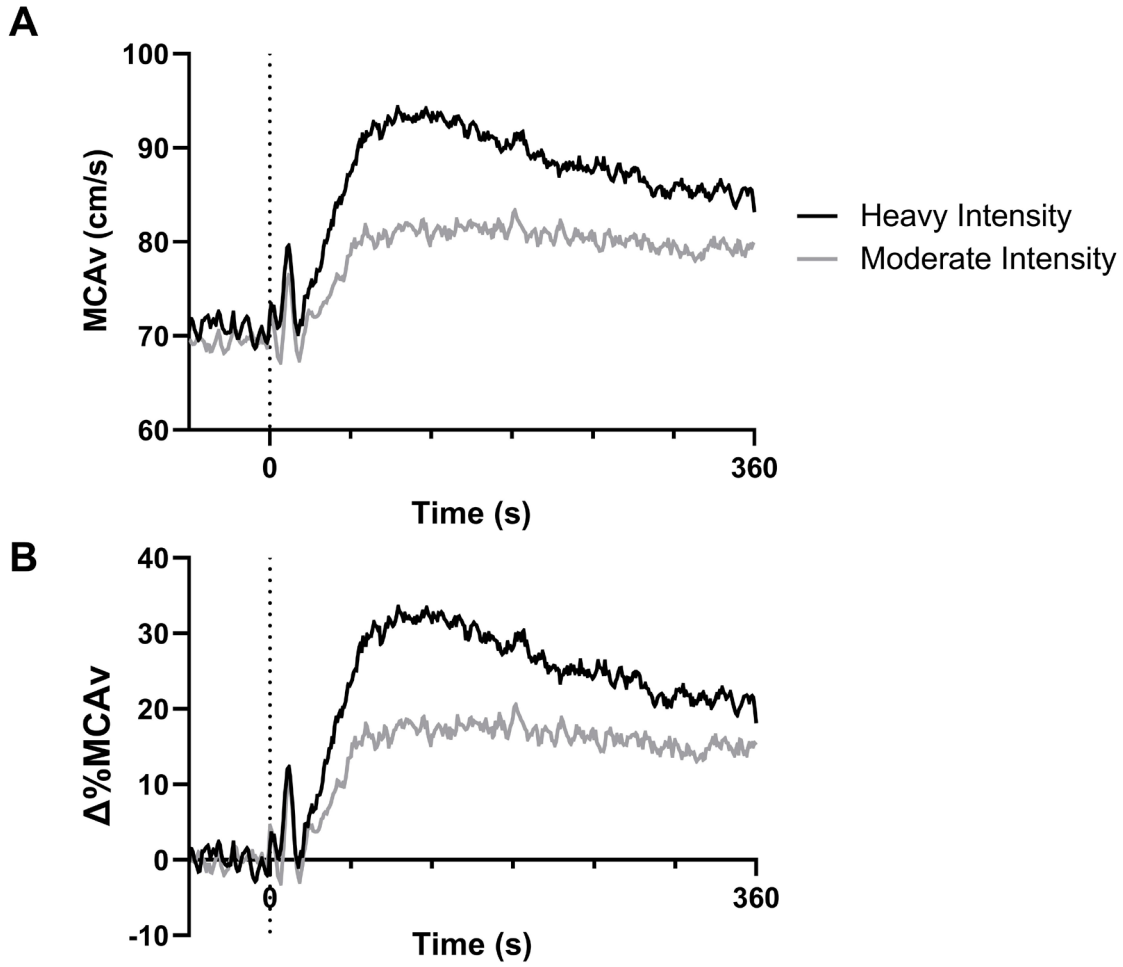

Fig 1. The absolute (A) and relative (B) MCAv response to moderate and heavy intensity cycling exercise in adults (n=17). Dashed line indicates exercise onset.

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PC18

Differences in cerebrovascular regulation and ventilatory responses during ramp incremental cycling in children, adolescents and adults

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Regulation of cerebral blood flow during exercise in youth is poorly understood. This study investigated the cerebrovascular and ventilatory responses to a ramp incremental cycle test to exhaustion in 14 children (mean ± SD age: 9.4±0.9 y), 14 adolescents (12.4±0.4 y) and 19 adults (23.4±2.5 y). Middle cerebral artery blood velocity (MCAv), partial pressure of end-tidal CO_2 ($P_{ET}CO_2$) and ventilatory parameters were analysed at baseline, gas exchange threshold (GET), respiratory compensation point (RCP) and exhaustion. Ventilatory efficiency was calculated as the increase in minute ventilation relative to CO_2 production during exercise (V_E/VCO_2 slope). Relative change from baseline (Δ%) in MCAv was lower in children, compared to adolescents and adults at GET (15±10% vs 26±14% and 24±10%, respectively, P≤0.03, effect size (*d)*=0.9) and RCP (13±11% vs 24±16% and 27±15%, respectively, P≤0.05, *d*≥0.8). Δ%MCAv was similar in adults and adolescents at all intensities, and similar in all groups at exhaustion. The magnitude of the V_E/VCO_2 slope was negatively associated with Δ%MCAv at GET, RCP and exhaustion across all participants (P≤0.01, r=-0.37 to -0.58). Δ%$P_{ET}CO_2$ was smaller in children and adolescents compared to adults at GET and RCP (P≤0.05, *d*≥0.6). In children, Δ%$P_{ET}CO_2$ and Δ%MCAv were not associated from baseline-GET ($\bar{r}$=0.14) and were moderately associated from RCP-exhaustion ($\bar{r}$=0.49). These relationships strengthened with increasing age, and were stronger in adolescents (baseline-GET: $\bar{r}$=0.47, RCP-exhaustion: $\bar{r}$=0.62) and adults (baseline-GET: $\bar{r}$=0.66, RCP-exhaustion: $\bar{r}$=0.78). This is the first study to compare cerebral blood flow responses during incremental exercise in children, adolescents and adults. Similar increases in cerebral blood flow were observed in adolescents and adults, with smaller increases in children. This study also provides the first evidence on the development of the regulatory role of end-tidal CO_2 on cerebral blood flow during exercise during the transition from childhood to adulthood.

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PC19

Data Acquisition Systems Improve Students' Learning of Physiology and Laboratory Experimentations

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Data acquisition (DAQ) systems are widely applied in Physiology teaching and research (1, 2). This study investigated the impacts of using the PowerLab system (ADInstruments) on learning and laboratory experimentations in Physiology by medical students. This study was carried out among 55 pre-clinical medical students of Nile University of Nigeria, Abuja. A pretested self-administered questionnaire was used to evaluate the impacts of using the PowerLab system on students' learning and laboratory experimentations in Physiology. The data were analysed using IBM SPSS Statistics (Windows) Version 22.0. Frequency distribution and summary statistics were analysed by descriptive analysis. Fifty-five (n=55) out of 90 pre-clinical students participated in this study. Fifteen (27.3%) respondents were males and 40 (72.7%) were females, with a mean age of 18.5 ± 1.0 years. More than 90% of the respondents are aware of the PowerLab system (n=51) and have used it to learn and conduct laboratory experiments (n=53). Majority of the respondents agreed that the use of the PowerLab system enhances their understanding of physiology experiments (n=50; 91%); stimulates imaginative and critical thinking skills (n=39; 71%); augments learning and comprehension of physiological concepts (n=45; 82%); boost engagement in learning and laboratory experimentations (n=47; 85.5%); and promotes active learning (n=50; 90.9%) amongst others. The use of the PowerLab system enhances pre-clinical medical students' learning and laboratory experimentations in Physiology. This DAQ system provides students with state-of-the-art hands-on exposure to current teaching and research trends in the medical sciences and is now indispensable tools for quality medical education.

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PC20

Application of Educational Technologies in Physiology Education amidst the COVID-19 lockdown: a Cross-sectional study of some Nigerian Universities

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Due to the emergence of the COVID-19 pandemic, numerous medical schools around the world, and a few in Nigeria shifted from conventional to virtual learning. Though online education is now a chief teaching alternative, new challenges about how to teach via this new normal are constantly evolving (1). This study explores the application of educational technologies in Physiology education amidst the COVID-19 lockdown among the few Nigerian universities that transited to online learning. An online descriptive, cross-sectional study was performed between July and September 2020. Present undergraduate medical students aged 17 years or older across five private-owned Universities who engaged in virtual learning formed the study population. A web-based questionnaire (Google forms) was served to participants through WhatsApp messenger. The questionnaire was established using validated questions from earlier published studies. Generally, out of 205 participants who responded (response rate = 70%), 190 responses were valid. Forty-eight (n = 48, 25.3%) participants were males, and 157 (82.6%) were in the age group of 17-20 years. During the nationwide lockdown period, all the participants (n = 190) from various medical-related disciplines engaged in learning Physiology online. Of the total, 78 (41.1%) were studying Bachelor of Medicine and Bachelor of Surgery (MBBS) program, 69 (36.3%) and 16 (8.4%) were studying Bachelor of Pharmacy (B.Pharm) and Bachelor of Science in Physiology (Majors; B.Sc.) respectively amongst others. Findings from the ownership of device(s) used for online learning revealed that the majority of the students (n = 172, 90.5%) and (n = 124, 65.3%) owned smartphones and laptops respectively, while only 4 (2.1%) and 1 (0.5%) used desktop computers and television respectively for online learning during the school closure. The popular platforms used by participants for online learning of Physiology were Zoom meeting (n = 161, 84.7%), WhatsApp (n = 135, 71.1%), Google Classroom (n = 117, 61.6%), while the least used was Microsoft Teams (n = 9, 4.7%). More than half (n = 99, 52.1%) of the participants were able to easily access the internet while at home; however, 48 (25.2%) students agree to the need for support in navigating online learning platforms. Regarding the assessment of participants after online lectures in Physiology, the popular assessment type used by students were test/quiz (n = 147, 77.4%), graded assignments (n = 108, 56.8%), and examinations (n = 55, 28.9%). The sensitization and training of students and faculty on e-learning and the use of existing educational technologies and learning platforms are important to improve the attitude and use of e-learning for Physiology education.

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PC22

Antidiabetic, anti-dyslipidemic and antioxidant effects of L-citrulline supplementation in high-fat diet- and dexamethasone-induced type-2 diabetes mellitus in Wistar rats (Rattus norvegicus).

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Introduction: Diabetes mellitus, once described as the epidemic of the 21st century affects over 415 million people globally. The beneficial effects of most conventional anti-diabetics wanes-off with time aside their various side effects. Newer and effective anti-diabetics with little or no side effect are therefore necessary. Scanty works exist on the effect of L-citrulline especially in the setting of type-2 diabetes mellitus (T2DM). Aim: This study aimed to determine the effect of L-citrulline in high-fat diet (HFD)- and dexamethasone-induced T2DM in male Wistar rats. Methods: Thirty male Wistar rats, 8-12 weeks old, weighing 200-250 grams were randomly assigned into six groups of five rats each. Group I received normal diet throughout the experiment, while T2DM was induced in groups II-VI with HFD for 21 days and dexamethasone intraperitoneally (1 mg/kg) for 7 days. After the induction, group III received metformin 100 mg/kg/day orally, while groups IV-VI received L-citrulline 200 mg/kg/day, 400 mg/kg/day and 800 mg/kg/day respectively for 21 days. Fasting blood glucose (FBG) level, lipid profile and some biomarkers of oxidative stress were determined. Results were expressed as mean±S.E.M and values at $P<0.05$ were considered statistically significant. Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (Approval No.: ABUCAUC/2020/71). Results: The FBG levels were significantly reduced by L-citrulline in a dose-dependent manner (192.5±3.4 mg/dL, 181.8±1.2 mg/dL and 174.8±2.8 mg/dL at 200, 400 and 800 mg/kg/day respectively). L-Citrulline markedly lowered the total cholesterol levels at 200 mg/kg (55.2±0.64 mg/dL), 400 mg/kg (57.8±1.19 mg/dL) and 800 mg/kg (63.1±1.50 mg/dL) compared to the diabetic control (149.8±2.68 mg/dL). There were similar decreases observed in the LDL and triglyceride levels. Significant elevations in the HDL levels were also seen at all doses of L-citrulline compared to diabetic control (24.6±1.1 mg/dL). Compared to the diabetic group (42.0±0.42 µmol/L), L-citrulline markedly decreased the MDA concentrations (20.7±0.81 µmol/L, 22.2±0.75 µmol/L and 22.1±0.39 µmol/L at 200, 400 and 800 mg/kg/day respectively). The depletions of SOD observed in the diabetic group (13.0±0.44 µmol/mg), was ameliorated by L-citrulline at all does especially at 800 mg/kg/day (23.7±0.78 µmol/mg; $P<0.001$). Similar increase was also recorded in the CAT activity at 800 mg/kg/day (14.6±0.54 ng/dL; $P<0.001$) compared to the diabetic group (10.8±0.41 ng/dL). The GSH level was augmented by L-citrulline at all doses, especially at 400 mg/kg/day (46.4 ± 0.53 µmol/mg protein; $P<0.001$), compared to the diabetic group (22.9±0.69 µmol/mg protein). Conclusion: L-Citrulline possesses a hypoglycemic, anti-dyslipidemic and antioxidant effects in HFD- and dexamethasone-induced T2DM in male Wistar rats.

Key words: Antioxidant, L-citrulline, anti-dyslipidemic, type-2 diabetes mellitus.

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Reference 4 :- N/A

Reference 5 :- N/A

Acknowledgements :- N/A

PC23

The Effects of Menstrual Cycle Phase on Exercise Performance in Eumenorrheic Women: A Systematic Review and Meta-Analysis

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Background: Concentrations of endogenous sex hormones fluctuate across the menstrual cycle (MC), which could have implications for exercise performance in women. At present, data are conflicting, with no consensus on whether exercise performance is affected by MC phase.

Objective: To determine the effects of the MC on exercise performance and provide evidence-based, practical, performance recommendations to eumenorrheic women.

Methods: This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Four databases were searched for published experimental studies that investigated the effects of the MC on exercise performance, which included at least one outcome measure taken in two or more defined MC phases. All data were metaanalysed using multilevel models grounded in Bayesian principles. The initial meta-analysis pooled pairwise effect sizes comparing exercise performance during the early follicular phase with all other phases (late follicular, ovulation, early luteal, mid-luteal and late luteal) amalgamated. A more comprehensive analysis was then conducted, comparing exercise performance between all phases with direct and indirect pairwise effect sizes through a network meta-analysis. Results from the network meta-analysis were summarised by calculating the Surface Under the Cumulative Ranking curve (SUCRA). Study quality was assessed using a modified Downs and Black checklist and a strategy based on the

recommendations of the Grading of Recommendations Assessment Development and Evaluation (GRADE) working group.

Results: Of the 78 included studies, data from 51 studies were eligible for inclusion in the initial pairwise meta-analysis. The three-level hierarchical model indicated a trivial effect for both endurance- and strength-based outcomes, with reduced exercise performance observed in the early follicular phase of the MC, based on the median pooled effect size (ES0.5 = − 0.06 [95% credible interval (CrI): − 0.16 to 0.04]). Seventy-three studies had enough data to be included in the network metaanalysis. The largest effect was identified between the early follicular and the late follicular phases of the MC (ES0.5 = − 0.14 [95% CrI: − 0.26 to − 0.03]). The lowest SUCRA value, which represents the likelihood that exercise performance is poor, or among the poorest, relative to other MC phases, was obtained for the early follicular phase (30%), with values for all other phases ranging between 53 and 55%. The quality of evidence for this review was classified as "low" (42%).

Conclusion: The results from this systematic review and meta-analysis indicate that exercise performance might be trivially reduced during the early follicular phase of the MC, compared to all other phases. Due to the trivial effect size, the large between-study variation and the number of poor-quality studies included in this review, general guidelines on exercise performance across the MC cannot be formed; rather, it is recommended that a personalised approach should be taken based on each individual's response to exercise performance across the MC.

PC24

Effects of honey on glycemic and hematological indices in lead-induced toxicity in male Wistar rats

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Introduction: Lead is an environmental toxicant known to cause glucose intolerance and hematological disorders through oxidative stress damage. Studies have reported the antioxidative potential of honey. However, the mechanisms of its antioxidative activity have not been fully elucidated.

Aim: This study was designed to investigate the effects of honey on glycemic and hematological indices in lead induced toxicity using male Wistar rats.

Methods: Twenty-five (25) Wistar rats were randomly divided into five (5) groups (n=5) as follow; group 1 served as control which received deionized water, group 2 served as lead acetate group (40 mg/kg), groups 3, 4 and 5 served as lead acetate groups co-administered with honey (1 ml/kg), vitamin C (100 mg/kg) and honey+vitamin C (1 ml+100 mg/kg) respectively. The treatments were orally administered for 28 days. The rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally, blood and organ samples were collected and thereafter the rats were

euthanized through cervical dislocation (Alemán-Laporte et al., 2020; AVMA, 2020). Body and liver weights, blood glucose and insulin concentration were determined using analytical weighing balance, glucose oxidase and ELISA methods respectively. Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) were determined using spectrophotometry procedure. Hematological indices were measured using auto-analyzer. The pancreas histology were assessed using hematoxylin and eosin staining technique. Data were analyzed using ANOVA and results expressed as mean ± S.E.M., at $p<0.05$.

Results: A significant increase was observed in body weight, liver weight, insulin concentration, SOD, CAT, GPx, TWBC, lymphocytes, monocytes, granulocytes, RBC count, HGB concentration and PCV in lead acetate groups co-administered with honey, vitamin C and both when compared to lead acetate only (Tables 1, 2&3) . However, fasting blood glucose decreased significantly in lead acetate groups co-administered with honey, vitamin C and both compared to lead acetate only (Table 1). Histological evaluation of the pancreas showed areas of necrotic lesions in lead acetate only compared to lead acetate groups co-administered with honey, vitamin C and both.

Conclusion: Honey exhibits its antioxidative potentials by increasing the activities of antioxidant enzymes, improving glycemic control and maintaining hematological profile in Wistar rats.

Keywords: Lead, Honey, Glycemic indices, Hematological indices, toxicity.

Ethical Standards: The study was approved by the Institution Review Board of the University of Medical Sciences, Ondo City, Nigeria and conducted according to the guidelines for the use of animal by the National Institute of Health.

Table 1. Glycemic indices in lead toxic rats co-administered with honey and vitamin C

Glycemic indices	Group 1	Group 2	Group 3	Group 4	Group 5
Body weights (g)	196.3±6.9	151.0±4.5*	185.2±4.2	191.5±7.2	202.4±10.2
Liver weights (g)	6.6±0.5	2.4±0.3*	5.2±0.5	5.8±0.9	6.2±0.7
Glucose conc. (mg/dL)	69.7±3.9	135.2±4.1*	97.6±4.6	107± 5.4	83± 5.8
Insulin conc. (µU/mL)	5.3±0.5	1.9±0.3*	5.4±0.6	3.8±0.5	5.9±0.6

Group 1 (Control), Group 2 (lead acetate only), Group 3 (lead acetate + honey), Group 4 (lead acetate +Vitamin C), Group 5 (lead acetate + honey + Vitamin C) * show values significantly different from control and the treatment groups, (n=5; $p<0.05$).

Table 2. Antioxidant enzymes activities in lead toxic rats co-administered with honey and vitamin C

Antioxidant enzymes	Group 1	Group 2	Group 3	Group 4	Group 5
SOD (U/L)	6.5±0.4	2.3±0.2*	7.3±0.5	7.5±0.8	6.8±0.5
CAT (U/L)	6.1±0.8	2.6±0.2*	5.6±0.4	6.7±0.6	8.2±0.9
GPx (U/L)	8.7±0.5	3.1±0.4*	8.1±0.7	7.2±1.0	8.5±0.5

Group 1 (Control), Group 2 (lead acetate only), Group 3 (lead acetate + honey), Group 4 (lead acetate +Vitamin C), Group 5 (lead acetate + honey + Vitamin C) * show values significantly different from control and the treatment groups, (n=5; $p<0.05$). SOD = Superoxide dismutase, CAT = Catalase, GPx = Glutathione peroxidase

Table 3. Hematological indices in lead toxic rats co-administered with honey and vitamin C

Hematological parameters	Group 1	Group 2	Group 3	Group 4	Group 5
TWBC (10^9/L)	5.7±0.6	2.1±0.2*	5.5±0.4	5.1±0.6	4.7±0.4
Lymphocytes (10^9/L)	3.8±0.3	1.5±0.2*	4.0±0.3	3.2±0.5	2.8±0.5
Monocytes (10^9/L)	0.8±0.04	0.2±0.03*	0.5±0.02	0.8±0.02	0.9±0.01
Granulocytes (10^9/L)	1.1±0.06	0.4±0.05*	1.0±0.05	1.1±0.03	0.9±0.06
RBC count (10^12/L)	4.7±0.6	1.8±0.5*	4.8±0.3	4.3±0.5	5.1±0.7
HGB Conc. (g/dL)	15.6±2.0	8.4±1.6*	15.8±2.1	15.2±1.8	15.5±2.4
PCV (%)	38.9±3.3	11.2±1.7*	25.0±2.3	20.7±3.5	29.6±2.8

Group 1 (Control), Group 2 (lead acetate only), Group 3 (lead acetate + honey), Group 4 (lead acetate +Vitamin C), Group 5 (lead acetate + honey + Vitamin C) * show values significantly different from control and the treatment groups, (n=5; $p<0.05$).TWBC = total white blood cell count, RBC = red blood cell, HGB Conc. = hemoglobin concentration, PCV = Packed cell volume.

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PC25

MAGNESIUM TRANSPORTERS EXPRESSION AND PROGNOSTIC IMPACT IN DIGESTIVE CANCERS

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Introduction: Digestive cancers are the most frequent and lethal cancers in the world [1]. Many epidemiological studies suggest that a bad diet and lifestyle could increase the risk of developing digestive cancer [2-4]. It is shown notably that magnesium (Mg^{2+}) intake decreases over the years and could be linked to the incidence of some digestive cancers, like pancreatic cancer [5]. Mg^{2+} is essential for cellular physiology, as it regulates a lot of cellular processes. Its homeostasis is regulated by membrane transporters, such as TRPM6, TRPM7, MAGT1, CNNM4, SLC41A1, and MRS2. However, their distribution in tissues from digestive cancers has not been exhaustively studied.

Aim: This work aims to study Mg^{2+} transporter expression in digestive cancers and their impact on patient survival.

Methods: We analyzed the Mg^{2+} transporters *TRPM6*, *TRPM7*, *MAGT1*, *CNNM1-4*, *SLC41A1*, and *MRS2* mRNA relative expression from the TCGA transcriptomic datasets to investigate their expression pattern, combinatory correlation, and their impact on survival in esophageal carcinoma (ESCA), stomach adenocarcinoma (STAD), pancreatic adenocarcinoma (PAAD) and colorectal adenocarcinoma (COADREAD). Genotype Tissue Expression (GTEx) and Cancer Genome Atlas (TCGA) datasets were analyzed in this study, using GEPIA2 and RStudio tools.

Results: By comparing non-tumoral and tumoral tissues, we observed an alteration of Mg^{2+} transporters expression in most of digestive cancers. *MAGT1* and *CNNM4* are overexpressed in all digestive cancers ($p < 0.01$) and are negatively correlated with overall-survival and in disease-free in PAAD patients ($p < 0.05$; $n = 171$). High *TRPM6* was correlated with a better outcome in those patients ($p < 0.01$). Interestingly, we identified a gene signature involving *MAGT1*, *CNNM4* and *TRPM7* ($0.19 < R < 0.16$ for ESCA; $0.2 < R < 0.27$ for PAAD, $p < 0.01$). This signature is associated with poor prognosis in some digestive cancers, like PAAD ($p < 0.001$; $n = 176$), ESCA ($p < 0.001$; $n = 184$) or COADREAD ($p < 0.001$; $n = 466$).

Conclusion: Our work suggests the importance of Mg^{2+} transporters such as MAGT1, TRPM7, and CNNM4 as potential new biomarkers in digestive cancers. More analyses are required to evaluate the functional interaction among those proteins and their impact on cancer processes such as cell proliferation, migration, or invasion.

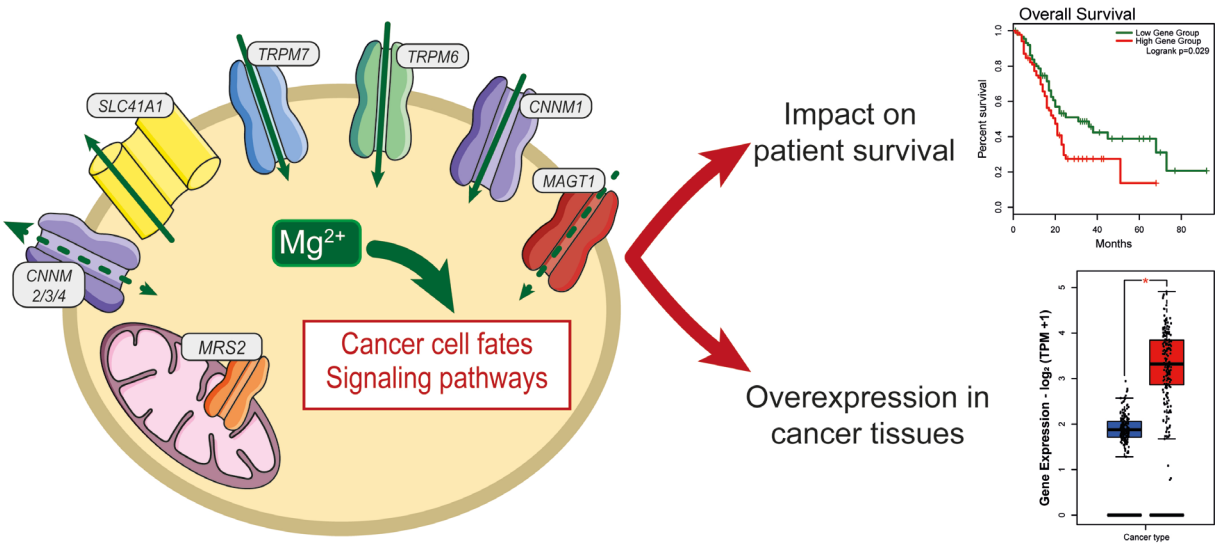

Fig. 1: Graphical abstract

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PC26

Orai3 channel regulates cell migration and adhesion of human breast cancer cells

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Breast cancer is the most common cancer in women worldwide. Despite improved screening and treatment in the early stages of tumor development, it remains difficult to treat stages of cancer when metastatic processes are underway. Metastatic development is based in particular on the acquisition by cancer cells of migration capacities, involving remodeling of the cytoskeleton, which is highly dependent on the intracellular calcium ion concentration. While research has focused on the involvement of membrane ion channels in migration processes, the role of the Orai3 calcium channel in migration processes remains unknown.

By cationic imaging and live-cell imaging, we showed that the Orai3 channel constitutively allows the entry of calcium essential in the migration of two breast cancer lines: MDA-MB-231 and MDA-MB-231 BrM2 ($n = 3$; Student t-test and ANOVA test; $p < 0.001$). We found that Orai3 silencing decreased cell migration along with a decrease in the basal calcium entry into these lines. Furthermore, the pharmacological activation of Orai3 with 2-Amino-Ethyl Borate (2-APB) leads to an increase in cell migration.

Moreover, we demonstrated the impact of Orai3 expression in breast cancer cell lines morphology through the modulation of the cytoskeleton. Indeed, Orai3 knockdown greatly decreased the polymerization of F-actin filaments, and cells lacking Orai3 expression presented a rounded cell morphology and failed to exhibit stress fibers compared to cells expressing Orai3 ($n = 3$; ANOVA test; $p < 0.001$).

Finally, we investigated the mechanisms of cell adhesion through the study of the activity of calpain. Our results showed that Orai3, through its ability to modulate calcium entry allows for the activation of calpain, which is required to modulate cell adhesion and migration processes. This indicates that Orai3 drives intracellular calcium dynamics necessary for migration processes and for cytoskeleton remodeling of breast cancer cells ($n = 3$; ANOVA test; $p < 0.001$). Interestingly, we also noticed the adhesion forces to the extracellular matrix, which were determined by single-cell force spectroscopy, were not altered in any way ($N = 3$; Student t-test), suggesting a regulation of adhesion and migration processes through a signaling pathway underlying Orai3.

In conclusion, our results reveal a key role of Orai3 in breast cancer cell migration by regulating actin cytoskeleton remodeling and cell adhesion correlated with the modulation of basal calcium entry.

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PC27

Vitamin D Receptor activation does not protect against cytokine-induced apoptosis in colonic epithelial cells

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Background: The vitamin D receptor (VDR) is a nuclear receptor that is expressed in many tissues throughout the body, but is particularly abundant within the colon. Despite this, there is not much known about the specific role VDR plays in the gut, although its activation by calcitriol has been shown to have both cytoprotective and anti-inflammatory actions. VDR is activated by the active form of vitamin D, calcitriol as well as the secondary bile acid lithocholic acid (LCA). Vitamin D deficiency is common in patients with Inflammatory Bowel Disease (IBD) and may be a key factor underlying loss of intestinal barrier function during IBD pathogenesis.

Aim: To determine if VDR activation by calcitriol is protective against cytokine-induced apoptosis in colonic epithelial cells.

Methods: Using the cytokines TNFα and IFNγ, cytokine induced apoptosis was modelled in polarised monolayers of T_{84} colonic epithelial cells. Calcitriol (1-100nM) was used to activate VDR and the VDR target protein, CYP24A1, was used to verify activation of the receptor. Barrier function and apoptosis were measured as changes in TER (transepithelial electrical resistance) and expression of the apoptosis marker, cleaved PARP, by western blot. All experiments have at least an n=3. GraphPad Prism 8 was used to do the statistical analysis for each experiment. The paired t-test was used for the comparison of paired treatments between 2 groups. While for more than 2 groups, ANOVA was used.

Results: Gene expression of CYP24A1, a marker of VDR activation, significantly increased with treatment of calcitriol, whereas the activity of another nuclear bile acid receptor, farnesoid x receptor (FXR) was not altered. Barrier function in cytokine-treated colonic epithelial cells (as measured by TER) was significantly reduced compared to controls. Cytokine treatment also increased levels of cleaved PARP indicating that apoptosis was occurring. In cells pre-treated with 1-100nM calcitriol, cytokine-induced reductions in TER were not altered and PARP cleavage was not inhibited.

Conclusion: Activation of the VDR by calcitriol does not prevent cytokine-induced epithelial apoptosis or loss of barrier function in vitro. Thus, previously reported protective effects of VDR in colitis are likely due to other actions, such as mucosal immune suppression or modulation of the microbiome. Further studies to elucidate the anti-inflammatory effects of VDR in the colon are required.

Acknowledgements :-

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PC28

Development of a novel method to measure oxidative stress in single cells

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Oxidative stress is associated with the pathogenesis of many diseases. To better understand this, it is often necessary to measure relative levels of oxidative stress in cells. Biochemical methods can be highly quantitative but tend to be undynamic given they usually require fixation or destruction of the cell. Oxidative stress-sensitive fluorophores such a CellROX, can be used in living tissue and with certain apparatus which permit dynamic measurement. To do this in single cells currently requires the use of flow cytometry; which isn't suitable for many cell types, or conventional photometry; which is laborious and limits time course of single experiments. To overcome these limitations, we developed the use of the Biotek Cytation cell imaging system with CellROX fluorophores. Here, we present the early optimisation of the technique.

A HEP G2 cell line was maintained in culture and plated in 96 well plates. Cells were loaded with 5µM CellROX deep red for 30 minutes. A Cytation cell imaging system (Biotek, USA) was used to capture brightfield and fluorescent images. To model oxidative stress, we exposed cells to 20 µM hydrogen peroxide for 30 min. To measure the effectiveness of our optimisation steps, relative fluorescence was quantified using ImageJ software. Fluorescent values are given as mean ± S.E.

We first tested the effect of CellROX loading order. When cells were loaded *before* exposure to H_2O_{2-}, we observed no increase in fluorescence. However, we observed a considerable increase in fluorescence when cells were loaded *after* exposure to H_2O_{2-} (Control (n=162); 1.15 ± 0.07, Before (n=104); 1.00 ± 0.07, After (n=104); 2.05 ± 0.18, MAU, $p < 0.001$. Our next experiment sought to establish the effect of well cell density on fluorescent intensity. Exposure to H_2O_2 produced an inverse sigmoidal relationship between cell density (250 - 30,000 cells per well) and fluorescent intensity ($n = 104$, $p < 0.001$). For subsequent experiments we used a density of 5000 cells per well, which offered an ideal practical compromise. Our final experiment was designed to test the sensitivity of the method. Cells were exposed to a range (0-50 µM) of H_2O_2 concentrations which produced a concentration-dependent increase of fluorescence, which saturated at 20µM ($n = 90$, $p < 0.001$).

These data suggest the Cytation cell imaging system when used with CellROX fluorophores may offer a convenient way to measure oxidative stress in living cells. CellROX loading order and cell density are important considerations for experimental design. Our next experiments will optimise the technique for use with primary cardiac myocytes.

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PC29

What role, if any, Serine/threonine-protein phosphatase 6 catalytic subunit (PPP6C) have in G2 to M phase transition?

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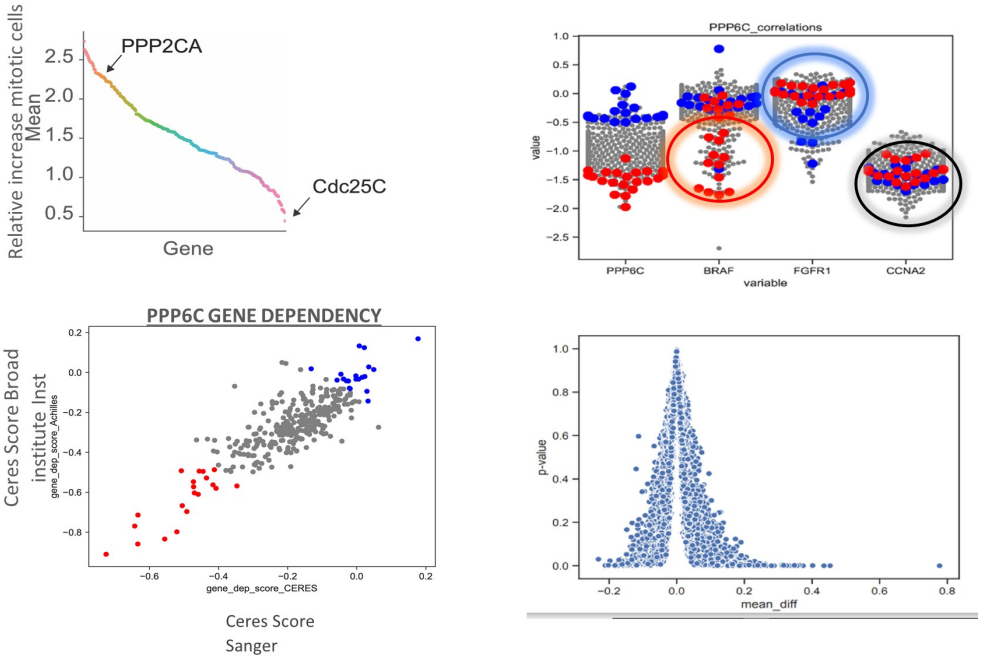
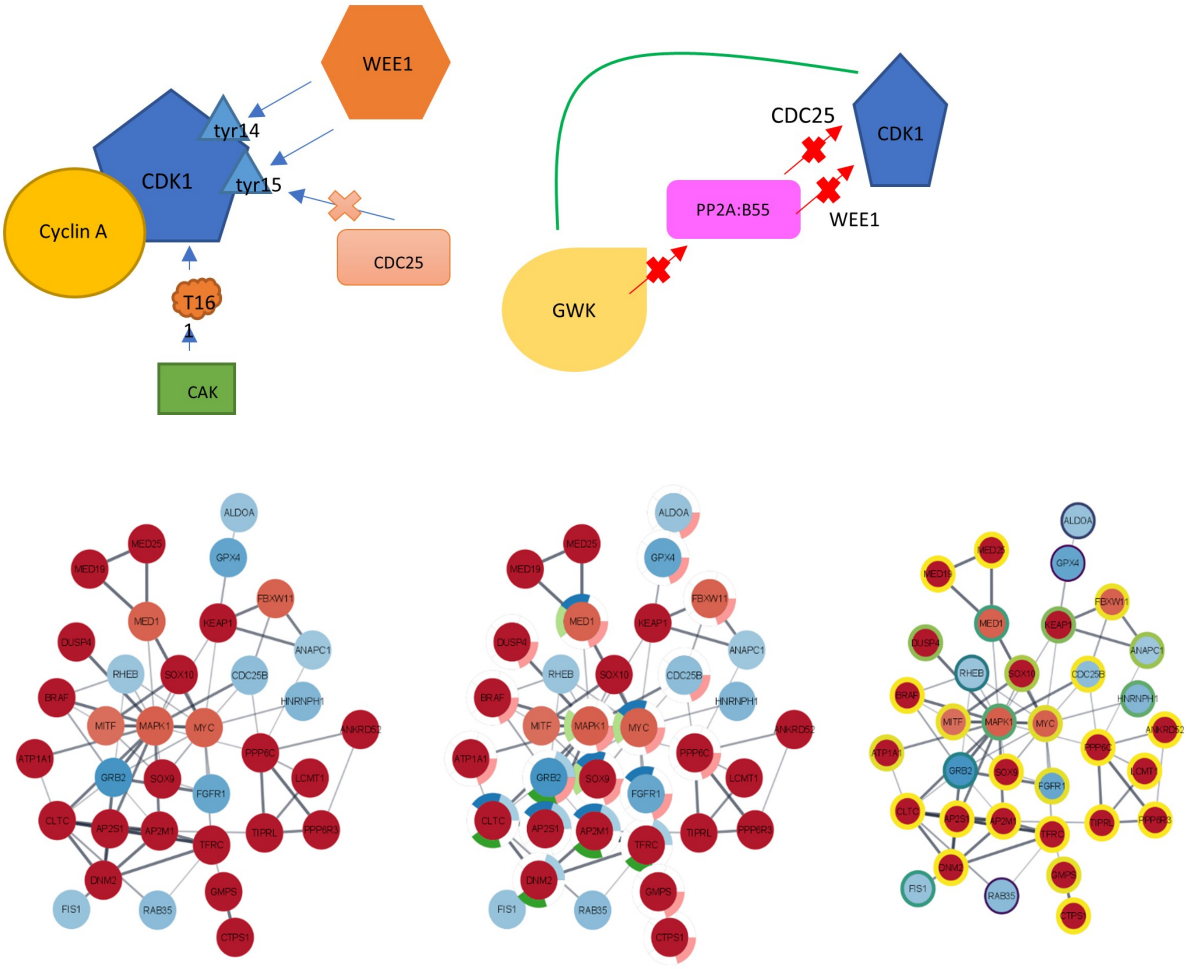
The cell cycle allows for the growth and progression of cells within organisms; G2 to Mitotic (M) phase transition under the control of kinases and phosphatases is involved in this. The dysregulation of the cell cycle is associated with cancer pathology and finding new phosphatases or kinases would help in understanding this and could provide new grounds for treatment. A genetic screen carried out prior to this project identified the catalytic subunit of protein phosphatase 6 (PPP6C) as a potential negative regulator of the G2 to M phase transition. The aim of this project was to analyse gene dependency correlations of PPP6C based on large scale CRISPR depletion screens in the cancer cell line encyclopaedia data sets. I used a novel based on distance correlation for cells sensitive or resistant to PPP6C depletion and performed a network and gene ontology enrichment analysis of the identified genes. The top 30 and bottom 20 correlating genes were analysed in Cytoscape to find biological processes, correlations, and confidence within the gene network. My results suggest a relationship between PPP6C and genes including: CDC25B, BRAF, MYC and MAPK. In conclusion, mechanisms shared by these genes may give light to the role of PPP6C in G2 to M phase transition. However, this needs to be explored further based on experimental validation.





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PC30

Sarcoplasmic reticular Ca^{2+}-ATPase inhibition paradoxically upregulates murine skeletal muscle $Na_v1.4$ function

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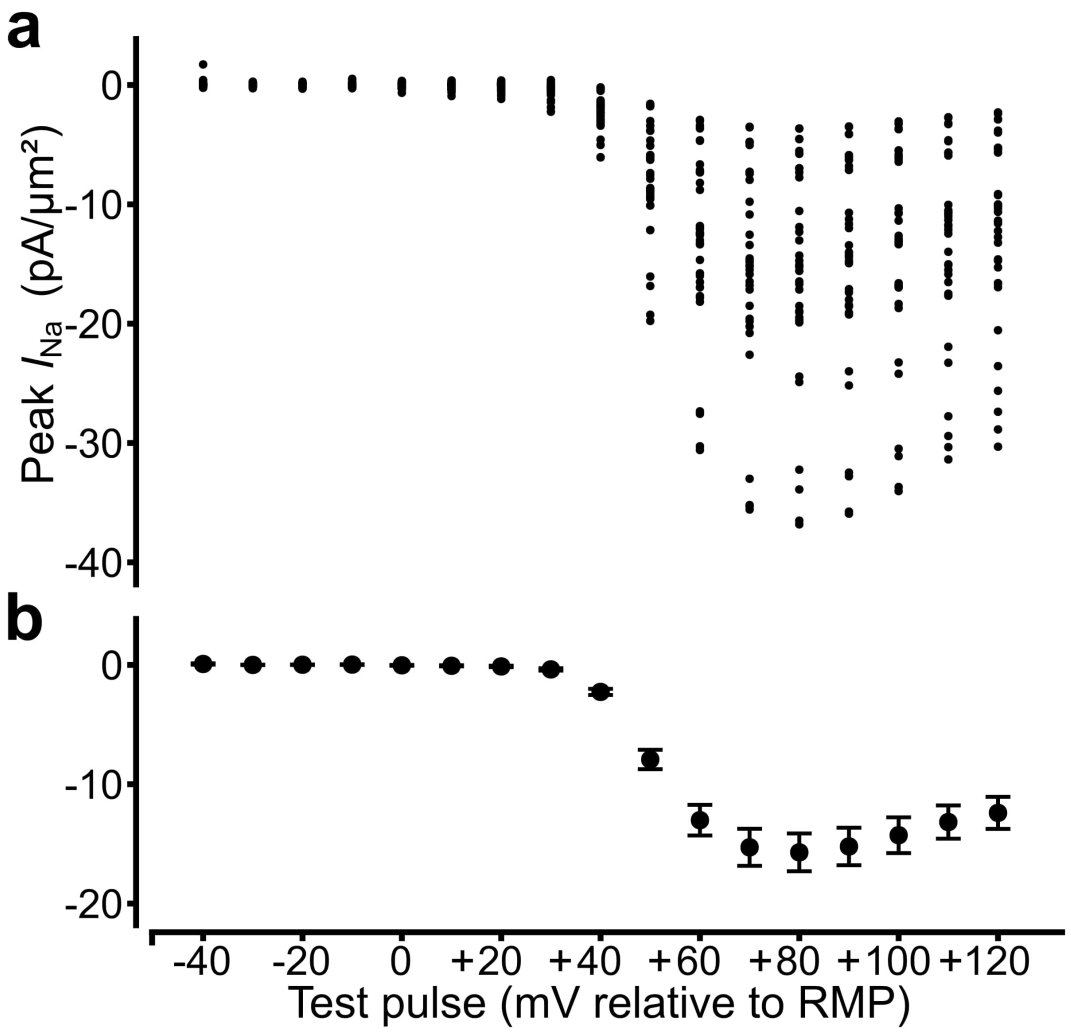
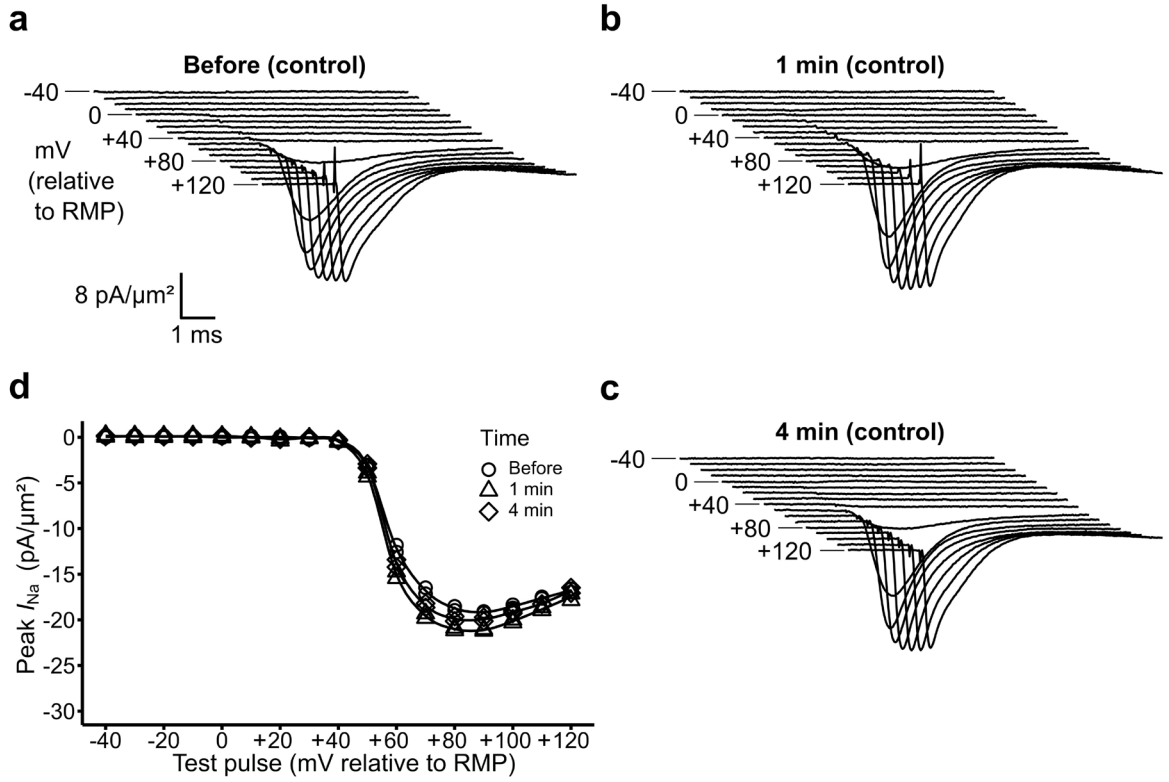
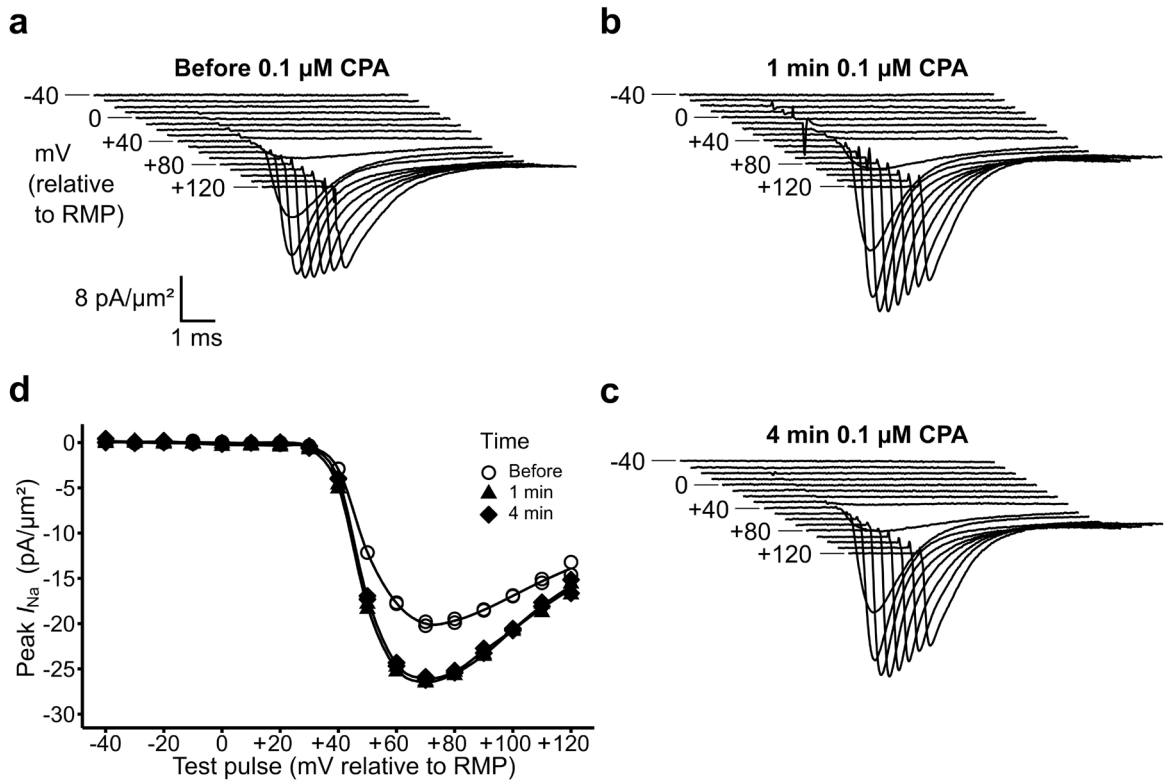
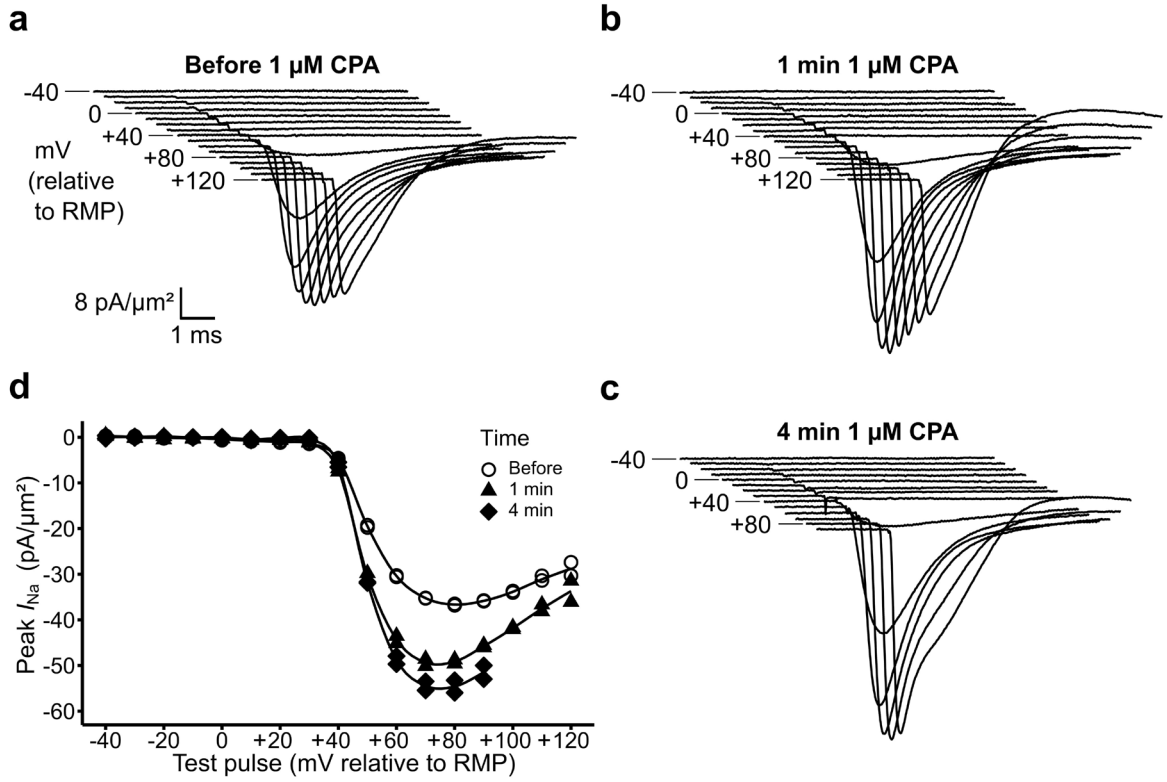
Skeletal muscle Na^+ channels possess Ca^{2+}- and calmodulin-binding sites implicated in Nav1.4 current (I_{Na}) downregulation following ryanodine receptor (RyR1) activation produced by exchange protein directly activated by cyclic AMP or caffeine challenge, effects abrogated by the RyR1-antagonist

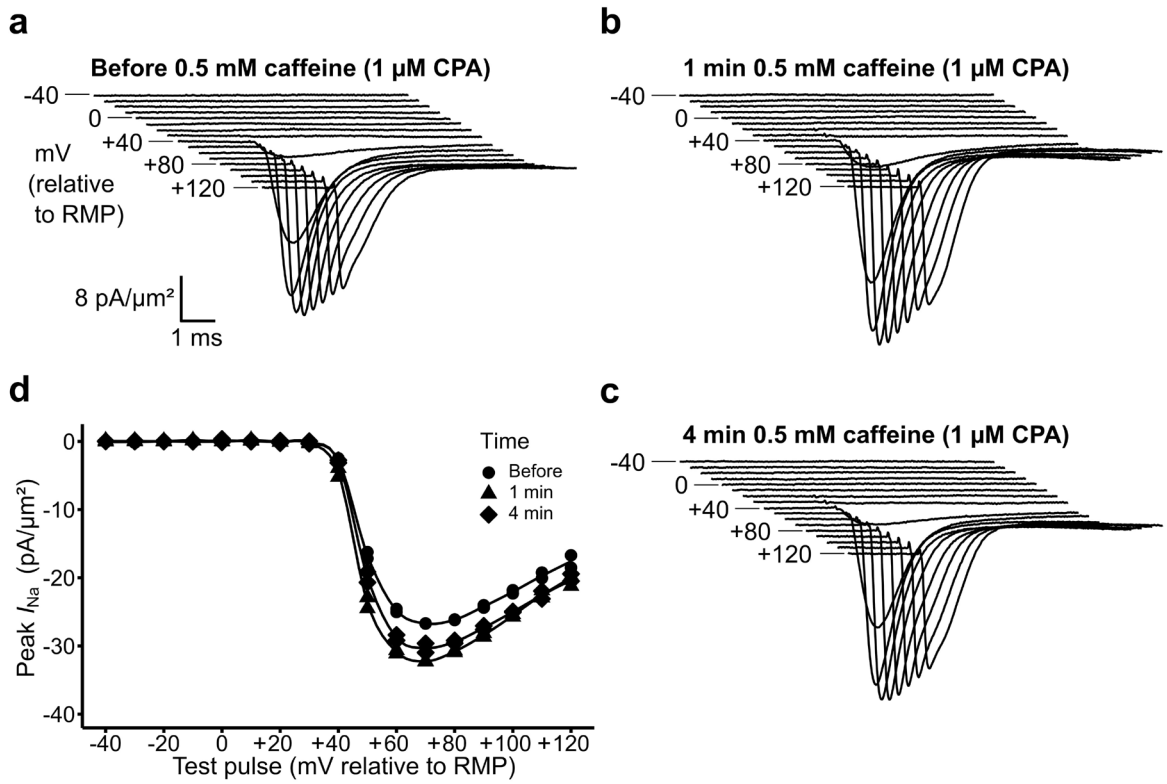
dantrolene which itself increased I_{Na}. These findings were attributed to actions of consequently altered cytosolic Ca^{2+}, $[Ca^{2+}]_i$, on Nav1.4. We extend the latter hypothesis employing cyclopiazonic acid (CPA) challenge, which similarly increases $[Ca^{2+}]_i$, but through contrastingly inhibiting sarcoplasmic reticular (SR) Ca^{2+}-ATPase. Loose patch clamping determined Na^+ current (I_{Na}) families in intact native murine gastrocnemius skeletal myocytes, minimising artefactual $[Ca^{2+}]_i$ perturbations. A bespoke flow system permitted continuous I_{Na} comparisons through graded depolarising steps in identical stable membrane patches before and following solution change. In contrast to the previous studies modifying RyR1 activity, and imposing control solution changes, CPA (0.1 and 1 µM) produced persistent increases in I_{Na} within 1-4 min of introduction. CPA pre-treatment additionally abrogated previously reported reductions in I_{Na} produced by 0.5 mM caffeine. Plots of peak current against voltage excursion demonstrated that 1 µM CPA increased maximum I_{Na} by ~30%. It only slightly decreased half-maximal activating voltages ($V_{0.5}$) and steepness factors (k), by 2 mV and 0.7, in contrast to the $V_{0.5}$ and k shifts reported with direct RyR1 modification. These paradoxical findings complement previously reported downregulatory effects on Nav1.4 of RyR1-agonist mediated *increases* in *bulk* cytosolic $[Ca^{2+}]$. They implicate possible *local* tubule-sarcoplasmic triadic domains containing *reduced* $[Ca^{2+}]_{TSR}$ in the observed upregulation of Nav1.4 function following CPA-induced SR Ca^{2+} depletion.

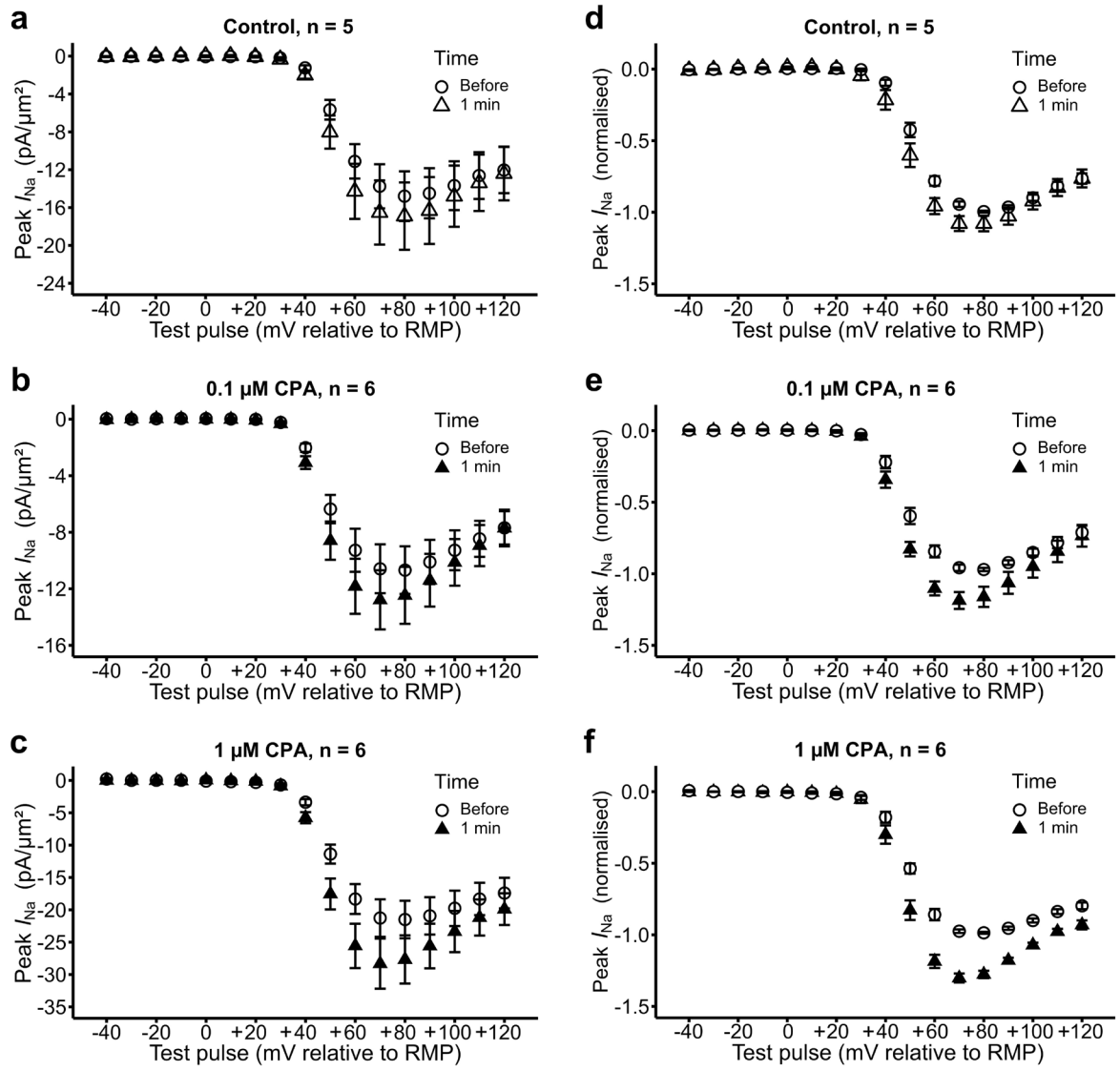


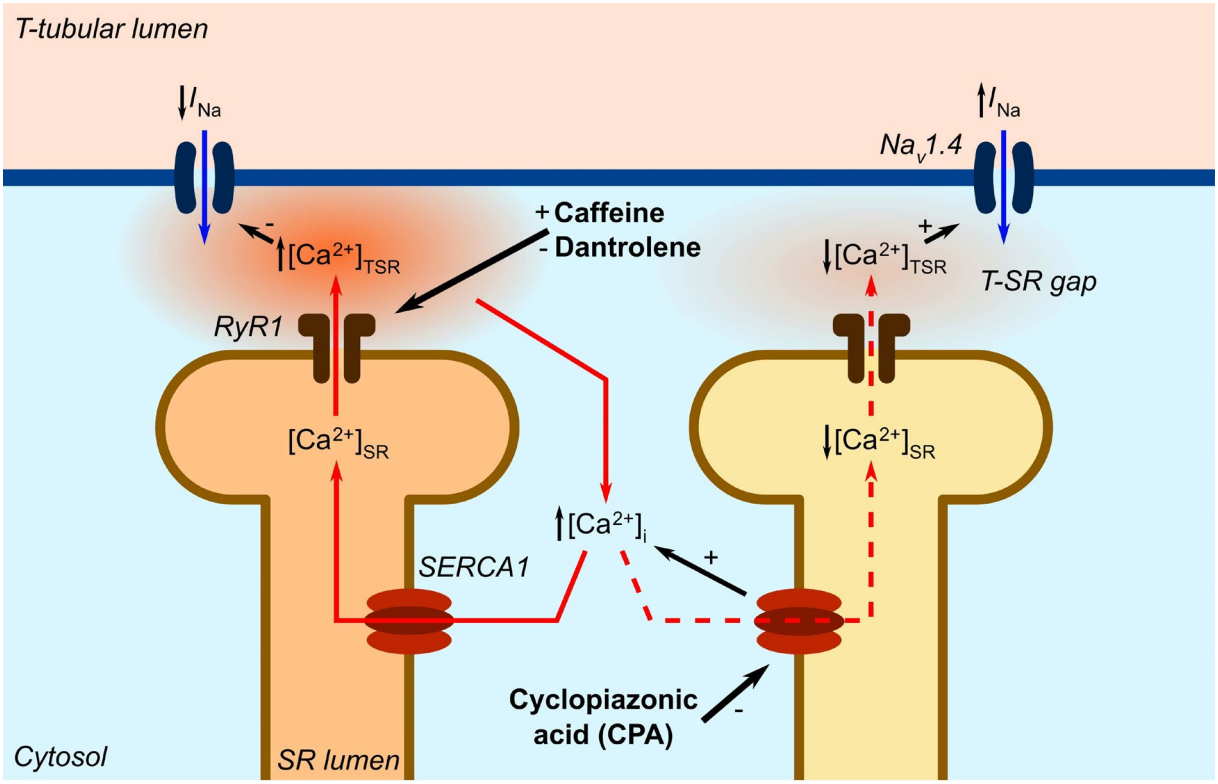

a
Before (control)
-40
0
+40
+80
+120
mV
(relative
to RMP)
8 pA/μm²
1 ms
b
1 min (control)
c
4 min (control)
d
Time
Before
1 min
4 min
Peak I_{Na} (pA/μm²)
0
-5
-10
-15
-20
-25
-30
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)

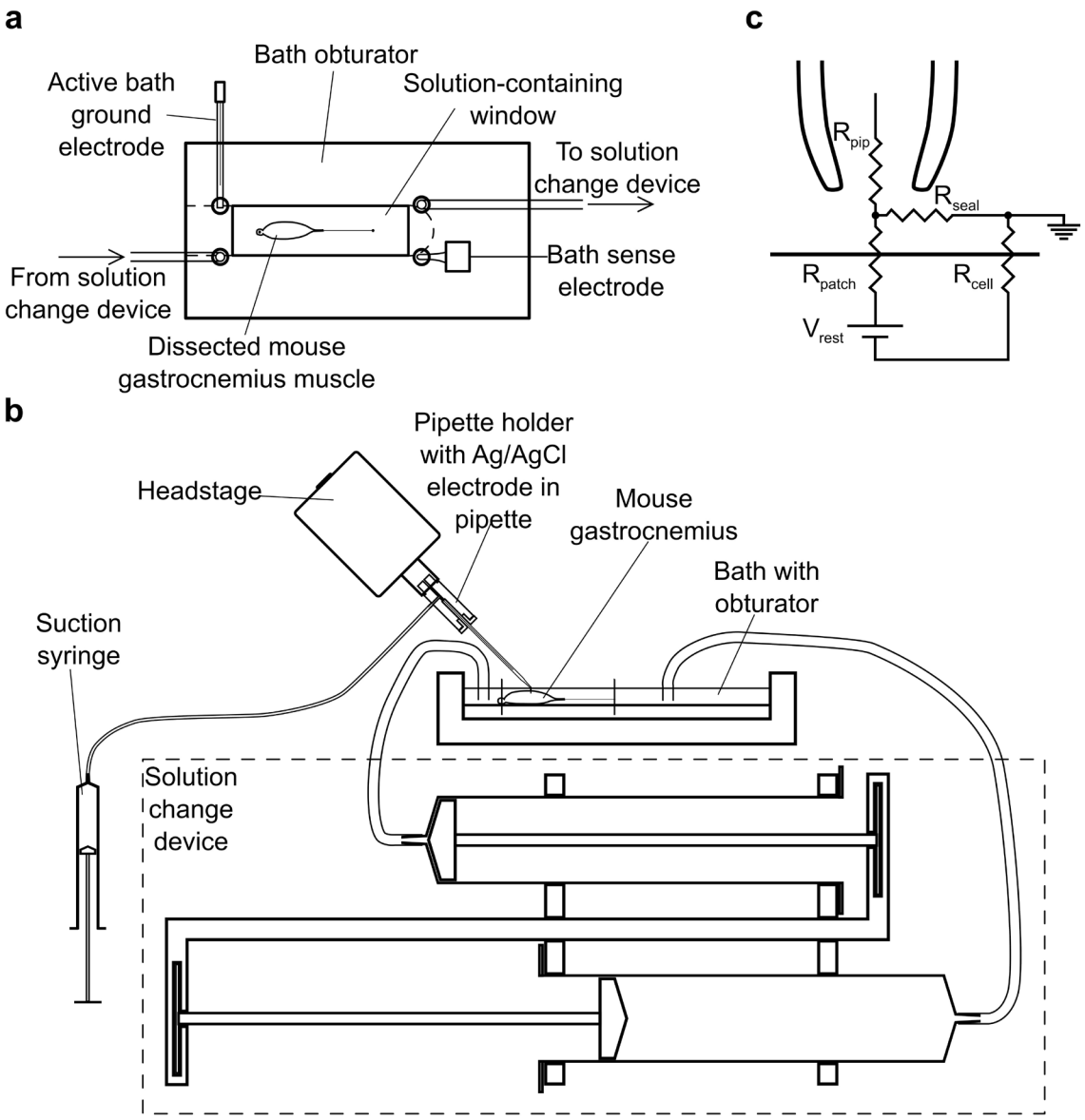

a
Before 0.1 μM CPA
-40
0
+40
+80
+120
mV
(relative
to RMP)
8 pA/μm²
1 ms
b
1 min 0.1 μM CPA
c
4 min 0.1 μM CPA
d
Time
Before
1 min
4 min
Peak I_{Na} (pA/μm²)
0
-5
-10
-15
-20
-25
-30
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)


a
Before 1 μM CPA
-40
0
+40
+80
+120
mV
(relative
to RMP)
8 pA/μm²
1 ms
b
1 min 1 μM CPA
c
4 min 1 μM CPA
d
Time
Before
1 min
4 min
Peak I_{Na} (pA/μm²)
0
-10
-20
-30
-40
-50
-60
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)


a
Before 0.5 mM caffeine (1 μM CPA)
-40
0
+40
+80
+120
mV
(relative
to RMP)
8 pA/μm²
1 ms
b
1 min 0.5 mM caffeine (1 μM CPA)
c
4 min 0.5 mM caffeine (1 μM CPA)
d
Time
Before
1 min
4 min
Peak I_{Na} (pA/μm²)
0
-10
-20
-30
-40
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)


a
Control, n = 5
Time
Before
1 min
Peak I_{Na} (pA/µm²)
0
-4
-8
-12
-16
-20
-24
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)
b
0.1 µM CPA, n = 6
Time
Before
1 min
Peak I_{Na} (pA/µm²)
0
-4
-8
-12
-16
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)
c
1 µM CPA, n = 6
Time
Before
1 min
Peak I_{Na} (pA/µm²)
0
-5
-10
-15
-20
-25
-30
-35
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)
d
Control, n = 5
Time
Before
1 min
Peak I_{Na} (normalised)
0.0
-0.5
-1.0
-1.5
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)
e
0.1 µM CPA, n = 6
Time
Before
1 min
Peak I_{Na} (normalised)
0.0
-0.5
-1.0
-1.5
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)
f
1 µM CPA, n = 6
Time
Before
1 min
Peak I_{Na} (normalised)
0.0
-0.5
-1.0
-1.5
-40 -20 0 +20 +40 +60 +80 +100 +120
Test pulse (mV relative to RMP)


T-tubular lumen
I_{Na}
I_{Na}
$Na_v1.4$
+ Caffeine
- Dantrolene
$[Ca^{2+}]_{TSR}$
$[Ca^{2+}]_{TSR}$
T-SR gap
RyR1
$[Ca^{2+}]_{SR}$
$[Ca^{2+}]_{SR}$
$[Ca^{2+}]_i$
SERCA1
Cyclopiazonic acid (CPA)
Cytosol
SR lumen
a
b
c


a
Bath obturator
Active bath ground electrode
Solution-containing window
To solution change device
From solution change device
Bath sense electrode
Dissected mouse gastrocnemius muscle
c
R_{pip}
R_{seal}
R_{patch}
R_{cell}
V_{rest}
b
Pipette holder with Ag/AgCl electrode in pipette
Headstage
Mouse gastrocnemius
Bath with obturator
Suction syringe
Solution change device
d
Test pulse
Voltage (mV relative to RMP)
120
100
80
60
40
20
0
-20
-40
Hyperpolarising pre-pulse
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Time in cycle (ms)

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PC31

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

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[1]Department of Molecular Medicine, The Royal College of Surgeons in Ireland, Dublin, Ireland
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Introduction and Aims: CFTR, a transmembrane Cl^- channel important in regulating intestinal fluid and electrolyte secretion, is implicated in the pathogenesis of a number of intestinal disorders. Bile acids, classically known for their roles in lipid digestion, are now also recognised as important enteric hormones that regulate many aspects of epithelial function. Previous studies from our laboratory have shown that, at physiological concentrations, bile acids, acting via the nuclear receptor, farnesoid x receptor (FXR), inhibit colonic epithelial CFTR expression. Dietary phytochemicals have been reported to have the capacity to modulate FXR signalling. Here, we set out to investigate mechanisms underlying FXR regulation of epithelial CFTR expression, and the potential for therapeutically targeting the receptor with dietary phytochemicals.

Methods: T_{84} colonic epithelial cells were cultured as monolayers on permeable supports and treated with the FXR agonist, GW4064 (5 μM), in the absence or presence of the plant phytochemical, designated here as KFS1 (5 μM). Expression of CFTR and FXR were measured by qRT-PCR and immunoblotting. Expression of NF-κB, FOXA1, HNF1A, and CDX2, transcription factors thought to be involved in the regulation of CFTR expression, were also measured by qRT-PCR. Nuclear translocation of NF-κB was measured by immunoblotting. Electrophysiological studies of T_{84} cells were conducted in Ussing chambers.

Results: Treatment of T_{84} monolayers with GW4064 significantly downregulated CFTR mRNA to 0.51 ± 0.06 fold after 12 hrs ($n = 12$; $p < 0.001$) and protein levels to 0.28 ± 0.0 6 fold after 48 hrs, compared to controls ($n = 8$; $p < 0.001$). Electrophysiological studies in Ussing chambers showed that GW4064 treatment for 48 hrs inhibited Cl^- secretory responses to the Ca^{2+}-dependent agonist carbachol (CCh; 100 mM) and the cAMP-dependent agonist, forskolin (FSK; 10 mM) by 79.9 ± 7.5 % and 74.2 ± 8.9 %, respectively. Transcriptomic analysis of human colonic enteroids revealed FXR to be robustly expressed in secretory (crypt-like) cells, that its levels were inversely correlated with those of CFTR, and that its activation also induced CFTR downregulation. FXR activation did not alter the expression or phosphorylation of the p65 subunit of NF-κB, or inhibit its translocation to the nucleus. GW4064 downregulated FOXA1 mRNA expression by 33.2 ± 5.2% after 3 hrs ($n = 4$; $p < 0.05$), but had no effect on HNF1A or CDX2 expression. Treatment with the phytochemical, KFS1 (5 mM; 24hrs),

upregulated FXR mRNA and protein expression in T_{84} cells and enhanced GW4064-induced downregulation of CFTR mRNA by 0.28 ± 0.05 fold (n = 8; $p < 0.01$) and protein by 0.25 ± 0.11 fold (n = 4) after 24 hours. KFS1 also enhanced FXR downregulation of Cl^- secretory responses in Ussing chambers.

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

Acknowledgements :- This work was funded by Science Foundation Ireland.

PC32

DIFFERENCES IN THE ACTIVATION OF LEUKOCYTES AT BLOOD CONTACT WITH SKT-6A-HF SORBENTS AND SILOCHROME S-120 IN VITRO

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[1]V.A. Almazov National North-West Medical Research Center, Ministry of Health of the Russian Federation, Saint-Petersburg, The Russian Federation

Introduction. Activation of leukocytes is an important component of the therapeutic effect of the low-volume hemoperfusion method, which consists in contact induction of bioactive substances and activated cells in the blood and delivery of the altered spectrum of activity to the lesion focus. In this method, we used the SKT-6A-VCh carbon sorbent, tested in the clinic with a positive effect when it is included in the treatment regimens for limb diseases(1). According to the results of the studies, Silochrome S-120 has the best characteristics of hemocompatibility and the possibility of further modification, which is significant for the development of new technologies for low-volume hemoperfusion(2),(3),(4).

Materials and methods. Experiments to study the activation properties of foreign surfaces were carried out in bench conditions. As blood-contact preparations, we used: carbon hemosorbent of gloom SKT-6A-VCh and a sorbent based on silica - Silokhrom S-120. SKT-6A-VCh is a high-purity medical carbon hemosorbent approved for use in clinical practice as a hemosorbent and as a hemoactivator in the procedure of low-volume hemoperfusion. Silochrome S-120 - irregular white granules, 0.3-0.5 mm in size. The specific surface of the granules is 130 m2 / g, the pore size is 28 nm. Donor blood was taken from healthy volunteers from the cubital vein into a 9.0 ml vacuum tube with heparin. The activation of leukocytes was assessed using the method of lucigenin-induced chemiluminescence on a chemiluminometer "Lum-1200". 14 experiments were performed, 7 with each of the studied sorbents. Luceginin prepared by dissolving the luceginin matrix in Hanks solution in a ratio of 1:25 was added to a test tube with a volume of 3 ml and a diameter of 12 mm. The matrix was prepared by diluting 5.8 mg of luceginin with 10 ml of dimethyl sulfoxide. Then the Hanks solution was added to the test tube to 0.9 ml and 0.1 ml of donor heparinized (16 IU per 1 ml) blood.

The assessment of the activity of leukocytes was carried out within 1 hour from the moment of contact of the blood with the sorbent according to the maximum chemiluminescence and light sum per granulocytes. Granulocyte count was performed by flow cytometry on hematologic analyzer SySmex XT 1800i. Statistical analysis was performed in "RStudio" and "Excel 2019" by parametric methods: Shapiro-Wilk test, unpaired Student's t-critrea. Differences were considered significant at $p<0.001$.

Results.

• Upon contact of blood with the drug "Silochrome S-120", the maximum chemiluminescence was 0.252±0.059 kPPS in the time interval 14.48 ± 1.583 min and the light sum was 81,8±18,85 kPPS*s per granulocytes*10^9.

• Upon contact of blood with the drug with SKT-6A-VCh, the maximum chemiluminescence was 0.257±0.077 kPPs in the time interval of 9.05 ± 2.500 min and the light sum was 92,3±25,06 kPPS*s per granulocytes*10^9.

Conclusion. Contact induction of activated leukocytes in the blood on the preparation Silochrome S-120 does not differ from the reference sorbent SKT-6A-VCh, but the activation of leukocytes on this preparation occurs later in time, which must be taken into account when using it in medical technology.

Reference 1 :- . Treatment of Critical Ischemia of Lower Extremities by the Method of the Target Small Hemoperfusion. Methodical Recommendation. SPb, 2003. p. 7. In Russian

Reference 2 :- Kuznetsov S.I., Kirichuk O.P., Burkova N.V., Postnov V.N., Yurev G.O., Romanchuk E.V. The use of granules of silica sorbent "Silokhrom S-120" as a contact hemoactivator of blood cellular elements. - 2020.

Reference 3 :- Kuznetsov S.I., Kirichuk O.P., Burkova N.V., Davankov V.A., Postnov V.N., Litvinenko E.V. REACTION OF THE CELLULAR ELEMENTS OF THE BLOOD TO THE CONTACT WITH THE GRANULATED HYPERCROSS-LINKED POLYSTYRENE AND SILICA. *Translational Medicine*. 2017;4(4):43-55. (In Russ.) https://doi.org/10.18705/2311-4495-2017-4-4-43-55

Reference 4 :- Burkova N.V., Kirichuk O.P., Romanchuk E.V., Davankov V.A., Postnov V.N., Kuznetsov S.I. Changes in plasma spectral characteristics during the in vitro contact of human venous blood with granulated sorbents. *Almanac of Clinical Medicine*. 2018;46(8):772-777. (In Russ.) https://doi.org/10.18786/2072-0505-2018-46-8-772-777

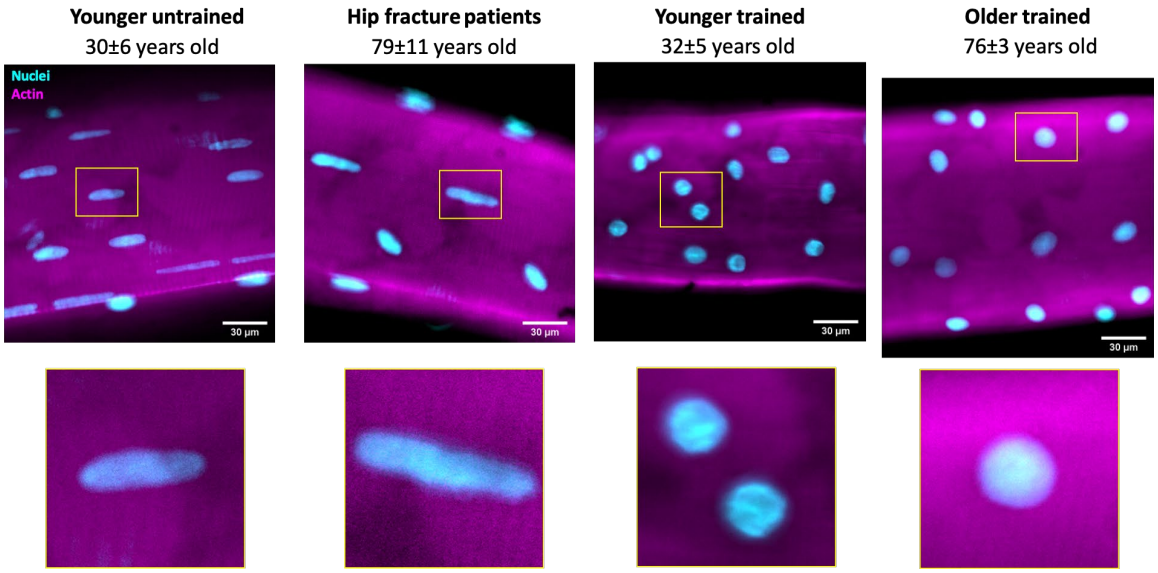
PC33

The Missing LINC to Human Healthy Ageing

Ed Battey[1], Stephen Harridge[1], Ross Pollock[1], Norman Lazarus[1], Matthew Stroud[2], Julien Ochala[1, 3, 4]

[1]Centre for Human and Applied Physiological Sciences, King's College London, King's Health Partners, London, United Kingdom [2]British Heart Foundation Centre of Excellence, School of Cardiovascular Medicine and Sciences, London, United Kingdom [3]Department of Biomedical Science, University of Copenhagen, Copenhagen, Denmark [4]Randall Centre for Cell and Molecular Biophysics, London, United Kingdom

Age-related declines in cellular structure and function can be initiated or accelerated by physical inactivity. Ageing is associated with aberrant nuclear morphology, architecture and translation of forces to biochemical signals (mechanotransduction); this project focuses on whether, in skeletal muscle, these changes are influenced by endurance training status. Nuclear morphology and the distribution of nuclear envelope proteins important for nuclear architecture and mechanotransduction (SUN1) were studied in isolated human muscle fibres from individuals of different ages and endurance training statuses. So far, the data indicate that in humans, changes in myonuclear morphology occur in response to exercise regardless of age, indicated by a 6-10% reduction in myonuclear sphericity and 25-30% reduction in myonuclear aspect ratio in young and older exercise-trained individuals compared to inactive young and older counterparts (n = 6 per group, $p < 0.05$ one-way ANOVA). No differences in the distribution of SUN1 were revealed through super-resolution microscopy. Endurance training therefore appears to influence nuclear morphology in a manner unaffected by age; alterations to nuclear envelope proteins other than SUN1 may be involved in this process. Ongoing research is focused on whether these changes are recapitulated in mice and if the distribution of nuclear envelope proteins Lamin A and Nesprin-1 and muscle-specific cytoskeletal protein Desmin are altered with endurance training. Whether alterations to myonuclear mechanics influences gene transcription will also be assessed through cell microharpooning and RNA sequencing.



Acknowledgements :- We thank the Medical Research Council for funding this project.

We also thank the following collaborators for generously providing us with highly valuable skeletal muscle samples to analyse, without which we could complete our studies:

Professor Stephen Harridge and colleagues Professor Norman Lazarus and Dr Ross Pollock for kindly providing young sedentary, master cyclist and hip fracture patient skeletal muscle biopsy samples

Dr Jamie Pugh and Professor Graeme Close for kindly providing marathon runner skeletal muscle biopsy samples

Professor Christoph Handschin and Dr Regula Furrer for kindly providing mouse PGC-1 α mKO skeletal muscle samples

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PC34

Exploring the utility of handgrip strength as a tool to predict lower limb function in masters athletes.

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Introduction

Handgrip strength (HGS) is a widely used method to assess physical function in older adults and has been suggested as a biomarker of aging [1]. It is easy to perform and has been suggested to be a reliable indicator for vitality and several risk factors related to human ageing [2]. However, its utility in highly athletic older people is under-reported, particularly across different athletic disciplines. The purpose of this study was to determine the association between HGS and lower limb power in masters sprinters and endurance athletes, and healthy age-matched controls.

Methods

A total of 187 masters athletes consisting of 38 sprinters (71.1 ± 6.7 years; 28M 10F) and 149 endurance athletes (69.7 ± 5.5 years; 111M, 38F), and 58 age-matched controls (73.7 ± 5.1 years, 28M, 30F) were recruited from the UK. HGS was assessed using a handheld dynamometer (Jamar) and lower limb muscle power was measured using a Leonardo Jump Mechanography Platform during a countermovement vertical jump. One-way ANOVA analysis, adjusted for sex, was used to identify differences among groups. Mixed effects linear regression models, adjusted for sex, were performed to determine relationships. Significance was assumed when $p<0.05$.

Results

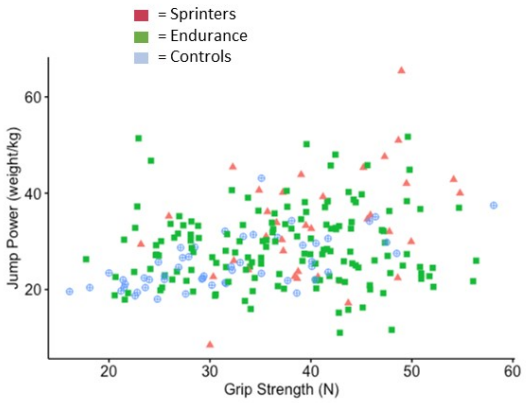
HGS in sprinters (40.28 ± 7.42 kg) was not statistically different to endurance runners (37.15 ± 8.87 kg) ($p=0.106$). However, both athletic groups had higher HGS than controls (31.89 ± 8.83 kg) (both $p<0.05$). Jump power in sprinters (33.3 ± 10.97 W/kg) was greater than in endurance athletes (28.8 ±

7.89 W/kg) and controls (25.18 ± 5.47 W/kg) (both $p<0.05$), with no significant difference between endurance athletes and controls (p=0.181).

In regression models adjusted for sex, HGS was not associated with jump power in sprinters (β [95% CI]: 0.44 [-0.09 to 0.97], p=0.103) or endurance athletes (0.03 [-0.11 to 0.17], p=0.640). However, in controls there was a positive relationship between HGS and power (0.41 [0.2 to 0.63], p=0.000).

Discussion and Conclusion

These data demonstrate that HGS is a good predictor of lower limb function, as assessed by jump power, in non-athletic older adults only. These findings question the utility of HGS as a biomarker of physical function in masters athletes. However, given the known health-related benefits of life-long exercise [3], the clinical utility of this assessment tool to assess those most 'at risk' of functional deficits (and for example, falls and fractures) is supported by the relationship observed in our controls [4]. The reason for this dissociation in the utility of HGS as a biomarker of ageing must be explored further, so the correct tools to assess aspects of health-status can be used for individuals.



Reference 1 :- Sayer AA, Kirkwood TB. Grip strength and mortality: a biomarker of ageing? Lancet. 2015 Jul 18;386(9990):226-7. doi: 10.1016/S0140-6736(14)62349-7. Epub 2015 May 13. PMID: 25982159.

Reference 2 :- Labott BK, Bucht H, Morat M, Morat T, Donath L. Effects of Exercise Training on Handgrip Strength in Older Adults: A Meta-Analytical Review. Gerontology. 2019;65(6):686-698. doi: 10.1159/000501203. Epub 2019 Sep 9. PMID: 31499496.

Reference 3 :- Galloza J, Castillo B, Micheo W. Benefits of Exercise in the Older Population. Phys Med Rehabil Clin N Am. 2017 Nov;28(4):659-669. doi: 10.1016/j.pmr.2017.06.001. PMID: 29031333.

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PC35

Physiological networks of response to high-altitude trek: porters vs trekkers

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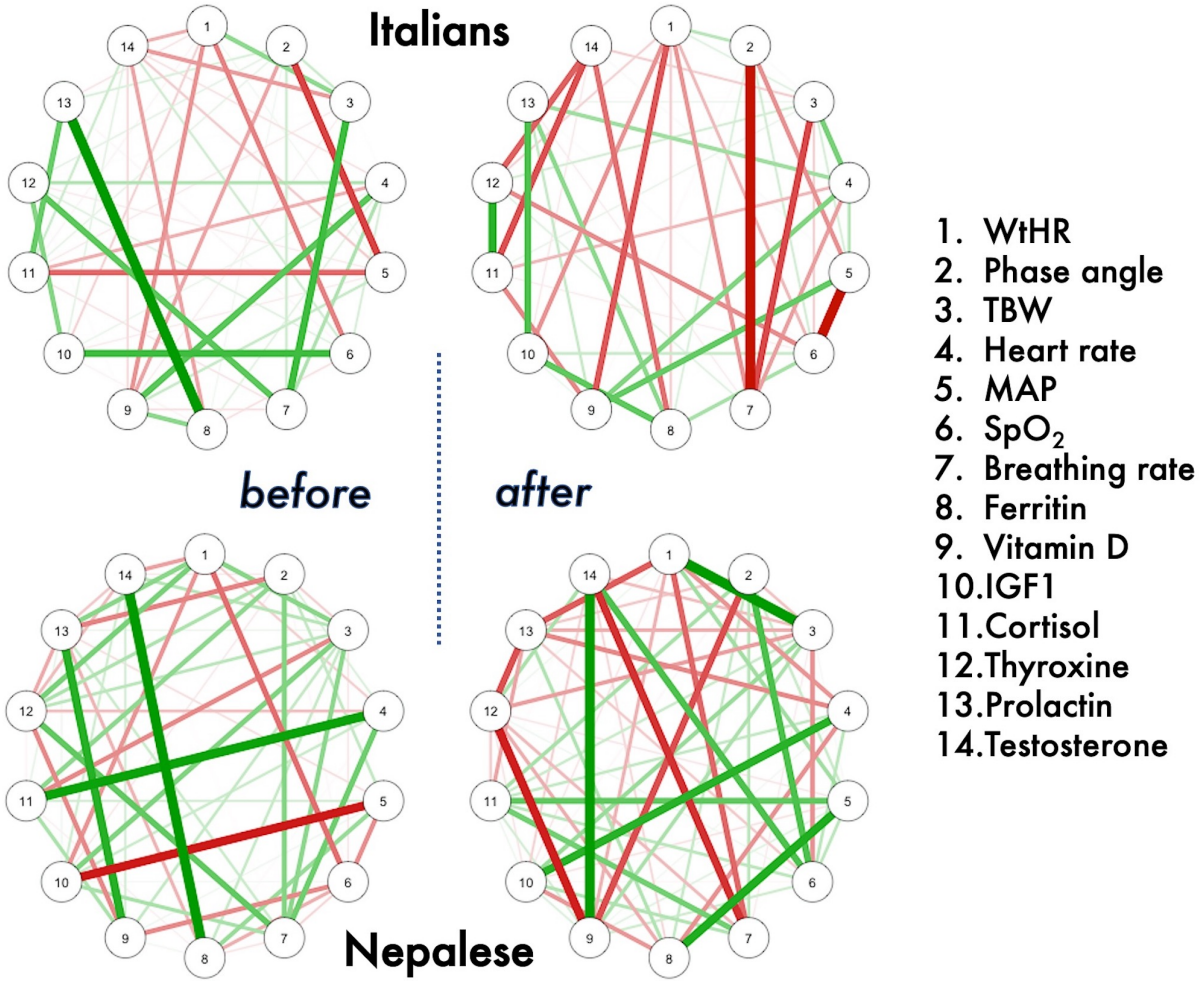
Interconnectedness and adaptiveness paved the way for network physiology, as the science devoted to the analysis of the structure that links the components of complex physiological systems (1). Network science approaches rise the possibility of unveiling novel and meaningful insights for translational medicine and applied physiology. We used a network approach to evaluate ethnic differences, at baseline and in response to an altitude hypoxia trek (300 km distance in 19 days, up to 5,140 m of altitude), of five Italian trekkers (45 ± 17 years, BMI = 25.96 ± 3.61 kg/m^2) and six Nepalese porters (30 ± 8 years, BMI = 24.36 ± 4.70 kg/m^2), all males, taking part to the Himalayan expedition “Kanchenjunga Exploration & Physiology” (2). A subset of 14 out of 21 variables initially included were selected,consisting of anthropometric, endocrine and cardiorespiratory data. To reduce the number of variables, we checked Shapiro-Wilk's test for normality and Leven's test for homoscedasticity and run a linear mixed model obtaining Akaike and Bayesian information criterion (IC); then, among variables of the same physiological subsystem, we selected those ones with the lowest IC. Analyses were carried out with RStudio Version 1.1.456; comparisons were made with the "NetworksComparisonTest" package, and graphs were made with the "qgraph" package. Four graphs were built and compared; nodes were represented by physiological variables and edges by partial correlation coefficients. Aspects of invariant network structure and invariant global strength (3), along with the centrality measures of strength and expected influence, were calculated. The hyperparameter γ of EBICglasso estimation was set at 0.1 (4). The number of iterations of the permutation method for the significance test was set at 100. The most likely finding was the difference in pre vs post networks of Italians about invariance measures (p=.09 for both network structure and global strength). The difference for centrality measures was far from being significant. The low sample size, as a common pitfall in field studies at high-altitude, may have limited the robustness of comparisons about the differential response of centrality, for what concerned SpO_2 and body fluids dynamics. It can be speculated that the physiological interconnectedness of Italian trekkers, rather than Nepalese porters, was more affected by the altitude trek. For further models, it can be recommended to consider both significance and coefficients' threshold, dealing with the assumption to obtain an adequate balance for density/sparsity of graphs. Statistical testing of topological, whole structure, and centrality measures represents a valuable approach to compare correlation graphs, with possible original and meaningful insights for high-altitude physiology.



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PC36

Chronobiological Immunology: The Influence of Diurnal Variation on Circulating Lymphocytes

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There has been increasing interest surrounding the impact of the 'biological clock' on immunological responses. In 2017, the Nobel Prize in Physiology or Medicine was awarded to a team that discovered the genetic molecular mechanisms which regulate circadian rhythms. Many physiological events including immune responsiveness are linked to the circadian rhythm. Previous studies have demonstrated that a majority of patients with allergic rhinitis experience their most severe symptoms in the morning. A study of 765 patients in France with allergic rhinitis determined that the major symptoms of sneezing, stuffy nose and runny nose occur in the morning in approximately 70% of patients (1). While the increased severity of symptoms of allergic rhinitis with regards to circadian rhythm has been established, there is minimal evidence on the link between the symptom presentation and immune cell variation. One study analysed the diurnal variation of lymphocyte subsets of 25 healthy Caribbean participants, finding that CD4+ helper T-cell counts increased throughout the day, CD56+ and CD16+ natural killer cell counts showed no change and CD8+ cytotoxic T-cells and CD19+ B-cell counts increased between 8:30am and noon, demonstrating no change during the afternoon (2). While this study indicates some immune cell fluctuations, there is a lack of literature regarding the effect of time on lymphocyte subsets during the day. Due to the lack of data on the effect of diurnal variation on lymphocyte subsets, it is not clear whether the time of day should be a consideration during common blood tests. The aim of this study is to determine any variations between morning and afternoon blood collection on the prevalence of circulating CD3+, CD3+CD4+, CD3+CD8+, CD19+, CD16+ and CD56+ cells. 25 human participants will provide blood samples at 8:30am and 4:30pm timepoints in a single day. Participants will be randomly recruited and will consist of both males and females between 18 – 35 years of age. Samples will be analysed using flow cytometry (3) and live-cell gating will be conducted using propidium iodide assessment. A Student's paired *t*-test will be applied as the primary form of statistical analysis to assess for any relevant diurnal variation induced changes. The literature suggesting that lymphocyte counts may increase during the night-time, and remain elevated throughout the morning also supports the observation that several immune conditions are exacerbated in the morning. As such, it is hypothesised that the prevalence of lymphocyte subpopulations will be the greatest in the morning sample. This study is being conducted as part of an Honours Research program with data collection ongoing.

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PC37

The acceptability, feasibility, and effectiveness of wearable activity trackers for increasing physical activity in children and adolescents: A systematic review.

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Wearable activity trackers (wearables) may offer an affordable and accessible way of increasing child and adolescent physical activity (PA). Wearables have been shown to successfully increase adult PA, however less is known about their impact on child and adolescent PA. The aim of this review was to examine the acceptability, feasibility and effectiveness of wearables, and their potential mechanisms of action, for increasing PA, in 5- to 19-year olds. A systematic search was conducted of 7 databases (from the start date of each database to December 2019). The following eligibility criteria were used: (a) published in English; (b) included participants aged 5- to 19-years; (c) examined the use of a wearable within an intervention, acceptability or feasibility study; and (d) measured PA and/or experiences of using a wearable. The presence or absence of behaviour change techniques (BCTs), were coded, using the behaviour change technique taxonomy v1. A narrative review (effectiveness findings), and a thematic synthesis (acceptability and feasibility findings) were conducted. The heterogeneity of study samples, designs, and PA outcomes did not permit a meta-analysis. Thirty-three studies were included. There was some evidence that wearables increase steps, and moderate-to-vigorous-intensity PA and reduce sedentary behaviour. Some evidence suggested that multi-component interventions may be more effective than using a wearable alone. On average, 7.8 BCTs (range:2-12) were present in studies exploring the effectiveness of wearables. From the thematic synthesis, four analytical themes (14 subthemes) were identified: (1) perceived facilitators and barriers of using a wearable may impact device use, (2) affective attitude: feelings towards using wearables, (3) wearables ease of use, understanding of PA outputs, and perceived impact on PA varies between devices and individuals, and (4) perceived mechanisms of action underlying wearables impact on PA. Children and adolescents reported wearables increased their PA, via wearable features that promote self-monitoring, goal setting, feedback, and competition. However, barriers of wearable use (e.g. technical difficulties, novelty effect) were present. Thus, there is some evidence that wearables are an acceptable, feasible and effective way of increasing PA, in 5- to 19-year olds. Future interventions may benefit from incorporating multiple intervention components and more BCTs, and aiming to overcome barriers of wearable use.

This review is registered with PROSPERO (CRD42020164506) and has not been published in a scientific journal.

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PC38

A family-based acceptability and feasibility study, using wearable activity trackers to increase physical activity in 5- to 9-year old children.

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The family environment is an important social correlate of child physical activity (PA), with parent, and sibling PA levels positively correlated with child PA. Wearable activity trackers (wearables) incorporate numerous behaviour change techniques (feedback, self-monitoring, goal-setting) that has previously been identified as mechanisms of increasing child PA, in a family-setting. There is some evidence that wearables can increase steps, moderate-to-vigorous-intensity PA, and reduce sedentary behaviour in children and adolescents, however few studies have explored wearables in a family-setting (*n*=3 identified in our previous systematic review). The aim of this study was to explore the acceptability and feasibility of wearables to increase PA in 5- to 9-year olds, in a family-setting. Families were recruited via social media, staff emails and existing connections, and were eligible if they had: at least one child, aged 5- to 9-years, living in the same household, a smartphone/computer device with Bluetooth/USB port, and the internet/Wifi. Physical activity and perceived barriers of wearable use and PA were measured in week one and five. Family members wore the ActiGraph GT3X+ on their right hip for 7-consecutive days, and a parent/guardian completed a questionnaire (20 questions), which was developed using the COM-B behaviour change model, and Theoretical Domain Framework, and explored families' capability, opportunity and motivation for PA, and using a wearable to increase child PA. Family members wore the Fitbit Alta HR for 4-weeks, and completed weekly surveys, which assessed the acceptability of using the Fitbit. Family members participated in a focus group to discuss their experiences using the Fitbit. Data collection is currently ongoing, with (to date) 12 families participating (*n*= 15 parents, *n*= 14 target children, *n*=5 siblings), and six families expressing interest in taking part. A thematic synthesis will be conducted for qualitative data, and changes in perceived acceptability (weekly surveys), barriers to wearable use and general PA (pre- and post-barriers questionnaire), and PA levels (pre- and post-ActiGraph data) will be presented. Statistical tests will not be conducted, given the primary aim of the study is to assess feasibility, and sample size calculations were not performed. By the time

of this conference, at least 12 families will have completed data, and their findings available to present. The findings of this study will inform the development of future family-based wearable interventions. The use of the COM-B model and Theoretical Domains Framework to develop the barriers questionnaire, enables researchers to use the Behaviour Change Wheel to identify future intervention functions and policies that may increase PA.

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PC39

Proportionate age-related declines in muscle strength, respiratory function and performance in Master Track Cyclists

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Background: There is an age-related decline in respiratory and musculoskeletal function, irrespective of physical activity levels, but it is not known to what extent each system contributes to decreasing performance in ageing master track cyclists.

Objective: To determine 1) whether the age-related rate of decline in respiratory function, respiratory muscle strength and muscle power in master track cyclists are uniform and 2) to what extent the respiratory and skeletal muscle system contribute most to the age-related reduction in athlete performance.

Methods: Sixty men (mean age 61±11) and 15 women (mean age 50±12) master cyclists were recruited during the Track Cycling Masters World Championship 2019 in Manchester. Respiratory function and respiratory muscle strength were determined using spirometry and measurement of maximal mouth pressures, respectively. The architecture of the vastus lateralis muscle was determined using ultrasonography, and muscle power by countermovement jump.

Results: Forced expiratory volume in the first second, forced vital capacity, peak expiratory flow, maximal inspiratory and expiratory pressures, fascicle length, muscle thickness, take-off velocity,

jump power, jump power per body mass, handgrip strength, haemoglobin concentration and performance correlated negatively with age ($p \leq 0.043$). The age-related rate of decline did not differ significantly between parameters ($p=0.124$), but it was slower for haemoglobin concentration ($p=0.041$). Take-off velocity was the major determinant of performance in 200m, 500m and 2000m track cycling disciplines ($R^2_{adj}=0.675$, 0.786 and 0.769 respectively; $p<0.001$).

Conclusion: Pulmonary function, peripheral skeletal muscle function and cycling performance showed a similar relative age-related decline in master track cyclists. It appeared that the major determinant of performance in master track cycling was the take-off velocity during a countermovement jump.

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PC40

Peripheral Innocuous Cold Stimulation in Humans: Investigating the Thermogenic Response and Thermal Sensation in Women and Men

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Background: As endotherms, humans face the vital challenge of maintaining an elevated and constant body temperature under varying environmental conditions, through the metabolic production of heat (thermogenesis). However, despite many decades of research aimed at understanding the thermoregulatory responses under varying cold stresses, very little is known regarding the variability in the thermogenic responses and the influence of skin temperature in this variability. Therefore, the aim of this study is to assess the relationship between skin cooling and the resulting heat production, shivering responses, and the resulting oxidative fuel selection in cold-exposed men and women to better understand the determinants in the cold-defence responses in humans.

Methods and Results: By combining indirect calorimetry with surface electromyography, we examined substrate utilization, shivering intensity and muscle fiber recruitment when clamping skin temperature at 3 different temperatures (31°C, 29 °C and 27 °C) using a liquid-conditioned suit to provide a controlled uniform stimulus. Using this paradigm, we were able to carefully characterize the effect of skin temperature on thermogenesis and fuel selection in lean, healthy men (n=12) and

women (n=12) and investigate its relationship with the subjective evaluation of thermal sensation. In addition, we examined whether sex-differences in cold-induced shivering intensity and fuel selection persist when exposing men and women to the same mean skin temperature. Our results showed a skin temperature-dependent increase in shivering intensity and metabolic heat production ($P < 0.0001$), including sex-dependent difference in heat production at all three skin temperatures (31°C: $P = 0.0003$; 29°C: $P = 0.0009$; 27°C: $P = 0.0041$). Using an 11-point thermal comfort like scale, participants reported feeling progressively colder with every 2°C decrease in mean skin temperature compared to the baseline period ($P < 0.0001$). At a skin temperature of 27°C the shivering burst rate (27 vs. 31: $P = 0.0097$; 27 vs. 29: $P = 0.0376$), burst shivering intensity (27 vs. 31: $P < 0.0001$; 27 vs. 29: $P = 0.0002$) and continuous shivering intensity (27 vs. 31: $P = 0.0001$; 27 vs. 29: $P = 0.0172$) were all significantly higher than at a skin temperature of 29°C and 31°C. Nevertheless, our results show that although participants were all clamped at the same skin temperature there was significant variability in both metabolic heat production and shivering intensity between individuals.

Conclusions: These data suggest that heat production, shivering intensity, shivering pattern, and thermal comfort change in a temperature-dependant fashion. Apart from the metabolic heat production, these responses are similar between men and women. Despite clamping mean skin temperature at 3 different temperatures, there was tremendous inter-individual variability in the means used to defend their core temperatures. While whole-body heat loss and the consequent increase in metabolic heat production appears to be driven primarily by physical properties such as differences in body mass, body composition or body surface area, the varied shivering responses may be determined by factors such as genetics, training status or regular cold exposure. Further work is required to examine the thermogenic mechanisms that are recruited under these various skin temperatures.

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PC41

Fatigue and quality of life in chronic venous insufficiency patients: a French survey

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Introduction. Lower extremity chronic venous disease (CVD) is a common cause of lower extremity pain and discomfort in the adult population of Western countries. The typical self-reported symptoms of CVD are aching, cramping, fatigue, heaviness, restlessness, and swelling. Physical activity is often evoked as a non-pharmacological and non-surgical intervention that can prevent venous disease progression and complications, as well as reduce symptoms and improve quality of life in CVD sufferers (1).

Objectives. The aim of this study was to quantify fatigue and quality of life in CVD sufferers within a large cohort representative of the French population. Moreover, the link between physical activity and fatigue/quality of life of CVD sufferers was also further investigated.

Methods. A survey was conducted on a representative sample of the adult French population, which comprised 3,008 subjects aged 18-93 years. All the procedures were approved by a local institutional ethics committee and performed in accordance with the Declaration of Helsinki (except for registration in a database). The venous symptoms were scored using a customized questionnaire to classify the participants into two groups: non-CVD subjects and CVD sufferers. Physical activity data were acquired using the Godin-Shephard Leisure-Time Physical Activity questionnaire (2). Fatigue was scored using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) questionnaire (3). Quality of life scores of CVD subjects were acquired using the CIVIC-14 questionnaire (4). Stepwise procedures of selection of variables by using AIC and BIC criteria were performed. The beta coefficient (β) is the degree of change in the outcome variable for every one unit of change in the predictor variable.

Results. In the French population, 32% of people were classified as CVD sufferers. Fatigue score was worst in CVD sufferers than in non-sufferers (35.0 ± 11.0 vs 40.4 ± 9.3, respectively; $\beta = 5.49$; 95% CI 4.73 to 6.24; $p < 0.001$). Within CVD sufferers, there was a relationship between fatigue score and quality of life score ($\beta = 1.25$; 95% CI 1.20 to 1.31; $p < 0.001$). Physical activity influenced the fatigue score in CVD sufferers (37.1 ± 10.4 vs 34.4 ± 11.1 in active and inactive, respectively; $\beta = 3.38$; 95% CI 1.68 to 5.82; $p < 0.001$). Physical activity affected the quality of life score in CVD sufferers (74.7 ± 20.1 vs 71.4 ± 21.5 in active and inactive, respectively; $\beta = 3.90$; 95% CI 0.56 to 7.26; $p = 0.022$).

Conclusions. Fatigue, assessed using a validated questionnaire (i.e. FACIT-F), can significantly discriminate between CVD sufferers and non-sufferers. The fatigue score was also a good predictor of quality of life in CVD sufferers. Active CVD sufferers were less fatigued and their quality of life was less impacted. Therefore, physical activity might be considered as a treatment to alleviate CVD-induced symptoms of fatigue.

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PC42

Thirty minutes of standing recovery enhance post-exercise hypotension.

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Effective control of vascular conductance after exercise is essential to avoid post-exercise hypotension (1). Such a vascular control is due to a balance between sympathetic vasoconstriction and metabolic vasodilation (2). Previous evidence suggests that immediately after exercise there may be relatively greater SNS effects on vascular control (3) associated with persisting vasodilation and reduced vasodilator function (4). As the recovery progresses, sympathetic vasoconstriction effects become less evident and vasodilator function normalizes. Indeed, although most documented hypotension episodes occur within 10 minutes after exercise, athletes often report presyncope symptoms over 30 minutes of recovery (5). Thus, this project assessed the cardiovascular adjustments to posture changes immediately after exercise than after a 30 min recovery. In compliance with the ethical standards, 15 male subjects practicing different sports (weightlifting, tennis, soccer, cycling, running) were passively tilted twice (1 min supine and 1 min upright) before and after a 30 min standing still recovery (REC30; if not interrupted by pre-syncopal symptoms). This was performed at REST as well as after a 1-min Wingate test (WNG) and after a long-lasting incremental test to voluntary exhaustion (INC; 15W / min). Such exercises were chosen to obtain two different levels of metabolic vasodilation in the immediate post-exercise period. During all phases of the experiments, beat-by-beat blood pressure (BP) and heart rate (HR) were collected continuously. Cardiac stroke volume (SV) was calculated upright just before both tilt tests through cardio-impedance analysis. Baroreceptor gain (BG) was calculated as ΔRR / ΔSAP. Percentage changes in systolic (SAP), mean (MAP), and diastolic (DAP) BP as well as in HR, and BG from supine to standing position were analyzed via 2-way repeated measures ANOVA. DAP and SV were considered related to peripheral resistance. Among all subjects, syncope (1) or presyncope (3) appeared only in weightlifters. In the other 11 subjects, REC30 consistently increased the heart rate buffering response to standing inclination (+5Δ%, +16Δ%, +15Δ%; all $p<0.05$; REST, WNG, INC) and augmented the SAP drop to the upright tilting (+5Δ%, +16Δ%, +15Δ%; all $p<0.05$; REST, WNG, INC). Pre-exercise MAP rose to standing transition more before (+2.5±0.8%) than after REC30 (-2±0.2%; $p<0.05$). Post-exercise MAP to standing transition fell immediately after exercise (-2±2.1%, -12±4.3%; WNG, INC), but such a fall was much greater after REC30 (-12±1.1%, -22±1.4%; all $p<0.05$; WNG, INC). DAP increased to standing inclination at REST and after WNG, but dropped after INC (-5±4.9%) to a greater extent after REC30 (-14±1.1%; $p<0.05$). Post-recovery BG was lower ($p<0.05$) after INC only. SV was consistently lower ($p<0.05$) after INC than REST and WNG throughout the entire recovery period. Our data show that the standing transition from supine induces augmented hypotension after a 30 min standing recovery than immediately after exercise, which cannot be effectively counteracted despite an effective HR buffering response. Such an effect is greater after a long-lasting strenuous aerobic exercise compared to a short bout of supramaximal exercise and, according to DAP and SV data, enhanced hypotension may be due to ineffective control of the peripheral resistance.

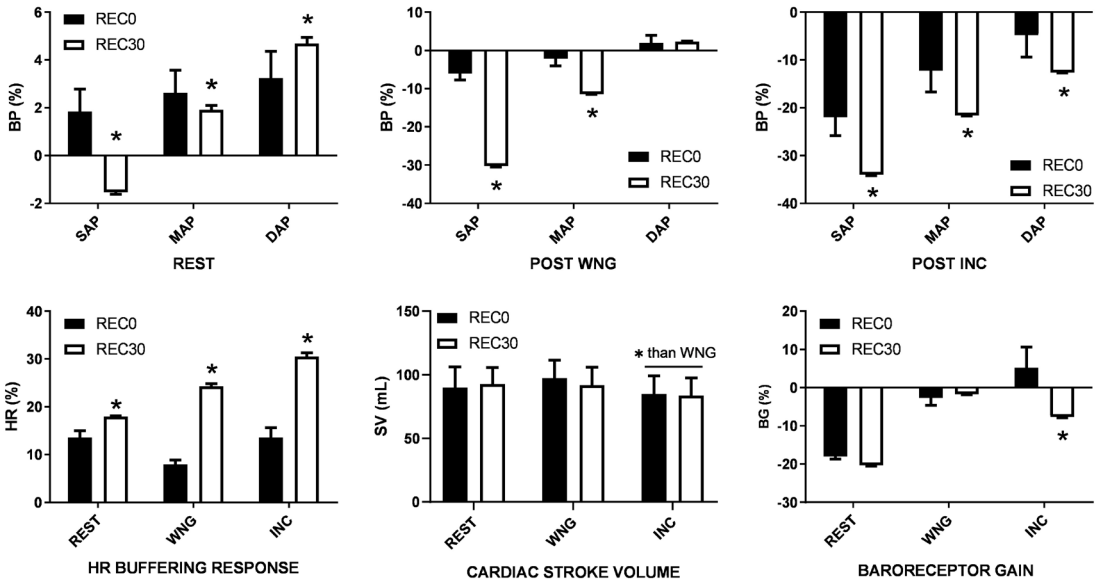

Figure shows percent changes in blood pressure, heart rate, cardiac stroke volume, and baroreceptor gain during the transition from supine to standing posture before (REC0, black bars) and after (REC30, white bars) a 30 min standing recovery at rest, after a 1 min Wingate test (WNG), and after a long lasting incremental test to exhaustion (INC). * p<0.05.

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PC43

ENDURANCE TRAINING-INDUCED INCREASE IN MUSCLE OXIDATIVE CAPACITY WITHOUT LOSS OF MUSCLE MASS IN YOUNGER AND OLDER RESISTANCE-TRAINED MEN

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While concurrent training is regularly used in older populations, the inverse relationship between fibre size and oxidative capacity suggests that endurance training in resistance-trained individuals may result in some loss of resistance training-induced gains in muscle mass, which may be more pronounced in older people. We investigated the impact of superimposed endurance training in younger (28.5 ± 4.8 years; n=8) and older (67.5 ± 5.5 years; n=7) highly resistance-trained men. Participants underwent a 10-week endurance cycling training programme consisting of five 6-min intervals at 75% max heart rate (HRmax) separated by 4-min intervals at 90% HRmax. The anatomical cross-sectional area (ACSA) of the thigh muscles, as determined with MRI, was 24% smaller in older compared to younger participants ($p<0.001$). Although maximal oxygen consumption (VO2max) was also lower in the older group ($p<0.001$), VO2max per kg body mass did not differ significantly between younger and older participants. Histological analyses of biopsies of the m. vastus lateralis showed that endurance training induced an increase in succinate dehydrogenase activity in both younger and older participants ($p\leq0.043$), and an increase in the number of capillaries around type I fibres ($p=0.017$). The superimposed endurance training did not induce a significant decrease in thigh ACSA, fibre cross-sectional area, or knee extensor maximum voluntary isometric force. These observations indicate that adding endurance training to resistance training can lead to positive endurance-related adaptations without negative consequences for muscle size and strength in older and younger resistance trained people.

PC44

Exploratory factor analysis of relationships between latent training load and overreaching variables in mixed martial arts

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Effective athlete preparation requires an understanding of the dose-response relationship between training load (TL) and fatigue. Acute overreaching (OR) related to fatigue may present as muscle soreness and changes to mood(1). Non-functional overreaching (NFOR) may present as disturbed sleep(2) and inhibited psychomotor speed(3). Understanding the link between TL and fatigue can allow NFOR to be avoided(1). Inherent difficulties in measuring the latent physiological responses to exercise means multiple measures of fatigue may be required to understand the effects of a given TL. Exploratory factor analysis (EFA) may provide insight to which measured variables best reflect these latent responses. Mixed martial arts (MMA) is a cross-discipline combat sport requiring multiple forms of training. Previous research has demonstrated that MMA training does not feature weekly changes in TL or fatigue, making identification of a dose-repose relationship difficult(4). Therefore the aim of this study was to determine the relationship between the latent variables related to TL and the latent variables related to OR and NFOR in MMA training using EFA(5). Following institutional ethical approval n = 14 human MMA participants (age = 22 ± 4.4 years; habitual mass = 71.3 ± 7.7 kg; stature = 171 ± 9.9 cm) took part in this study for 8 consecutive weeks. Participant's TL (sessional rating of perceived exertion, strain and monotony) was recorded after every training session(6). At

the end of each day participants recorded soreness using a CR10 scale for the following body regions: head and neck; shoulders and arms; upper torso; lower torso; legs. Fatigue score was measured via short questionnaire of fatigue at the end of each day(1). The following variables were measured as central fatigue proxies at the start of each week: overall sleep quality and sleep efficiency via Pittsburgh Sleep Quality Index (PSQI)(2); choice reaction time (CRT) and CRT correct count via Deary-Liewald CRT test(3). EFA was completed in JASP 0.14.1.0 following established protocols(5). Relationships between factors were assessed via Pearson's correlation coefficient. 112 observations of 15 variables were included in the model which was found to have acceptable RMSEA = .069 and good TLI = .969 whilst explaining 64% of total variance in the data. Three factors (Figure 1) were identified via scree plot inflection point and an acceptable number (max=5) of factor loadings ≥±.30. Factor 1 may be labelled 'Load' and contains relationships between each TL variable apart from strain. Factor 2 may be labelled 'Fatigue and Soreness', containing relationships between body region soreness (apart from legs) and fatigue score. Factor 3 may be labelled 'Central Effects', containing relationships between sleep quality, sleep efficiency and CRT correct count. Factor 1 was found to have a moderate, negative relationship with Factor 3 (r= -.431), and a trivial positive relationship with Factor 2 (r=.095). Factor 2 also had a trivial, negative relationship with Factor 3 (r= -.021). The three factor model identified suggests static MMA TL causes central fatigue effects that may be indicative of the onset of NFOR in this population, despite an absence of acute changes in OR measures(4).

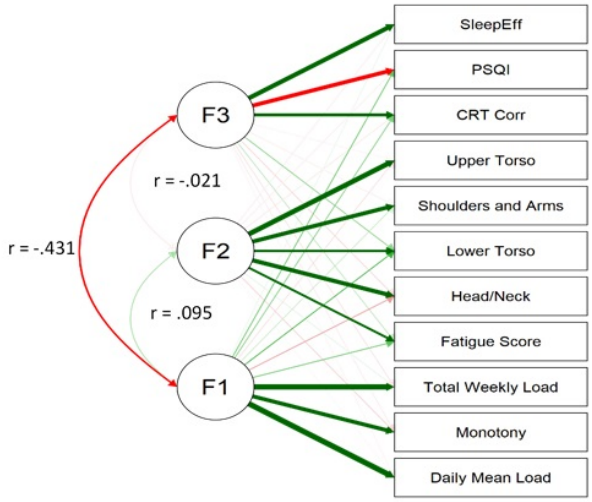

Figure 1 – Model showing the interactions of F1 (Load), F2 (Fatigue and Soreness) and F3 (Central Effects) and associated loading variables
Nb. Green lines indicate positive relationships between variables; red lines indicate negative relationships between variables

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PC45

The Effects of Acute High Altitude Exposure and Arterial Blood Gas Manipulation on Neurovascular Coupling in Healthy Humans

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Neurovascular coupling (NVC) is the temporal and spatial link between local neuronal activity and regional cerebral blood flow. High altitude elicits several vasoactive stimuli, which alter cerebrovascular tone through changes in arterial blood gases (ABGs). Previous work from our group found NVC remains intact during ascent to high altitude. However, given the incremental nature of the ascent profile, NVC was assessed in individuals that were fully acclimatized. This study aimed to assess the effects of 1) rapid ascent to and residence at high altitude (pre vs. post acclimatisation) and 2) the isolated and combined effects of ABG manipulation on NVC response magnitude. We hypothesized that acute exposure to high altitude would impair NVC.

12 healthy participants (5 female) were recruited from a large expedition. Arterial blood draws from the radial artery and NVC were measured at baseline (1045m) and on days 2 and 9 at altitude (3800m). End-tidal gas challenges were used to elicit the isolated and combined effects of blood gas manipulation, specifically isocapnic-hypoxia and hyperoxia gas challenges. Cerebral blood velocity was measured through the posterior cerebral artery (PCAv) using transcranial Doppler ultrasound. NVC was measured as the peak (Δpeak) and mean (Δmean) changes in PCAv during intermittent visual stimulation (VS; strobe light). In addition, NVC was quantified as the change in total area under the curve (ΔtAUC) during VS. Lastly, the NVC waveform was compartmentalised into three distinct temporal regions (0-10, 11-20- and 20-30-seconds post-stimulus onset) to assess mechanistic involvement during the NVC response.

Immediate and chronic exposure to high altitude elicited hypoxic-hypocapnia, evidenced by reductions in both PaO_2 and $PaCO_2$ ($P<0.001$). However, arterial pH was unchanged following acute exposure to high-altitude ($P=0.72$) due to reductions in arterial bicarbonate ($P< 0.001$), indicative of a

renally-mediated compensatory metabolic acidosis. No significant differences were observed for Δpeak, Δmean, ΔtAUC NVC response between baseline and days 2 and/or 9 at altitude (P>0.05). Moreover, neither the independent effects of hypoxia and hypocapnia, nor combined hypoxic-hypocapnia affected Δpeak, Δmean or ΔtAUC NVC response (P >0.05). Finally, no significant differences in the magnitude of the haemodynamic response across temporal regions of the NVC response were observed during acute and sustained exposure to high altitude (P>0.05) and/or isolated and combined manipulation of ABGs (P>0.05).

Our results reveal remarkable stability of the NVC response magnitude during pronounced physiological stressor associated with rapid ascent and residence at high altitude.

PC46

Validity and reliability of physiological and perceptual responses during a novel treadmill-based Soccer Referee Simulation (SRS)

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Soccer refereeing represents a highly demanding activity, requiring referees to perform complex decision-making processes under challenging physiological conditions (Weston et al. 2012). Growing interest therefore exists with respect to the physical and physiological demands imposed during match play (Castagna et al. 2007). However, as large levels of inter-match variability are evident in the activity profiles of referees, the detection of real systematic changes in performance outcomes between matches is difficult (Weston et al. 2011). Simulation protocols that mimic match play represent alternative approaches that have been used to standardise the internal and external demands imposed on soccer players (Russell et al. 2011). Nonetheless, a valid and reliable protocol does not currently exist for soccer referees. The current study therefore sought to explore the validity and reliability of the physiological and perceptual responses elicited during a novel treadmill-based Soccer Referee Simulation (SRS). Following the collection of baseline measures and habituation procedures, eight male soccer referees (age: 30.1 ± 3.8 years; stature: 178.4 ± 8.8 cm; body mass: 77.1 ± 10.7 kg; $\dot{V}O_{2max}$: 53.2 ± 4.1 mL·kg·min^{-1}) completed a single trial of the SRS whereby measures of heart rate (HR), oxygen uptake ($\dot{V}O_2$), blood lactate concentrations, and differential ratings of perceived exertion (RPE) were obtained. Referees' HR responses were also monitored during a series of competitive matches (5 match observations per referee). For the reliability aspect of the investigation, eight well-trained males (age: 25.1 ± 4.2 years; stature: 177.6 ± 9.0 cm; body mass: 79.6 ± 12.0 kg; $\dot{V}O_{2max}$: 50.6 ± 4.8 mL·kg·min^{-1}) were initially habituated, and thereafter completed three separate trials of the SRS during which the reliability of the selected outcome variables were ascertained. Trials were separated by 3-7 days and performed under standardised conditions (temperature: 19°C; humidity: 40%). Paired sample t-tests explored any differences in measures of HR between the SRS and match play, with ANOVAs used to detect any systematic differences in physiological and perceptual responses between reliability trials. Measures of relative and absolute reliability were ascertained through the calculation of intraclass correlation coefficients

(ICC) and coefficients of variation (CV), respectively. Data are presented as mean ± SD. No differences were evidenced between the SRS and match play in relation to measures of mean HR (SRS: 79.6 ± 6.6%; Match Play: 80.6 ± 6.0%; *P*=0.444; ES=0.29), peak HR (SRS: 90.0 ± 7.1%; Match Play: 93.8 ± 3.5%; *P*=0.074; ES=0.74), or HR-based training impulse (SRS: 58.3 ± 20.2 au; Match Play: 61.6 ± 14.1 au; *P*=0.498; ES=0.25). Additionally, no systematic differences were detected between reliability trials for any of the measured outcome variables (P≥0.293), whilst good levels of reliability were observed for measures of mean HR (ICC=0.94; CV=3.1%), peak HR (ICC=0.93; CV=2.2%), HR-based training impulse (ICC=0.95; CV=10.0%), mean $\dot{V}O_2$ (ICC=0.95; CV=2.6%); blood lactate concentrations (ICC≥0.89; CV≤11.5%), and differential RPE (ICC≥0.94; CV≤15.1%). Together, the present findings have demonstrated the SRS to be a valid and reliable protocol that closely replicates the physiological and perceptual responses elicited by soccer referees during match play.

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Acknowledgements :-

The authors would like to thank each of the participants for their commitment to this study, as well as Mr. Joan Aguilera-Castells for his support with data collection. The authors also wish to thank the Scottish Football Association who provided funding to support a PhD studentship for Gary McEwan.

PC47

Self-reported physical functional health predicts future bone mineral density in EPIC-Norfolk cohort

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Background

Osteoporosis is a growing – yet preventable – public health issue, affecting more than 1 in 3 women. Costing the NHS an estimated £4.4 billion per year, early detection of low bone mineral density (BMD) and prevention of osteoporosis in mid-adulthood is considerably more effective than pharmaceutical intervention in later life. Failure to reach and maintain peak BMD during adulthood (e.g. by regular weight-bearing exercise) is a critical factor in determining future osteoporosis risk. Self-reported physical functional health, a measure determined by health and lifestyle factors such as physical activity, has previously been shown to predict health outcomes such as mortality and stroke risk. In this study, we seek to examine whether self-reported physical functional health may be predictive of future BMD.

Methods

The association between physical functional health and future BMD was investigated in participants aged 40-79 years enrolled in the European Prospective Investigation of Cancer-Norfolk study. Associations between a participant's SF-36 physical component summary (PCS) score, measured 18 months after baseline health check, and broadband ultrasound attenuation (BUA – a measure of BMD), measured 2-5 years after baseline, were examined using sex-specific multivariate linear and logistic regression analyses adjusting for age, BMI, medical co-morbidities, lifestyle and socioeconomic factors.

Results

Complete data from 10,203 participants (57.1% female), mean (SD) age 61.5 (8.9) years were analysed. Low PCS score was associated with a reduced BMD; in the linear regression analysis, for every one unit decrease in PCS score in men and women, there was a 0.1 dB/MHz ($p<0.001$) decrease in mean BUA. In logistic regression analysis, low PCS score (less than one standard deviation (1 SD) below the sex-specific mean) was associated with having a poor BUA value (less than 1 SD below sex-specific mean), which is indicative of osteopenia. Results were highly significant in women [odds ratio (95% confidence interval) 1.527 (1.263, 1.847); $p<0.001$], but lesser so in men [odds ratio (95% confidence interval) 1.406 (0.991, 1.994); $p=0.056$]. Furthermore, low PCS score was also shown to predictive of very low BUA (less than 2.5 SD below sex-specific mean) in women, which is indicative of osteoporosis [odds ratio (95% confidence interval) 5.960 (2.084, 17.049); $p<0.001$], although findings were less significant in men [odds ratio (95% confidence interval) 2.823 (0.966, 8.255); $p=0.058$].

Conclusion

Study findings indicate that self-reported physical functioning is a tool capable of predicting future BMD and identifying at risk individuals in an apparently healthy population, especially in women. In the current climate, where increased sedentary activity and a reduction of routine medical appointments will have implications on bone health, self-reported functional health may prove a useful and inexpensive indicator to stratify populations by risk of low BMD. Future research is needed to examine the clinical applications of self-reported physical functioning scores and its capability to identify poor BMD before further decline.

Acknowledgements :-

This work was supported by an institutional grant from the University of Aberdeen Academic Centre for Applied Clinical and Translational Research into Ageing (ACTRA). The role of this grant was to financially support a student researcher from November 2020 to January 2021.

PC48

Design of PerformGlove for Quantifying Performance of Scientific Fieldwork Tasks When Wearing a Mars Extravehicular Mobility Unit (MEMU) Glove

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Background: It is known that wearing extravehicular mobility unit (EMU) gloves decreases performance such as grip strength, dexterity, and time to completion of tasks. They may also cause fatigue and injury such as pain, swelling, abrasions, onycholysis, and even nerve impingement (Charvat, 2015). Astronauts will need to carry out scientific fieldwork tasks in partial gravity, extreme temperatures, and dusty conditions during extravehicular activities (EVAs) on the Martian surface. This contrasts with the current near pristine environment in space that current EMU gloves are designed for. Further, astronauts may undergo significant physiological deconditioning on the way to Mars. Overall, there are safety implications and the possibility of performance reduction or even no-go of EVAs. Currently, there is no publicly available Mars EMU (MEMU) glove testing protocol to choose the best of future designs from commercial companies.

Methods: A "data glove", named PerformGlove, was developed to measure performance (Figure 1).

Design Criteria:

- Provide protection for delicate sensors
- Minimally impact mobility, tactility, comfort
- Thin
- Breathable
- Lightweight
- Not impact on sensor performance
- Fit inside an EVA glove
- Allow some interchange of sensor size and type depending on individual anthropometry
- Give accessibility to wiring and for sensor placement
- Have pre-wiring ability
- Be multi-layered
- Be reusable
- Variability in anthropometrics

Sensors: Glove sensors are either Bluetooth or wired. Data will be collected via channels into a PowerLab (AD Instruments, NZ) or equivalent. A display monitor for calibration and real-time monitoring will also be utilised. All sensors will be calibrated via manufacturer specifications, and pre-study measurements and maximums taken of individual participants.

PerformGlove uses a variety of sensors and fabrics building on previous EMU glove testing efforts for previous generations of EVA glove (Korona, 2018; Reid & McFarland, 2015). Measurements taken include humidity (relative humidity %, mini humidity sensor), temperature (degrees Celsius, thermocouple sensor), nail strain (unitless μ-strain value, strain gauge sensor), fingertip blood

perfusion (perfusion units, Laser Doppler Perfusion Monitor sensor), pressure (mmHg, BeBop smart fabric), range of motion (% maximum, bend sensors), skin conductance micro Siemens, Galvanic Skin Response sensora), and barometric pressure (psia, mini barometric pressure sensor PS-2KC, Kyowa Electronics). Complementary measures include anthropometrics (3D scanning), palm moisture level (%, MoistSense meter), hand grip strength (lbs, Jamar hydraulic dynamometer), Rating of Perceived Exertion (Borg, 1982), and data from a comfort questionnaire.

Testing: EMU gloves can be tested independently or as part of a whole suit EMU study. Various analogues are implemented such as pressurised glove boxes, ground analogue missions, parabolic flight, partial/microgravity offload systems, neutral buoyancy pools, and underwater analogues (Norcross, 2020).

Mars EVA (MEVA) tasks reflecting the scientific fieldwork to be done on Mars should be selected primarily using the Generalisable Skills and Knowledge List for Exploration Missions (Stuster, 2019) including operating scientific instruments, tool use, collecting Martian samples, and dealing with Mars regolith contamination.

Impact and Future Directions: Standardised MEMU glove testing protocol for mEMU glove candidates in a variety of analogues. There are various clinical applications such as assessing weakness, mobility, and dexterity as well as monitoring rehabilitation after injury or surgery.

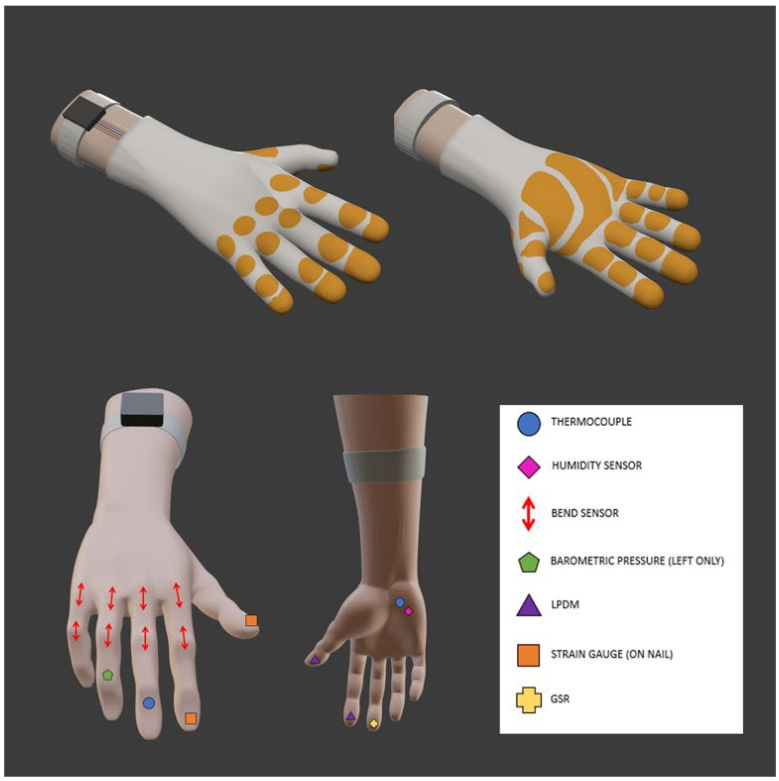

Figure 1: CAD mock-up of sensor placement within PerformGlove. LDPM = Laser Doppler Perfusion Monitor. GSR = Galvanic Skin Response.

Reference 1 :- Borg G (1982). Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* **14,** 377–381.

Reference 2 :- Charvat CM, Norcross J, Reid CR & McFarland SM (2015). Spacesuit Glove-Induced Hand Trauma and Analysis of Potentially Related Risk Variables. *45th Int Conf Environ Syst*1–23.

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Acknowledgements :- Please note this work was submitted for an MSc as a project plan (9,000 words) in lieu of a traditional experimental dissertation due to covid-19. PerformGlove was designed in detail, with description of experiments for it to be used in.

PC49

Exploring associations between bone health and muscle mass and function in masters athletes

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Introduction

Osteoporosis rehabilitation programs widely recognize the beneficial effects of weight-bearing exercise for long-term bone health. However, endurance running has been related to reduced bone mass and potential osteoporosis risk in older age [1], with greater average weekly mileage negatively correlated with lumbar spine BMD [2]. In younger individuals, lean mass is the main determinant of peak bone mineral density [3], and maximum jump power is positively correlated to whole body bone mineral density (BMD) [4]. However, data in older athletic populations are lacking. This study aimed to determine associations between bone health, total lean mass (TLM), and lower limb power in older competitive runners.

Methods

187 masters athletes were recruited as part of the VIBE study [5], consisting of 38 sprinters (71.1 ± 6.7 years; 28M) and 149 endurance runners (69.7 ± 5.5 years; 111M). Right hip BMD (YA T-score) was assessed using dual energy x-ray absorptiometry (DXA) and resulting T-scores recorded. TLM was assessed via DXA, with lower limb muscle power (normalised to body weight) measured during a countermovement vertical jump (Leonardo Jump Mechanography Platform). Participants repeated the jump sequence 3 times, resting for 60 seconds between efforts. The jump with the highest value for power was used for statistical analysis. Hip BMD T-scores, lean mass and jump power were compared across sprinters and endurance runners with unpaired t-test models adjusted for sex. Linear regression models adjusted for sex were performed on each cohort to account for within-

group variability and to determine the relationship between hip BMD T-score, TLM, and jump power. Statistical analysis was performed using Stata (V.16) and significance was assumed as $p<0.05$.

Results

Sprinters mean T-scores was greater than that of endurance runners (0.27±1.21 vs. -0.49±1.15, $p<0.001$). TLM was greater in power athletes compared to endurance athletes (54.11±8.19 vs. 50.88±7.72 kg, $p=0.024$). Lower limb power was also greater in the sprinters (33.31±10.97 vs. 28.80±7.86 W/kg, $p=0.0051$).

Linear regression models adjusted for sex revealed TLM was not associated with hip BMD T-score in sprinters (β [95% CI] 0.89 [-0.68 to 2.46], P=0.258) or endurance runners (-0.45 [-1.23 to 0.33], p=0.253). Jump power was not associated with hip BMD T-score in sprinters (1.96 [-1.166 to 5.088], p=0.211) or in endurance runners (0.097 [-1.028 to 1.22], p=0.865).

Discussion

Compared to masters endurance athletes of a similar age, masters sprinters have greater hip BMD T-score, TLM and lower limb power. However, contrary to what is commonly reported in young individuals, in neither group of masters athletes is there a clear relationship between bone health and lean mass. Power training, as commonly undertaken by sprinters, has unsurprisingly resulted in greater lower limb power, however, has also conferred benefits to bone health, when directly compared to endurance athletes.

These findings may inform practitioners when recommending physical activity for older adults, supporting the notion of encouraging resistance training in older age to improve parameters of bone health such as bone mineral density and decrease the risk of bone diseases.

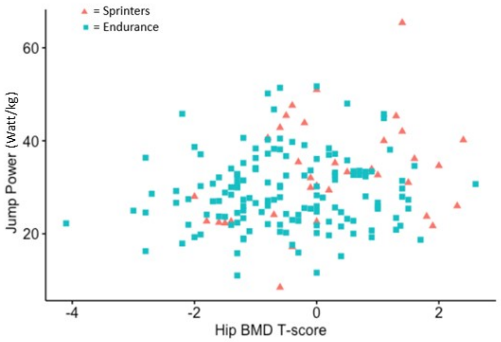

Figure 1: HIP BMD T-score and Jump power (Watt/kg) in sprinters (1) and Endurance runners (2)

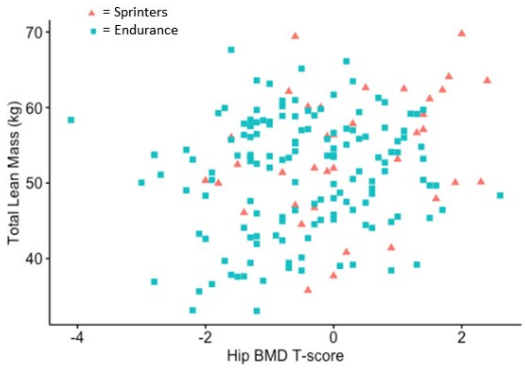

Figure 2: HIP BMD T-score and Total lean mass (kg) in sprinters (1) and controls (2)

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PC50

Acute effect of static handgrip exercise on cerebral artery flow mediated dilation

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Background: Cerebral blood flow and reactivity declines with older age, which increases the risk of cerebrovascular disease and cognitive decline. Cerebral disease is also associated with reduced vascular function and elevated blood pressure. Isometric handgrip (HGEx) training is emerging as an alternative approach for improving vascular function. However, the effect of acute HGEx on cerebral blood flow and function, including endothelial function, is not well understood. Cerebral flow mediated dilation (cFMD) is suggested as a novel and recent technique that provides an index of extracranial cerebrovascular endothelial function. This study aimed to assess the acute effect of isometric handgrip exercise on cFMD and intracranial cerebrovascular reactivity of the middle cerebral artery velocity (MCAv CVR) in middle-aged healthy adults.

Methods: Twelve middle-aged, healthy adults (8 females, age 54 ± 7.8 years; BMI 26±3.5 kg/m^2) performed isometric unilateral HGEx at 30% of maximal voluntary effort for three minutes. Intracranial (MCAv) and extracranial blood flow of the internal carotid artery (ICA) were measured continuously by Transcranial Doppler and Duplex ultrasound, respectively. At baseline and immediately following HGEx, cFMD and MCAv CVR was assessed using a hypercapnic breathing challenge (5% CO_2). Beat-by-beat blood pressure and cardiac output, heart rate and end-tidal of carbon dioxide ($P_{ET}CO_2$) were measured continuously throughout HGEx, cFMD and CVR assessments. Cerebral blood flow,blood pressure and $P_{ET}CO_2$, was analysed using a custom built edge detection and graphing software. Statistical analyses were performed using RStudio. All data are expressed as mean ±SD unless otherwise stated. Hemodynamic responses during HGEx are displayed as change from baseline (%).

Results: Resting mean arterial pressure was 89±10 mmHg, and resting heart rate was 56±7 bpm. HGEx significantly increased extracranial and intracranial cerebral blood flow (ICA diameter, 4.4±4.7%, p=0.025; shear rate, 28.2±18.3%, p≤ 0.001; MCAv, 24.9±21%, p≤ 0.005) and blood pressure (MAP 19.9±10.2%), p≤ 0.001). cFMD was higher following HGEx but this was not statistically significant (cFMD pre/ post: 4.7± 3.3 % vs. 6.7± 2.9 %, p= 0.11). MCAv reactivity was significantly higher following HGEx (CVR pre/ post: 16.3±14.4% vs 29.5±11.4%, p≤0.001).

Conclusion: These preliminary data reveal that isometric HGEx may be an effective exercise mode to promote acute increases in CVR. Our findings also support future larger investigations into the benefits of HGEx for improving cFMD. The effect of a HGEx intervention on cerebrovascular function is warranted.

PC51

Is laboratory-based research on energy availability ecologically valid? Heterogenous daily energy availability is dictated by, and inversely related to, exercise energy expenditure in male elite road-cyclists during pre-season training.

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Introduction:

Insufficient dietary energy to maintain normal physiological function results in a state of 'low energy availability' (LEA). LEA is believed to be the aetiological factor underpinning endocrine, metabolic and physiological dysregulations often observed in athletes[(1-4)], given laboratory-based controlled studies in humans show a causal role between LEA and endocrine dysregulations. However, these studies inflict a homogenous pattern of LEA that may not be ecologically valid.

Endurance athletes have been identified as at risk of suffering from the consequences of prolonged LEA due to persistently high daily exercise energy expenditure (EEE)[(5)]. However, to date there is no study characterising the relationship between the EEE and energy availability (EA) of these athletes in typical training conditions. The aim of this study was to characterise daily EEE and dietary energy intake (EI), to assess the EA of a cohort of elite male road-cyclists during seven consecutive days of pre-season training. We hypothesised that EA would be inversely related to EEE.

Methods:

Ten male, elite cyclists (22 ± 8 years, 75.1 ± 8.5 kg body mass [*BM*], 65.4 ± 7.4 kg estimated fat free mass [*FFM*], 1.84 ± .05 m, 5.27 ± 0.25 W•kg^{-1} 20 min Mean Maximal Power) were assessed during 7 consecutive days of pre-season training. Dietary intake was assessed using the remote-food photography method and EI calculated from macronutrient content of foods (*Nutritics^{TM}*, Ireland). EEE was estimated from cycling crank-based power-meter data, and by using METS for other exercise types. FFM was estimated from BM and team dual X-ray absorptiometry fat % records. Daily EA was calculated as (EI – net EEE)/FFM. The relationship between EA & EEE, EA & EI and EEE & EI were determined using linear regressions.

Results:

High variation in daily EA was observed, ranging from -21.9 to 76.0 kcal•kg FFM^{-1}•day^{-1}. Mean daily EA was 19.5 ± 22.0 kcal•kg FFM^{-1}•day^{-1}, reaching 'Low' EA (<30 kcal•kg FFM^{-1}•day^{-1}) in 71% (5/7) of training days. Daily EEE averaged 38.4 ± 25.9 (98.9 % of EEE from cycling) and EI 57.9 ± 13.3 kcal•kg FFM^{-1}•day^{-1}, with ranges of 0.0 to 99.8 and 28.5 to 95.0 kcal•kg FFM^{-1}•day^{-1} respectively. We show a very large, negative, linear relationship between EA and EEE (R^2 = .735; 95% CL: -1.155 & -.862; P < 0.001) but a small, positive, linear relationship between EEE and EI (R^2 = .278; 95% CL: .166 & .378; *P* <.001) (Figure 1).

Conclusions:

Large fluctuations in daily EEE appear to dictate heterogenous EA values observed during seven consecutive days of pre-season training in elite male cyclists. The majority of the sessions were

undertaken in what is typically deemed LEA. EI increased slightly with EEE but did not fully compensate for it. Periods of homogeneous LEA induced in laboratory-based research do not replicate the EA patterns observed in the field in this population.

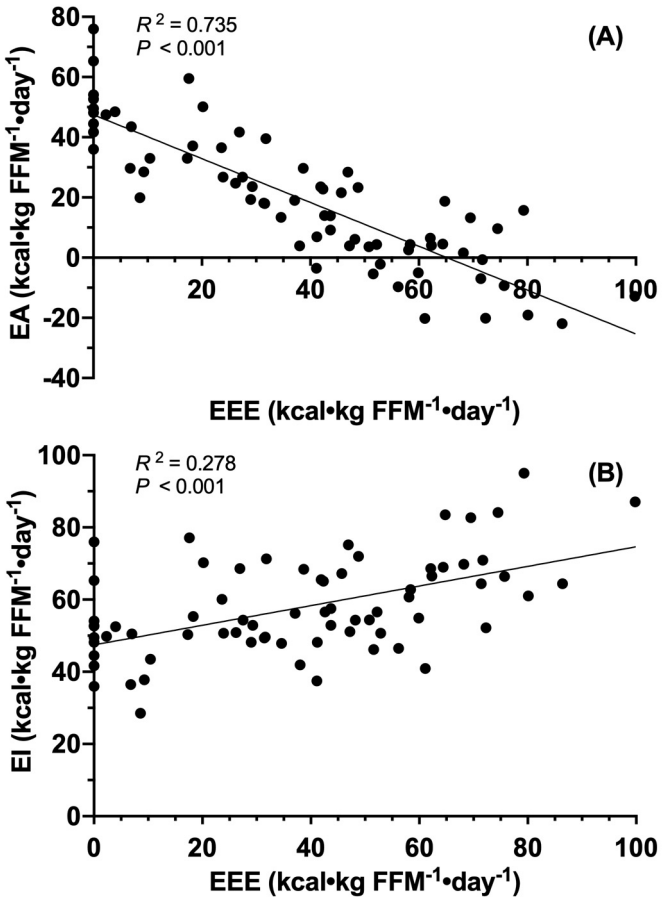

Figure 1. Relationship between (A) exercise energy expenditure (EEE) & energy availability (EA), and (B) exercise energy expenditure & energy intake (EI) in elite male road-cyclists over 7-day's pre-season training.

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PC52

Contraction mode and sex differences in neuromuscular function during isometric and dynamic quadriceps incremental tasks.

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Introduction. It has been well established that performance fatigability depends on the kind of task performed, sex, and the method of evaluation (maximal isometric vs. concentric torque loss). However, only a limited number of studies evaluated performance fatigability following isometric *vs.* dynamic fatiguing tasks in the same participants, and none of those studies reported changes in both maximal isometric and concentric (isokinetic) torque. Fatigability of men and women was also seldom compared in these conditions. The aim of this study was to compare performance fatigability of the knee extensors, assessed as isometric and concentric torque loss, between isometric and concentric fatiguing tasks in men and women.

Methods. Twenty healthy participants (25 ± 4 yr, 10 women) randomly performed the isometric and dynamic (isokinetic) quadriceps-intermittent-fatigue (QIF) tests with similar duty-cycles. The isometric QIF test consisted of 10-contractions (5-s on/ 5-s off) incremental stages, starting at 10% of maximal isometric torque with 10% increments for each stage. The dynamic QIF test consisted of 100-contractions ($120°.s^{-1}$ for 60° range-of-motion, 0.5-s on/0.5s off) incremental stages, starting at 10% of maximal concentric torque with 10% increments for each stage. Maximal isometric and concentric torque, voluntary activation and contractile function were quantified before, at the end of each stage and at exhaustion. Fatigue aetiology was investigated using peripheral nerve electrical stimulation during maximal voluntary isometric contractions and at rest. All the procedures were approved by the institutional ethics committee and performed in accordance with the *Declaration of Helsinki* (except for registration in a database).

Results. Greater number of stages (6.2 ± 0.7 *vs.* 4.9 ± 0.8; P < 0.001) and torque-time integral (TTI; 20,166 ± 7,821 *vs.* 11,285 ± 4,933 Nm.s; P < 0.001) were performed for the isometric compared to the dynamic QIF test. Maximal isometric and concentric torque, as well as voluntary activation, decreased along the tasks (P < 0.001) without differences between sessions. However, impairments in resting twitch amplitude were greater for the dynamic than the isometric QIF test (P < 0.001). Men were stronger and performed higher TTI than women (P < 0.001), but no sex differences were observed in the number of performed contractions (isometric: 62 ± 8 *vs.* 61 ± 5 contractions; dynamic: 521 ± 67 *vs.* 458 ± 76 contractions for men and women, respectively; P < 0.05). Women showed greater loss in maximal concentric torque independent of the session, while maximal isometric torque loss was similar between men and women.

Conclusions. These results indicated that, despite greater TTI after the isometric fatiguing task, no differences between sessions was noticed for performance fatigability quantified as both isometric and concentric maximal torque loss. Contractile function was more reduced in the dynamic QIF test, suggesting that those impairments manifest earlier during dynamic than isometric fatiguing tasks. Moreover, sex-related differences in fatigability depend on the contraction mode used to assess performance fatigability during and / or after the test.

PC53

Glucocorticoid depletes glutathione and adenosine in the liver of pregnant and non-pregnant

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This study aimed to investigate the effect of glucocorticoid exposure on hepatic glutathione and adenosine contents in pregnant and non-pregnant rats. Rats were grouped into four groups (n=6/group) respectively; pregnant (PRE) and age-matched non-pregnant (NPR) rats received vehicle, pregnant (PRE+DEX) and non-pregnant (NPR+DEX) rats treated with dexamethasone (DEX) received glucocorticoid (DEX; 0.2mg/kg, *per oral*) between gestational days 14-19 and six days respectively. Statistical analysis was performed using SPSS software (Version 22; SPSS Inc. IL., USA) and data were expressed as mean ± SEM of 6 rats per group. Student's t-test was used to compare the mean values of variables among the groups. Statistically significant differences were accepted at $p<0.05$. Data showed that GC exposure caused decreased hepatic glucose-6-phosphate dehydrogenase, glutathione peroxidase, GSH/GSSG ratio and adenosine content in both pregnant and non-pregnant rats. On the other hand, GC exposure led to elevated adenosine deaminase and xanthine oxidase activities, uric acid production, lactate dehydrogenase, aspartate aminotransferase, alanine transferase, alkaline phosphatase and gamma-glutamyltransferase. In sum, the present study indicates that liver damage caused by GC exposure is underlined by depleted hepatic adenosine and glutathione content and elevated markers of tissue inflammation and/or injury. The findings also suggest that the effects of GC exposure on hepatic health are not affected by pregnancy.

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PC54

Ferulic acid, atorvastatin and their combination ameliorate high fat diet-induced dyslipidemia, insulin resistance and steatohepatitis by modulating the Nrf2 - NF-κB pathways

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Backgound and Aims: Entire human race is now under the threat of Metabolic disorder pandemic. The current study evaluated a novel therapeutic efficacy of ferulic acid (FA), atorvastatin (AS) and their combined treatment against high fat diet (HFD) induced oxidative stress and non alcoholic steatohepatitis (NASH). ***Methodology:*** The swiss albino mice were administered HFD (52.37% fat, 11.4% of total energy, 33.6% carbohydrate from published protocol for 6 weeks. A robust experimental protocol with contemporary analytical tools were employed to validate the hypothesis. ***Statistical analysis:*** The values were given as Mean ± SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test using OriginPro2020 software. $p < 0.05$ was considered as the level of significance. Each data are representative of at least two independent biological repeats. ***Results:*** The HFD mice showed increased body weight, insulin resistance index, plasma and hepatic lipid profile, histological features of NASH with decreased phosphorylation of AMPK-α. HFD mice exhibited increased oxidative damage in liver concomitant with increased production of intracellular reactive oxygen species (ROS), lipid peroxidation with simultaneous inhibition of the antioxidant defence machinery. Increased liver apoptosis was noticed through the ROS induced apoptotic pathway, elicited by increased depolarization of the mitochondrial membrane, increased expressions of Cdc42, p-SAPK/JNK and Bax-Bcl2 ratio. There was relevant decrease in the phosphorylation status of the survival proteins like PI3K and Akt, synergistic to the decreased nuclear translocation of Nrf2. This study also tried to hone in to understand the status of the ROS mediated inflammatory pathway which showed increased nuclear translocation of NF-κB and simultaneous up-regulation of its downstream proinflammatory genes. Supplementation with FA or AS and unique combination of FA and AS in the diet significantly counteracted the HFD-induced inflammation and apoptotic effects. ***Innovation :*** our results showed for the first time that HFD induced oxidative damage to the liver and its apoptosis through the ROS mediated pathway which was ameliorated by FA, AS and their combination treatment. ***Conclusion:*** FA and AS or the unique combination of FA+AS conferred protection against HFD-induced oxidative stress, NASH and apoptosis by modulating Nrf2, NF-κB pathway.

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PC55

Early life oxygen dysregulation and Gram-positive bacterial challenge modulates steroid hormone expression

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Preterm infant immaturity is characterised by unstable respiratory control, with male infants exhibiting particular vulnerability. Animal models of oxygen fluctuations during early life, have been previously demonstrated to cause persistent alterations in the control of breathing. Very preterm infants are at an increased risk of early life infection as a result of their immaturity and prolonged hospital stays. Late onset infection is a major challenge in very preterm infants. Proteins from Gram-positive bacteria such as (Lipoteichoic Acid) and PGN (Peptidoglycan) are known to mediate systemic inflammation through activation of multiple receptors, such as Toll-like receptors. We hypothesised that chronic exposure to intermittent hypoxia and hyperoxia (cIHH) prior to a subsequent Gram-positive bacterial infection would cause cardiorespiratory dysregulation.

This study was conducted in line with European regulations and approved by local animal ethics committee at University College Cork and the national regulatory body, HPRA Ireland. Sprague Dawley rat litters (male and female pups) were exposed for 10 consecutive days to either Sham or cIHH treatment from postnatal day (PND) 3 to PND 12. On PND 13 blood samples were taken by cardiac puncture, one hour after i.p. administration of LTA&PGN. The steroid hormones oestradiol, progesterone and testosterone were measured in plasma. All data were analysed using a 3-way ANOVA (factors: sex, gas, drug). In a separate cohort in vivo respiratory function was assessed by whole body plethysmography.

Assessment of sex steroids revealed that oestradiol concentrations were not changed by cIHH or LTA&PGN treatment. There was a higher concentration of testosterone in male pups compared to female pups (sex factor $P<0.05$), which was suppressed in cIHH treated animals (Gas factor $P<0.05$) but the interaction (sex x gas) was not statistically significant. Progesterone was enhanced by LTA&PGN treatment ($P<0.05$), this effect was most evident in males (sex x drug interaction $P<0.05$) but independent of gas treatment.

We have revealed increased progesterone concentrations during infection in our animal model of early life stress. Although progesterone is a known respiratory stimulant, we have previously reported during normoxia, a decrease in both minute ventilation and CO_2 production in response to LTA&PGN such that the ventilatory equivalent, in both male and female pups, was normal in this animal model. We will examine the hypoxic and asphyxic ventilatory response in a follow-up study to determine if the drug induced progesterone is a protective homeostatic mechanism. Further research is needed to identify the physiological basis for both vulnerability and resilience to infection during early life.

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PC56

The effects of adropin on proliferation and differentiation of rat primary brown preadipocytes

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Abnormal glucose and lipid metabolism may result in obesity – a lifestyle disease which prevalence increases. The metabolism is regulated, among others, by various peptides. One of them is adropin, a product of *Enho* gene (Energy Homeostasis Associated), which expression occurs, for instance, in the liver and brain (1). Previous studies indicate that adropin contributes to the modulation of body mass as well as glucose and lipid metabolism (2). In contrast to white adipose tissue which unhealthy expansion may lead to adiposity (3), brown adipose tissue activation ameliorates glucose homeostasis (4). However, the potential role of adropin on rat primary brown preadipocyte biology was unknown. Therefore, our research aimed to evaluate the effects of adropin on rat primary brown preadipocytes, their proliferation and differentiation. For this purpose interscapular brown fat tissue was collected from male Wistar rats. BrdU incorporation was measured to assess cell proliferation. Moreover, cells were differentiated with adropin, and the expression of adipogenic genes (*C/ebpα, C/ebpβ, Pparγ, Prdm16*) was studied by real-time PCR. Data was analysed using one-way ANOVA followed by the Bonferroni post hoc test, and results are shown as mean ± SEM. All experiments were conducted at least two times. We found that the proliferation of rat primary brown preadipocytes was stimulated after incubation (24 h) with adropin 10 nmol/l (1.05±0.06) and 100 nmol/l (1.43±0.04) vs. 0.79±0.04. The effects were also observed after 48 h of incubation [adropin 10 nmol/l (0.49±0.06), 100 nmol/l (0.63±0.06 vs. 0.31±0.04 OD 450–690 nm, $p<0.05$]. Adropin suppressed the expression of adipogenic genes (cells were collected 7 days after the onset of differentiation process). Adropin (10 and 100 nmol/l) downregulated the expression of *C/ebpα* (0.73±0.06 and 0.69±0.1 vs. 1.04±0.07, $p<0.05$, respectively). Adropin (10 nmol/l) decreased the expression of *C/ebpβ* (0.43±0.02 vs. 0.58±0.04, $p<0.05$). Furthermore, similar effects were observed in the expression of *Pparγ* (adropin 10 nmol/l 1.62±0.25 and 100 nmol/l 1.33±0.38 vs. 3.20±0.44), and *Prdm16* (adropin 10 nmol/l 0.15±0.04 and 100 nmol/l 0.10±0.03 vs. 0.84±0.09, $p<0.05$). These results indicate that adropin peptide upregulates the proliferation of rat primary brown preadipocytes. Moreover, adropin contribute to suppression of preadipocyte differentiation. Obtained results suggest that adropin modulates brown adipogenesis.

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PC57

Changes in the component composition of the rat body after single and multiple intravenous administration of iron oxide magnetic nanoparticles synthesized by different methods

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Magnetic nanoparticles (MNP) based on iron oxide are currently considered as promising agents for solving a wide range of biomedical problems. Despite numerous studies devoted to the study of their biocompatibility, the mechanisms of interaction of nanoparticles with cells, tissues and the body as a whole have not yet been fully studied. The aim of the work was to study changes in the composition of the body under the conditions of single and multiple intravenous administration of MNP based on iron oxide synthesized by various methods to male and female rats.The work was performed on male and female Wistar rats weighing 220-280 g. The experiments were carried out in compliance with the principles of humane treatment of animals, regulated by the requirements of the European Convention on the maintenance, feeding and care of experimental animals. MNP (MNP1 and composite nanoparticles with a silicon dioxide shell - MNP2) were injected into the tail vein of rats: a single injection at a dose of 400 mg / kg (MNP1 and MNP2 (n = 16)); repeated administration at doses of 30 mg / kg and 60 mg / kg - MNP1, n = 16 and 10 mg / kg and 30 mg / kg - MNP2 (n = 12 and n = 14) daily for 7 days. In the control groups, saline was administered. Body composition was studied using an EchoMRI 500 magnetic resonance tissue analyzer. The hypothesis was tested in independent samples using the Mann-Whitney test. Male and female rats were analyzed separately. There was a significant decrease in the content of adipose tissue in females on the 31st day of the experiment against the background of a single intravenous injection of MNP1 and MNP2 (9.0 [7.0; 11.0]% - MNP1 and 9.2 [7.9; 12.1]% - MNP2, 16.1 [12.8; 18.1]% - control). The total amount of water in the body of male and female rats of all experimental groups did not change in the dynamics of the experiment, which was evidence of the absence of disturbances in the water balance. After the administration of MNP1 to female rats at doses of 30.0 and 60.0 mg/kg and MNP2 at a dose of 10.0 mg/kg on the 15th day after the last injection, significant ($p<0.05$ compared to control) a decrease in the content of adipose tissue ($p>0.05$ between the experimental groups), which persisted by the end of the study (day 31): 9.0 [7.1; 11]% - MNP1 at a dose of 30 mg / kg, 11.0 [7.0; 12.1]% - MNP1 at a

dose of 60 mg / kg, 10.0 [7.9; 12.0]% - MNP2 at a dose of 10 mg / kg, 17.2 [13.0; 19.1]% - control. Thus single and multiple intravenous administration to female rats of MNP coated with silicon dioxide in the range of the above doses provides a change in the composition of the body in the form of a decrease in the content of adipose tissue.

PC58

High-Fat Diet May Upregulate Apoptosis and Necrosis Mechanisms at the Gene Level in the Liver of C57BL/6J Mice

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INTRODUCTION and OBJECTIVE: C57BL/6J mouse strain is known to be susceptible to obesity and/or diabetes/ metabolic syndrome after a high-fat diet term. However, cellular mechanism to transformation into obesity is not fully understood yet. In addition to clinical studies, recent evidences indicate hope for cellular pathophysiology from *in silico* analysis. The aim of this study was to detect possible pathophysiological factors in diet-induced obesity on examining the expression levels of genes by using bioinformatics tools.

METHODS: GSE39549[1] dataset obtained from GEO (Gene Expression Omnibus) database was re-examined for this research. In the dataset, total RNA obtained from isolated liver tissues of C57BL/6J mice fed normal diet (27 samples) and high fat diet (24 samples) is recruited. After the gene expression levels in the dataset were re-analysed in the R program, gene set enrichment analyses were performed in Gene Ontology (GO) and ENRICHR tools.

APPROACH FOR STATISTICAL ANALYSIS: Expression levels of genes, commonly implicated to play a role in several biological processes, in GSE39549 dataset are re-analysed in R program. Based on Benjamini-Hochberg correction, adjusted p-values <0.05 were accepted as significant.

RESULTS and CONCLUSION: Gene expression levels indicated that BCL2 (B-cell lymphoma 2), CASP-1,4,6,7 (caspases), MCL1 (myeloid cell leukemia 2), BIRC2 (baculoviral IAP repeat-containing protein 2) [responsible for apoptosis] and TNF (tumor necrosis factor), TNFSF-10,13B (TNF superfamily members), TNFRSF-1B,8,10,11A,18,21,22 (TNF receptor superfamily members), TNFAIP-2,3,8 (TNF alpha induced proteins), TLR4 (Toll-like receptor 4) [responsible for necrosis] genes were up-regulated ($p<0.05$) in high-fat diet group, compared with normal diet group. Although *in vivo* and *in vitro* researches about diet-induced obesity are on-going, gene expression analysis and gene set enrichment analyses results implicate that apoptotic and necrotic mechanisms may cause diet-induced obesity.

Keywords: diet-induced obesity, C57BL/6J mice, apoptosis, necrosis, bioinformatics.

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PC59

Glucose administered intraperitoneally overestimates glucose tolerance in old C57BL/6J mice

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Glucose intolerance, commonly seen in the elderly, is the underlying cause of many metabolic disorders including diabetes, and is measured using the glucose tolerance test (GTT). Murine models are widely used to study the development of diabetes, obesity and cardiovascular disease during ageing. Similar to humans, the GTT is routinely used to determine tolerance to glucose in mouse models, particularly C57BL/6J. In contrast to the human situation, however, it has been observed that old mice have a better, rather than poorer, tolerance to glucose than young mice after an intraperitoneal injection of glucose (De Leon et al., 2018; Oh et al., 2016; Reynolds et al., 2019). This has been explained by adaptations in old mice, such as an increase in the size of beta cells (De Leon et al., 2018) and a rise in number of calcium receptors (Oh et al., 2016), which lead to better glucose metabolism. However, an intraperitoneal injection may be biased by possible age-related differences in glucose uptake from the peritoneum into the circulation. In humans, it has also been suggested that there are inconsistencies in results and lack of reproducibility when performing an oral GTT (Nelson, 1988), so results must be interpreted carefully. To circumvent potential age-related differences in the rate of absorption of glucose into the circulation, the best way to assess glucose tolerance is direct injection into the circulation. In line with this, it has been observed that 56% of pregnant women suggested to have abnormal glucose tolerance after an oral GTT had normal glucose tolerance when assessed with a venous GTT (Benjamin & Casper, 1966). We hypothesized that an intraperitoneal injection of glucose overestimates glucose tolerance in old but not in young mice. To investigate this, after a 16-h overnight fast male C57BL/6J mice received an intraperitoneal injection (IP: young n=7; old n=6), or an intravenous injection via the tail vein (IV: young n=7; old n=6) of glucose (2 g glucose/kg body mass). A glucometer (Glucocard X-mini plus, Japan) was used to measure blood glucose from a drop of blood taken from an incision in the tail vein at baseline (0 min) and at 15, 30, 60, 90 and 120 min after the injection. Experiments were approved by the ethics committee of the Lithuanian Republic Alimentary and Veterinary Public Office (#G2-90 in 2018).To determine the changes in blood glucose over 120 mins, a repeated-measures ANOVA with time as within factor, and age and route of injection as between factors was used. The time * route interaction in old animals ($p=0.003$) was reflected by higher glucose concentrations at all time points after an intravenous than an intraperitoneal injection. The absence of a time * route interaction ($p=0.090$) in young animals indicated that the time course of the change in glucose concentrations

after IP and IV glucose injections was similar in young animals. Our data therefore indicate that especially in old animals an intraperitoneal injection of glucose overestimates glucose tolerance and that an intravenous injection of glucose is advised to determine glucose tolerance.

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PC60

Lipidomic profile of caspase-4 positive non-small cell lung cancer (NSCLC) patients.

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The alteration of the metabolomic profile in lung cancer represents one of the hallmarks for tumor cells which opportunistically hijack the physiological pathways in favor of carcinogenesis. In our previous study, we found circulating and tumor-associated caspase-4 as a novel diagnostic, predictive and prognostic biomarker for non-small cell lung cancer (NSCLC) patients. According to the altered metabotype of lung cancer patients, the main goal of the actual study was to understand any correlation between caspase-4 and the metabolomic profile of NSCLC patients (n=104, 60±10 (mean±S.E.M) years of age), stratified as caspase-4 positive or negative. Metabolomic profiles have been obtained by using gas chromatography coupled to mass spectrometry (GC-MS analysis), carried out on both lung tumor tissues and plasma. We found that circulating caspase-4 was correlated to lactate dehydrogenase (LDH), in that 82.69% of caspase-4 positive patients (86 out of 104) had high plasma levels of LDH, widely recognized as a marker associated to tumor progression and poor prognosis, compared to healthy subjects (n=61) ($p<0,0001$; according to Two-tailed Mann Whitney U test). However, this effect was not observed in caspase-4 positive tumor tissues, where instead, fatty acid biosynthesis was favoured in that the

malonic acid and the palmitic acid were higher than in non-cancerous ($p<0,0001$ and $p=0,0009$, respectively, according to One-Way ANOVA followed by Dunn's multiple comparison post-test) and caspase-4 negative tissues ($p=0,042$ and $p=0,0215$ respectively, according to One-Way ANOVA followed by Dunn's multiple comparison post-test). Moreover, caspase-4 positive tumor tissues had significantly higher levels of transaldolase ($p=0,0007$, according to Two-tailed Mann Whitney U test), pyruvate kinase ($p=0,002$, according to Two-tailed Mann Whitney U test) and fatty acid-binding protein ($p=0,0108$, according to Two-tailed Mann Whitney U test). Instead, malate dehydrogenase and phosphoglycerate kinase tended to decrease in tumor tissues, implying that higher consumption of glucose occurred. On the other hand, dysregulated glucose metabolism was counterbalanced by a higher presence of succinate dehydrogenase (SDHA) ($p=0,0381$, according to Two-tailed Mann Whitney U test) and by the gluconeogenic valine which supported the Krebs' cycle. In conclusion, we found that the recently identified caspase-4 positive subpopulation of NSCLC patients is characterized by an altered lipidomic profile accompanied by alternative pathways to guarantee glucose metabolism. This shifted equilibrium in favor of fatty acid biosynthesis, was balanced by the anaerobic LDH activity and valine/proline/SDHA-supported Krebs' cycle in order to provide ATP to the tumor cells allowing them to survive and proliferate.

Reference 1 :- N/A

Reference 2 :- N/A

Reference 3 :- N/A

Reference 4 :- N/A

Reference 5 :- N/A

Acknowledgements :- N/A

PC61

Temperature induced gill remodeling influences the thermal tolerance of reef fishes living on the world's hottest reefs

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It is suggested that many tropical coral reef fishes are living close to their upper thermal limits, with limited capacity to further acclimate to increasing sea surface temperatures (SSTs). Current maximum summer temperatures for the majority of coral reefs globally is 32°C, with an additional 3-4°C heating projected by 2100. However, the Persian/Arabian Gulf (PAG) is already experiencing summer maxima above 35°C, making it the hottest reef environment on earth, and creating a present-day example of end-of-century predictions. Here, we use PAG reefs as natural laboratories

for climate change to investigate thermal acclimation and adaptation in three species of locally common coral reef fishes (*Scolopsis ghanam, Cheilodipterus novemstriatus* and *Escenius pulcher*).

For each species we compared upper (CTmax) and lower (CTmin) thermal tolerance limits across populations from the environmentally extreme southern PAG, and populations from the more benign reefs in Gulf of Oman (GO) (annual temperature 22-32°C), focusing on five temperatures representing the existing seasonal PAG thermal range (18, 22, 27, 31.5, 35.5°C). Additionally, gill structure was assessed in *S.ghanam* to investigate thermally-induced remodeling of the cardio-respiratory system across 18°C, 27°C and 35.5°C. Fishes were collected throughout the year when SST matched that of experimental temperature, allowing an accurate representation of naturally occurring acclimation. As conditions in the GO do not reach the same seasonal extremes, fishes from this region were acclimated for >3 weeks to 18°C and 35.5°C before trials.

Our results reveal significant differences between species, and between populations. There was a significant positive effect of treatment temperature on CTmax for all species, from both populations. All three species from PAG reached a higher CTmax at 35.5°C than GO fishes, as well as reaching a lower CTmin at 18°C. Minimal differences in CTmin were seen between populations, although species specific values varied significantly. There was considerable variation in CTmax between all species, and between populations, suggesting varying strategies in coping with increasing temperatures. *C.novemstriatus* was significantly less tolerant of both high and low temperatures at each treatment than *S.ghanam* and *E.pulcher* in both populations.

Histological image analysis of *S.ghanam* gill structure across 18°C, 27°C and 35.5°C from PAG revealed a clear correlation between temperature and lamellar structure (n = 30). As temperature increased, lamellar length and perimeter significantly reduced, (ANOVA: $[F(2,58) = 38.937]$, $p = < 0.01$ and $[F(2,58) = 11.874]$, $p = <0.01$, respectively), whereas lamellar density significantly increased (ANOVA $[F(2,16) = 8.113$, $p = 0.004)$. *S.ghanam* from GO showed little difference in lamellar structure between temperatures. The presence of an interlamellar cell mass (ILCM) was detected in both populations at 18°C and 27°C, yet was not present at 35.5°C. The presence of ILCM represents a novel finding since it has previously not been documented in tropical reef fish species.

Our results revealed that fishes from PAG displayed a greater thermal tolerance, suggesting that thermal adaptation has occurred. The ability to remodel various aspects of their gill structure, facilitating greater O_2 uptake in higher temperatures, is likely a significant contributory factor. This study indicates that there may be capacity for some reef fish species to adapt to increasing SSTs.

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PC62

Functional ex vivo analysis by myography of the female rat rectus abdominis muscle

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Regenerative medicine, nanomedicine, and tissue engineering have progressed for the developing of new techniques and methods of analysis to study tissue regeneration and biological functions of organs and tissues. We investigated whether myography was a method of analysis to assess function of the rectus abdominis muscle (RAM). *Methods*: The project was developed according to the animal experimentation rules CONCEA, approved by the Ethics Committee of Botucatu Medical School (number 1234/2017-CEUA) Seven virgin young female rats (n=7), aged ~60 days (286 ± 14 g), were used to harvest the RAM after euthanasia by decapitation. RAM sections of ~1.5 cm were collected from the pubis bone towards the central portion and immersed in Krebs solution at 8ºC (mM: NaCl 118.5, KCl 4.7, $NaHCO_3$ 25, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 5.5 D-glucose, pH 7.4). RAM sections were mounted in the bath chamber of a myograph (Model 820MS, Danish Myo Technology) containing Krebs solution (37ºC) with constant bubbling (10% CO_2, 90% O_2). After 1 hour stabilization the response of RAM to electrical stimulation (20 Volts, 2 Hz, 1 ms pulse width, 500 ms pulse interval, bipolar pulse shape for 1 h:6 min:35 sec). *Results*: RAM responded to 85% ± 8 ($p \leq 0,01$, Tukey's post hoc test) of the applied pulses. An average of 19.4 ± 1.7($p \leq 0,01$,) mN (right RAM) and 18.7 ± 2.6 ($p \leq 0,01$,) mN (left RAM), an average for maximum contraction force was obtained and loss of strength (fatigue) was verified at the 52.7 ± 11.7 ($p \leq 0,01$,) min. *Conclusion*: Myography proved to be useful in analyzing RAM functionality. The muscle functionality (muscle contraction strength, fatigue and response to electrical stimulation) of RAM of young virgin rats could be properly assessed. The standardization of the myography analysis unprecedented in the literature has been successful. This approach may be useful in the characterization of muscle diseases and disorders as well as new therapies development.

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PC63

Methyl jasmonate rescues synaptic connectivity defects in the unpredictable chronic mild stress mouse model of depression

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Increasing evidence from human brain imaging studies indicates that depression alters structural and functional connectivity in brain regions governing cognition and emotions. Consistent with this, unpredictable chronic mild stress (UCMS), a mouse model of depression, causes dendritic atrophy and spine loss in neurons laying in cortical and limbic regions. Based on data showing that the plant anti-stress hormone methyl jasmonate (MJ) rescues the UCMS-induced depressive behavioral phenotype, here we examine whether the compound also prevents the occurrence of neuronal and synaptic connectivity defects in the hippocampus, prefrontal cortex, and amygdala. Male C57BL/6 mice (n=6) were injected with MJ (50 mg/kg) or saline (SAL) before each of the two daily exposure to UCMS administered over 14 days. On day 15, mice were sacrificed via cervical dislocation, and their brains were processed for Golgi-Cox staining, western blot, and immunohistochemistry analyses. Additional groups of UCSM-exposed mice were treated with MJ or SAL in a stress–free condition. Data were analyzed using descriptive statistics, Neurolucida, Image J, and ANOVA at $\alpha_{0.05}$. In the fear conditioning test, MJ significantly decreased freezing duration (20.53±4.00s) against stress (73.56±5.16s). Our results confirm that MJ prevents the manifestation of depressive-like behaviors and reveal that this effect persists after treatment cessation. Moreover, they show that, in the three regions of interest, UCMS-exposed mice treated with SAL exhibit a massive reduction in the dendritic arbor of the BLA (40.13±1.22) against SAL (100.30±5.11) and MJ (68.00±2.22); H-CA1 (22.13±2.42) against SAL (65.13±9.59) and MJ (48.13±4.57); and PFC (11.60±0.86) against SAL (42.75±3.48) and MJ (23.20±0.90); as well as spine density in the BLA (0.51±0.01) against SAL (0.94±0.02) and MJ (0.75±0.01), H-CA1 (0.47±0.02) against SAL (0.98±0.03) and MJ (0.85±0.03), and PFC (0.84±0.03) against SAL (1.53±0.05) and MJ (1.41±0.04). We also observed a significant decrease in CREB

expression level and a lower number of parvalbumin-positive cells than UCMS-non exposed mice injected with MJ or SAL. Remarkably, these alterations were entirely rescued by MJ treatment. Thus, in parallel with the alleviation of behavioral markers of depression, the compound preserves synaptic integrity in neural circuits via modulation of molecular and cellular regulators. It can offer, in this regard, a therapeutic alternative to treat this debilitative pathology.

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Study of Mechanism of Action of Repeated Transcranial Magnetic Stimulation to Modulate Pain in fibromyalgia Patients.

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Fibromyalgia (FM) is a chronic pain syndrome characterized by widespread musculoskeletal pain and tender points. It probably results from an altered pain perception due to aberrant pain modulation system. Despite extensive research, the pathogenesis and etiology of fibromyalgia is poorly understood. It is not a primarily a muscle disorder and other systems such as neuro-endocrine, central and autonomic nervous systems have been implicated in its causation. The aim of the present study is to investigate the effect of chronic exposure to low frequency transcranial magnetic stimulation (rTMS) given at right dorsolateral prefrontal cortex on pain and its modulation status in fibromyalgia patients utilizing both subjective (standard questionnaires and psychophysical methods) and objective (nociceptive flexion reflex, diffuse noxious inhibitory controls, endogenous opioid status) and find out whether any genetic link between COMT and 5HT2A receptor genes with chroinc pain in Fibromylagia patients. The study was approved by institutional Ethical review Committee prior to commencement of the experiment. All the ethical guidelines are followed required for the human study. All subjects were explained the experimental design and written consent was taken from all the subjects. Genetic polymorphism of COMT and 5HT2A receptor genes were studied in normal controls(n=79) and fibromyalgia (n=86). Female fibromyalgia patients were recruited according to American College of Rheumatology criteria 2010. They were randomly divided into Sham (n=36) and rTMS (n=49) groups. Real rTMS (1200 pulses at 0.5 Hz per day for 5 days per week for 4 week) and sham rTMS were given rTMS and Sham groups respectively. Pain and associated symptoms were recorded by subjective (visual analogue scale and McGill pain questionnaire for pain, Spielberger State-Trait Anxiety Inventory for anxiety, Beck Depression Inventory-II for depression, WHO quality of life BREF questionnaire for quality of life, Pain coping strategy questionnaire for coping strategy and Pain belief questionnaire for general beliefs) and objective methods (nociceptive flexion reflex). Pain modulation status was assessed by diffuse noxious inhibitory controls and indirect measurement of endogenous opioids in both the groups at pre-stimulation and 2, 6, 13 and 26 weeks post-stimulation follow up periods. To associate whether any link between chronic pain with COMT and 5HT2A receptor genes, single nucleotide polymorphism study was done in

fibromyalgia and normal controls. In our study, no association was observed between 5HT2A and COMT genes in FM patients. Significant reduction in pain and associated symptoms were observed from the beginning of week. Pain reduction preceded antidepressant effects. The beneficial effect of rTMS was sustained indicating a clinical application of rTMS.

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PC65

Infra-red laser light causes local hyperpolarization in rat optic nerve

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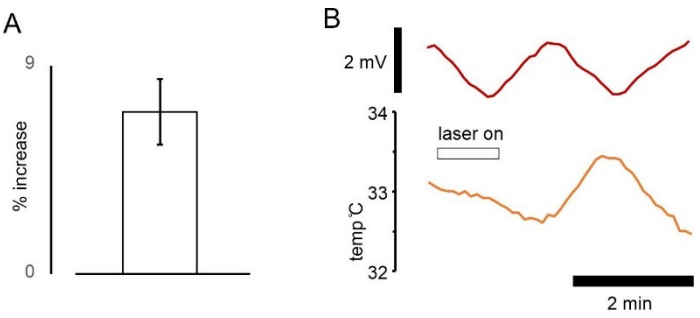
Introduction. Impulse conduction in demyelinated central axons may fail as the body temperature rises because faster Na^+ channel kinetics give rise to briefer action potentials, and a temperature dependent membrane potential moves the resting potential away from threshold (eg Coates et al 2015). Both mechanisms might contribute to conduction failure where the safety-factor for impulse conduction is lowered.

Aims and methods. We have investigated the effects of applying infra-red light, generated by a laser-diode (1550 nm), to undamaged rat optic nerve, via a 9 mm diameter fibre-optic light guide. Optic nerves were isolated from Wistar or Sprague Dawley rats following a Home Office approved schedule 1 procedure, and maintained in a nerve-bath. Axon excitability was repeatedly measured in response to a constant-current stimulus delivered via a suction electrode, using the technique of computer controlled threshold-tracking, and maintaining a 50 % maximal response. Changes in axon membrane potential were recorded across a grease gap.

Results. We report that the application of IR laser light reversibly increased threshold (400 mA diode driving current, n = 3, p = 0.038, one sample t-test, Fig. 1a). At constant light energy (350 mA driving current), the change in threshold was significantly raised by 99.81 ± 32.93 % following the application of 50 mM ZD7288, a blocker of I_h, (n = 5, p = 0.029, paired-t-test). These results are consistent with the laser light causing axonal hyperpolarization, that we have also recorded directly (Fig. 1b).

Conclusion. At least a part of the action of IR laser light on optic nerve is associated with locally raised temperature and a membrane potential hyperpolarization, explained by the previously hypothesized role for electroneutral Na^+ entry into optic nerve axons (Coates et al 2015; Kanagaratnam et al 2017).

Fig.1 IR light affects optic nerve. a) Laser light application (400 mA driving current) raised current threshold by 7.01 ± 1.42% (mean ± SEM). b) Temperature fluctuations in flowing buffer solution (lower panel) mimic laser light application (350 mA driving current) on axon membrane potential (upper panel).



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PC66

SYNAPTIC PLASTICITY IN DORSAL HIPPOCAMPUS REQUIRES OF A G-PROTEIN DEPENDENT MECHANISM THROUGH ADENOSINE A_1 RECEPTOR-ACTIVATED GIRK CHANNELS

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In the dorsal hippocampus, A_1 adenosine receptor-mediated GirK (G-protein-gated inwardly rectifying potassium) channels conductance is constitutively active contributing to the resting membrane potential of CA1 neurons and preventing from any excitation excess. GirK channel-dependent signaling disruption has been linked to the etiology of many diseases that involve neural excitability alterations, such as Alzheimer's disease, suggesting an important role of GirK channels for cognitive processes that depend on hippocampal neuronal activity.

In the present work, we aimed to explore the role of A_1 adenosine receptor-mediated GirK basal activity in the induction and maintenance of synaptic plasticity that supports dorsal hippocampus-dependent cognitive functions.

Towards this end, we pharmacologically modulated basal GirK channel conductance in the dorsal hippocampus by using A_1 adenosine receptor modulators (agonists: 2'MeCCPA and CPA; antagonist: DPCPX) or by directly manipulating channel activity using ML297, a selective GirK opener, and Tertiapin-Q, a specific GirK blocker, and we examined its involvement in controlling synaptic plasticity processes at different levels of complexity.

First, using dorsal hippocampal slices, we studied pharmacological A_1 receptor and GirK channel activity modulation effect on the induction and maintenance of long-term synaptic plasticity processes induced in CA1 by Schaffer collateral stimulation. On the other hand, using an *in vivo* approach, we performed acute intracerebroventricular injections of GirK modulators to study their contribution to CA3-CA1 synapse electrophysiological properties, synaptic plasticity, and non-associative learning and memory capabilities during an open field habituation task.

Our data shows that induction and maintenance of long-term synaptic plasticity processes in dorsal hippocampus involves a G-protein dependent mechanism through A_1 adenosine receptor-activated GirK, as both A_1 receptor and GirK channel activity modulation modified LTP/LTD induction threshold *ex vivo* (Vehicle (A_1 receptor), n = 10, 160 ± 2.4% of baseline, $p < 0.001$; 2'MeCCPA, n = 5, 95 ± 3.5%, $p = 0.806$; CPA, n = 5, 109 ± 2.1%, $p = 0.216$; DPCPX, n = 6, 61 ± 4.1%, $p = 0.003$; Vehicle (GirK channel), n = 21, 156 ± 1.7% , $p < 0.001$; ML297, n =12, 73 ± 2.6% of baseline, n = 12, $p < 0.001$; TQ, n = 13, 95 ± 2.6%, $p = 0.007$) and *in vivo* (Vehicle, 184 ± 9% of baseline; n = 9, $p = 0.003$; ML297, n = 6, 75 ± 8%, $p = 0.963$; TQ, n = 6, 58 ± 6%, $p = 0.508$), even switching HFS-induced LTP into LTD. Also, the disruption of such mechanism leads to hippocampal plasticity-dependent learning and memory deficits as shown during the open field habituation task (ML297, n= 8, *vs* vehicle, n = 10: $p = 0.032$, TQ, n = 7, *vs.* vehicle: $p = 0.012$).

These results support the contention that A_1 adenosine receptor-mediated GirK basal activity must take place in the hippocampus to sustain its correct functionality and that its dysregulation is detrimental for neural processes underlying cognitive function.

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Lycopene Supresses Palmitic acid-induced Neuro-oxidoinflammation via Attenuation of Oxidative stress and NF-κB-p65 activation in Female Rats

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Neuroinflammation can be triggered by certain nutrients. The effect of lycopene against palmitic acid-induced neuroinflammation in female rats has not been explored. This study evaluated the effects of lycopene against palmitic acid (PA)-induced neuroinflammation in rats. Twenty rats (weighing 150-200) g were randomised into six groups (n=5): Normal control, PA control, PA + lycopene (0.24 mg/kg), PA + lycopene (0.48 mg/kg), respectively. After seven weeks of PA challenge including two weeks of lycopene treatment, animals were sacrificed under Ketamine (60 mg/kg) and

Xylazine (20 mg/kg), and the brain was excised for biochemical analyses. Data were analysed using one-way analysis of variance, and the means were separated with Duncan Multiple Range Test (DMRT), with P< 0.05 considered significant. The PA-induced significantly ($p<0.05$) increased adenosine deaminase, monoamine oxidase-A, nucleotides tri-phosphatase, 5'-nucleotidase, acetylcholine esterase, myeloperoxidase activities, and malondialdehyde level, reduced significantly post-treatment. Conversely, catalase and glutathione peroxidase activities and reduced glutathione levels decreased (in PA control) by 43%, 34%, and 12%, respectively, compared with the Normal control. Also, PA triggered a decrease in the brain phospholipids (11.43%) and cholesterol (11.11%) levels, but increased triacylglycerol level (50%). Furthermore, upregulated expressions of IL-1β, IL-6, and NF-κB-p65 in the PA control were attenuated, while decreased IL-10 was upregulated after treatment dose-dependently. Severe vacuolation in PA control was normalized by lycopene. This study concludes that lycopene ameliorated PA-induced neuroinflammation, probably via attenuation of oxidative stress, and downregulation of TLR4/ NF-κB -p65 axis.

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Targeting G protein-gated inwardly rectifying potassium (GIRK) channels in the Central Nervous System

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G protein-gated inwardly rectifying potassium channels (Kir3/GirK) are important for maintaining resting membrane potential, cell excitability and inhibitory neurotransmission. Coupled to numerous G protein-coupled receptors (GPCRs), they mediate the effects of many neurotransmitters, neuromodulators and hormones contributing to the general homeostasis and particular synaptic plasticity processes, learning, memory and pain signaling. Here, we integrate recent findings on the physiology, function, dysfunction, and pharmacology of GirK channels in the central nervous system and highlight the relevance of GirK channels as a worthful potential target to improve therapies for related diseases. A search for articles on PubMed and Google Scholar was performed, using relevant search terms. GirK channels are assembled in a tetrameric unit, conformed by four essential subunits. They are broadly distributed in the central nervous system and have different expression patterns in different neurons and subcellular compartments. GirK channels are downstream targets for various neurotransmitters acting at respective GPCR: $GABA_B$, adenosine A_1, $5HT1_A$, endocannabinoid CB_1, D_1 and D_2-like and other receptors. Although many natural compounds and well-known drugs are found to modulate GirK channels, only few selective agents have been discovered. Behavioral and genetic studies suggest a critical role for the appropriate functioning of the central nervous system and excitatory-inhibitory balance maintenance, as well as their involvement in many neurologic and psychiatric conditions, such as Parkinson and Alzheimer's diseases, epilepsy, mood disorders, attention deficit hyperactivity disorder, schizophrenia, alcoholism and drug addiction. GirK channels emerge as a very promising tool to be targeted in the current scenario where these conditions already are or will become a global public health problem.

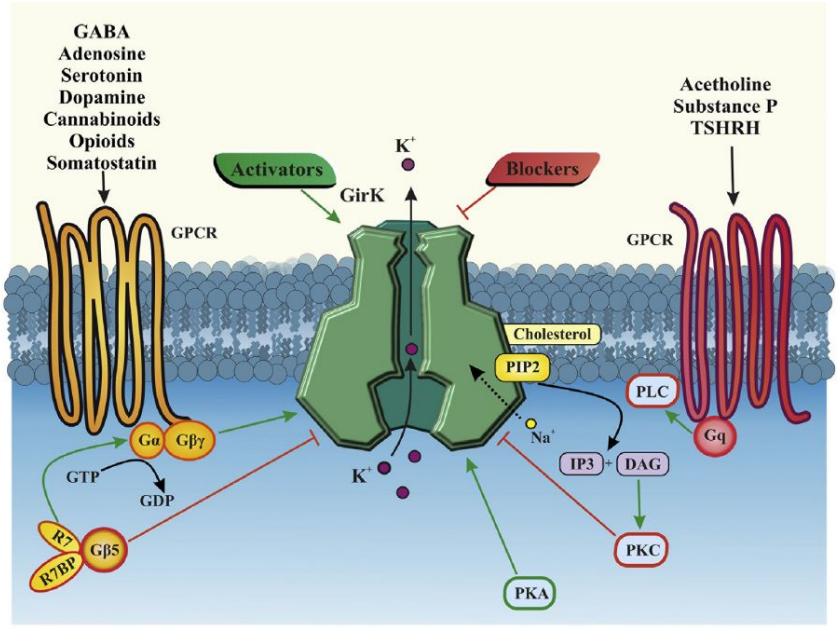

Fig. 3. GirK channels modulation Representative scheme of the different components involved in modulating the activity of GirK channels. Green arrows indicate activation, and red arrows inhibition. Abbreviations: ACh, Acetylcholine; DAG, diacylglycerol; GPCR, G-protein coupled receptor; R7BP - R7-binding protein: IP3, inositol triphosphate; PIP2, phosphatidylinositol 4,5-biphosphate; PKC, protein kinase C; PLC, phospholipase C; T-Q, tertiapin-Q; TSHRH, thyrotropin releasing hormone.

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PC69

SCIENTIFIC ANALYSIS OF ELECTROPHYSIOLOGICAL EFFECTS AND ANXIETY REDUCTION WITH THREE DIFFERENT INDIAN MELODIC SCALES - A RANDOMIZED CONTROL TRIAL

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Introduction: Music plays an integral part in our lives, in causing relaxation of body and the mind. Scientific evidence highlight beneficial influences of western music, but, unfortunately, evidence regarding the health benefits of Indian music is extremely meager. In this era of translational research, we studied the effect of Indian music melodic scales on health with special reference to electrophysiological parameters. As a part of *Sama Veda, Gandharva Veda,* various *ragas* & their health benefits have been enlisted. Notes description-Table 1;Ragas in (1,2). **Objectives:** To evaluate the acute effect of three selected *ragas* of Hindustani (Indian) music on, electrophysiological parameters [Heart rate variability (HRV) and Electroencephalography (EEG)] and to assess the relaxation effect of these ragas on Blood pressure (BP), salivary stress markers and anxiety. **Method**: After ethical clearance, a randomized controlled triple-blind trial with 3 *ragas* to 3 intervention groups (A-*raga Miyan ki Todi*,B-*raga Malkauns* & C-*raga Puriya* -n=35 in each;Table 2) and a control group (group D,n=35) was conducted. The music was alaap played on flute without percussion instruments for 10 minutes while the control group heard predominant silence with some natural sounds interspersed once every 2 minutes for 30 seconds. EEG, Event-related potential (P300), HRV analysis, BP & anxiety were tested prior to, during & after 10 minutes of music intervention & results computed & analyzed using ANOVA and other appropriate statistical methods using SPSS software. P≤0.05 was considered statistically significant. **Results and Conclusions:** State anxiety reduced significantly [Table 3] with maximum reduction using *raga Puriya* (3.94 reduction from baseline), followed by *raga Malkauns* (3.83) and *raga Miyan Ki Todi* (2.35). Insignificant changes were observed in BP. On HRV analysis, *raga Miyan ki Todi and Puriya* caused arousal effect [significant drop in SDNN,RMSSD, TP (ms^2), VLF (ms^2), LF (ms^2), HF (ms^2)] during the intervention and significant relaxation (increase in above parameters) after the intervention. R*aga Malkauns,* similar to the control group, caused a sustained rise in parasympathetic parameters of HRV over 30 minutes [Table 4]. A significant reduction was observed with all four interventions in salivary alpha-amylase. On EEG, *raga Puriya* caused a significant increase in delta & theta after the intervention [Figure 1], *raga Miyan ki Todi* showed a significant drop in Alpha, Beta1 during the intervention, whereas *raga Malkauns* showed a significant increase in theta after the intervention and reduced Alpha, Beta1 & Beta2 during the intervention. P300 amplitude reduced significantly with *raga Puriya* during the intervention, with more reduction after intervention with *raga Malkauns*. **Conclusions:** Indian Music produced a relaxation response similar to western classical music. Among the different *Hindustani ragas*, *raga Puriya* was found to be beneficial based on anxiety reduction, parasympathetic response after intervention, and theta power rise during the intervention. Nevertheless, *Raga Miyan ki Todi and Malkauns also* showed a relaxation response electrophysiologically, though the timing was different, one being after the intervention, while the other was during, respectively. Thus, further

studies are highly recommended to be taken up, in order to build a scientific foundation for the use of Indian music in medicine.

Table1: Names & equivalents of the 12 basic notes of Indian music

Carnatic					Western			Hindustani		
Full Name		Short forms		Alt[1]	Interval	Cents[2]	Note[3]	Full Name	Short forms	
1	Shadjam	Sa	S		P1	0	C	Shadj	S	S
2	Suddha Rishabham	Ri1	R1		m2	100	Db	Komal Rishab	kR	R1
3	Chatusruthi Rishabham	Ri2	R2		M2	200	D	Suddh Rishab	R	R2
	Suddha Gantharam[4]	Ga1	G1	G0			Ebb			
4	Shatsruthi Rishabham[4]	Ri3	R3		m3	300	D#	Komal Gandhar	kG	G1
	Saadarana Gantharam	Ga2	G2	G1			Eb			
5	Antara Gantharam	Ga3	G3	G2	M3	400	E	Suddh Gandhar	G	G2
6	Suddha Madhyamam	Ma1	M1		P4	500	F	Suddh Madhyam	M	M1
7	Prati Madhyamam	Ma2	M2		4	600	F#	Tivra Madhyam	tM	M2
8	Panchamam	Pa	P		P5	700	G	Panchm	P	P
9	Suddha Dhaivatham	Da1	D1		m6	800	Ab	Komal Dhaivat	kD	D1
10	Chatusruthi Dhaivatham	Da2	D2		M6	900	A	Suddh Dhaivat	D	D2
	Suddha Nishadham[4]	Ni1	N1	N0			Bbb			
11	Shatsruthi Dhaivatham[4]	Da3	D3		m7	1000	A#	Komal Nishad	kN	N1
	Kaisiki Nishadham	Ni2	N2	N1			Bb			
12	Kaakali Nishadham	Ni3	N3	N2	M7	1100	B	Suddh Nishad	N	N2

Table **2**: The three chosen Indian musical scales, the names of the notes in Hindustani music and Western scale

Svara / Note	Hindustani name	Staff note	Western scale Interval name
	Raga Miyan ki Todi (Scale A) (heptatonic, G appears in descent)		
S	Shadja	C	Perfect unison
r	Komal rishab	D♭	Minor second
g	Komal gandhar	E♭	Minor third
M	Tivra madhyam	F#	Augmented fourth
P	Pancham	G	Perfect fifth
d	Komal Dhaivat	A♭	Minor sixth
N	Shuddha nishad	B	Major seventh
	Raga Malkauns (Scale B) Ascent and descent same – pentatonic		
S	Shadja	C	Perfect unison
g	Komal gandhar	E♭	Minor third
m	Shuddha madhyam	F	Perfect fourth
d	Komal Dhaivat	A♭	Minor sixth
n	Komal nishad	B♭	Minor seventh
	Raga Puriya (Scale C) C, D♭, E, G♭, G, A/A♭, B (hexatonic)		
S	Shadja	C	Perfect unison
r	Komal rishab	D♭	Minor second
G	Shuddha gandhar	E	Major third
M	Tivra madhyam	F#	Augmented fourth
D	Shuddha Dhaivat	A	Major sixth
N	Shuddha nishad	B	Major seventh

Table 3: Comparison of Pre And Post STAI Score (STATE-Anxiety) between four groups

RANDOM GROUP		MEAN	SD	Difference in means	QUARTILES			P
					25	50	75	
A (N=37)	Pre	35.16	10.83	2.35	28.5	33	40	0.054*
	Post	32.81	10.74		24.5	30	39	
B (N=36)	Pre	34.92	12.28	3.83	24.5	30.5	44.3	0.057*
	Post	31.08	8.91		23.5	29	34	
C (N=36)	Pre	36.11	11.71	3.94	25.5	35.5	43.8	0.018*
	Post	32.17	10.26		25	29.5	37	
D (N=34)	Pre	34.21	7.15	0.32	30	32.5	37	0.781
	Post	33.74	8.32		28	30.5	40	

Note:

a) N is the number of subjects in each group.

b) All the values are in mean and standard deviation (SD).

c) P value < 0.05 was considered significant

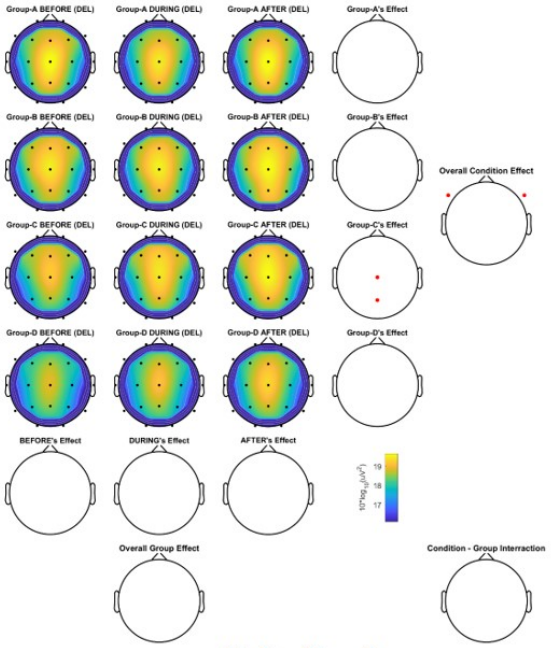
d) P calculated using paired t test.

Table 4: Log transformed Heart rate variability (HRV) parameters

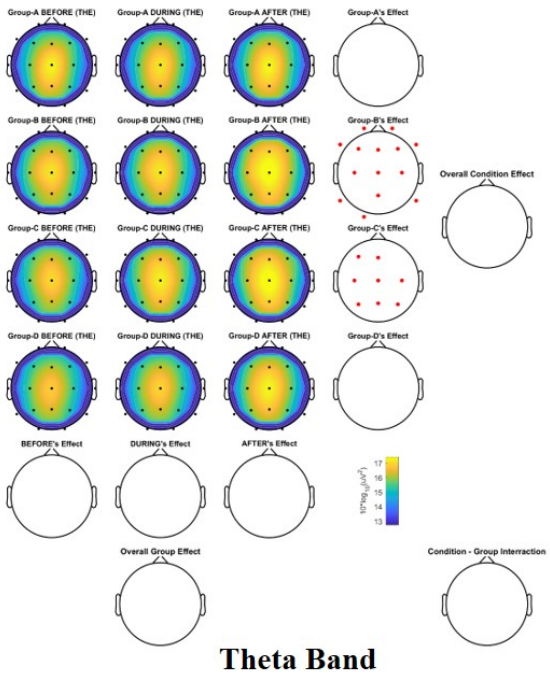
RANDOM GROUP	Pre	Dur	Post	
	Mean (SD)	Mean (SD)	Mean (SD)	P
Mean NN (ms)				
A	2.92 (0.06)	2.92 (0.06)	2.93 (0.06)	0.002
B	2.93 (0.07)	2.94 (0.07)	2.94 (0.07)	<0.0001
C	2.93 (0.06)	2.93 (0.06)	2.94 (0.06)	0.031
D	2.92 (0.06)	2.94 (0.06)	2.94 (0.06)	0.011
Mean HR (BPM)				
A	1.86 (0.06)	1.85 (0.06)	1.85 (0.06)	0.002
B	1.85 (0.07)	1.84 (0.07)	1.84 (0.07)	<0.0001
C	1.85 (0.06)	1.84 (0.06)	1.84 (0.06)	0.031
D	1.85 (0.06)	1.84 (0.06)	1.84 (0.06)	0.011
SDNN (ms)				
A	1.79 (0.18)	1.78 (0.19)	1.84 (0.19)	0.001
B	1.78 (0.19)	1.80 (0.21)	1.82 (0.21)	0.043
C	1.81 (0.20)	1.78 (0.19)	1.85 (0.20)	<0.0001
D	1.77 (0.16)	1.80 (0.15)	1.84 (0.16)	0.001
RMSSD				
A	1.75 (0.25)	1.72 (0.28)	1.78 (0.27)	0.104
B	1.71 (0.31)	1.73 (0.33)	1.75 (0.30)	0.118
C	1.75 (0.30)	1.74 (0.29)	1.80 (0.27)	0.003
D	1.72 (0.23)	1.76 (0.21)	1.79 (0.21)	0.013
TP ms^2				
A	3.56 (0.37)	3.51 (0.40)	3.65 (0.39)	0.002
B	3.52 (0.40)	3.55 (0.43)	3.61 (0.44)	0.062
C	3.60 (0.41)	3.53 (0.43)	3.67 (0.42)	<0.0001
D	3.51 (0.34)	3.57 (0.31)	3.63 (0.31)	0.007
VLF ms^2				
A	2.95 (0.29)	2.91 (0.36)	3.05 (0.35)	0.023
B	2.92 (0.38)	3.00 (0.36)	3.08 (0.42)	0.044
C	3.09 (0.39)	2.93 (0.44)	3.09 (0.43)	<0.0001
D	2.94 (0.36)	3.03 (0.29)	3.07 (0.29)	0.067
LF ms^2				
A	2.96 (0.42)	2.89 (0.42)	3.06 (0.44)	0.001
B	2.92 (0.40)	2.91 (0.44)	3.00 (0.42)	0.060
C	3 (0.40)	2.93 (0.40)	3.10 (0.42)	0.001
D	2.92 (0.38)	2.97 (0.37)	3.07 (0.34)	0.013
HF ms^2				
A	3.07 (0.52)	3.02 (0.51)	3.12 (0.53)	0.116
B	3.01 (0.57)	3.02 (0.63)	3.09 (0.57)	0.121
C	3.06 (0.53)	3.05 (0.53)	3.17 (0.49)	0.006
D	3.01 (0.45)	3.08 (0.39)	3.14 (0.42)	0.027

a)All the values are log converted and represented as mean and standard deviation (SD) – univariate ANOVA.; P value < 0.05 was considered significant – Levene's test of equality; *P calculated using RM-ANOVA.

b)SDNN (ms), i.e., Standard deviation of NN intervals; RMSSD (ms) (Root mean square of successive RR interval differences); NN50 (number of successive RR intervals that differ by more than 50 ms); and pNN50% (percentage of successive RR intervals that differ by more than 50 ms); Low frequency (LF) power (0.04–0.15 Hz); high frequency (HF) power (0.15–0.40 Hz) given in absolute power (ms^2/Hz); very-low-frequency (VLF) (0.0033–0.04 Hz).



Delta Band



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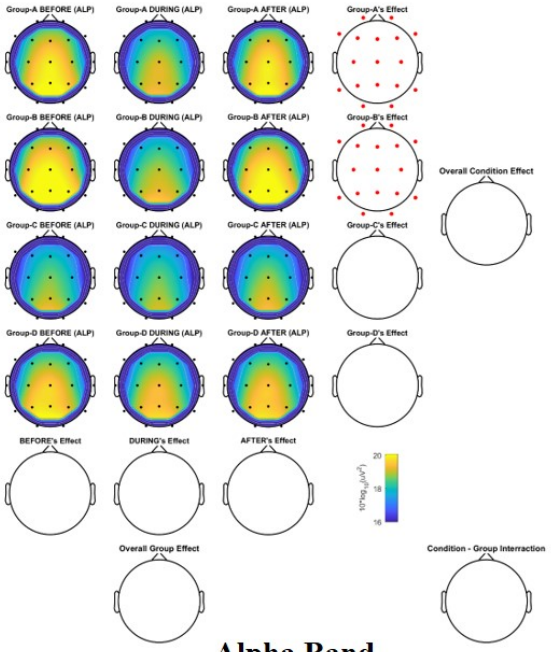

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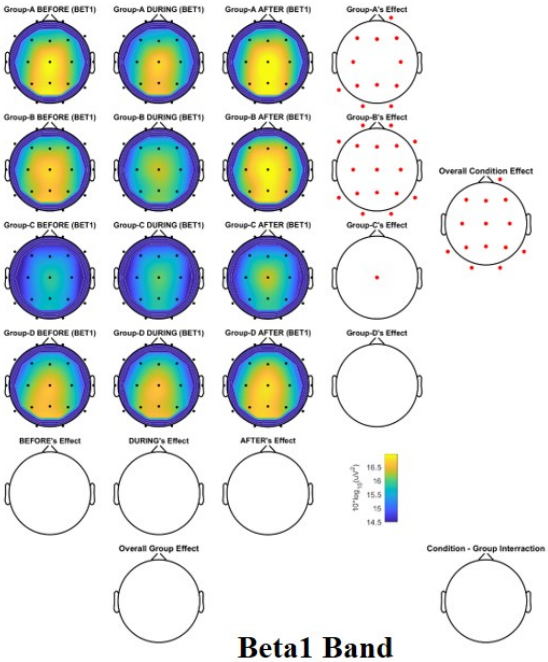

Beta1 Band

Note that for all the above figures the average power values across 19 electrodes are shown in the 9 central head maps, with warmer colour showing larger power values. Three conditions (Before music, During Music and After Music) are depicted along columns and three music groups (A, B and C) are shown along rows. The outer unfilled head maps show the FDR corrected p-values (red dots represent electrodes with significant p-values) for condition effect, interaction effect and group/*raga* effect respectively in the clock-wise direction (Permutation-based mixed effect ANOVA; 1000 permutations). The outermost layer showing overall effects of two-way ANOVA (condition, group and interaction effects) and the inner layer showing one-way ANOVA effects. The power spectral results for standard frequency bands (Delta: 1-4Hz, Theta: 4-7Hz, Alpha: 8-13Hz, Beta1: 13-20Hz, and Beta2: 20-30Hz), for all 19 EEG channels were taken into MATLAB software. They were subjected to permutation-based (1000 permutations) two-way and one-way ANOVAs; done using 'statcond' function of EEGLAB. Statistical threshold set at $p \leq 0.05$ (two-sided), after multiple comparisons between channels corrected using false discovery rate (FDR).

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Acknowledgements :- This project supported by Indian Council for Medical Research (ICMR), 2019-2020: RFC No.(P-10) HSR/Ad-hoc/9/2018-19,dated:03/12/2018.

PC70

Investigation of the neuroprotective effect of plasminogen activator inhibitor-1 antagonist in the rat model of traumatic brain injury

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Introduction: Traumatic brain injury (TBI) is defined as a change in brain function, or the presence of another brain pathology as a result of an external force (Menon et al., 2010). Oxidative stress, inflammation, and apoptosis following TBI lead to further deterioration of brain damage. Antiinflammatory and neuroprotective activity of the plasminogen activator inhibitor-1 (PAI-1) antagonist in neuroinflammatory animal models has been reported (Chan et al., 2018; Pelish et al., 2015).

Aims/Objectives: In this study, it was aimed to investigate possible protective effects of PAI-1 antagonist in a rat TBI model.

Method: Sprague Dawley male rats were grouped as sham (n=7), TBI (n=9), TBI + PAI-1 antagonist (5 or 10 mg/kg TM5441 or TM5484; n=6-7). Under anesthesia, TBI was induced by weight-drop model and the rats were decapitated after 24-hour. Before and 24-hour after trauma neurological examination, tail suspension, Y-maze, novel object recognition tests were performed. Activities of myeloperoxidase, nitric oxide levels, luminol- and lucigenin enhanced chemiluminescence were measured. Also, interleukin-1b, interleukin-6, tumor necrosis factor, interleukin-10, tumor growth factor-b, caspase-3, cleaved caspase-3, and PAI levels were measured with ELISA method in the brain tissue. Brain injury was graded histopathologically following hematoxylin-eosin staining. Western blot and immunohistochemical investigation for low density lipoprotein receptor, matrix metalloproteinase-3 ve nuclear factor-kB were also performed.

Results: Higher levels myeloperoxidase activity in the trauma group ($p<0.05$) were found to be suppressed in 5 and 10 mg/kg TM5441 treatment groups ($p<0.05$-$p<0.01$). The tail suspension test score was increased in trauma group ($p<0.001$), and decreased in all treatment groups ($p<0.05$-

0.001). The histologic damage score was increased statistically significantly in the cortex, dentate gyrus, and CA3 regions in the trauma group ($p<0.01$-0.001), decreased in the treatment groups in the cortex and dentate gyrus ($p<0.05$-0.001).

Conclusion: PAI antagonists, especially TM5441, are effective for some biological markers and behavioral tests after TBI. Examining the effects of PAI-1 inhibition on both pathophysiological and functional outcomes in TBI and obtaining promising results shed light on future clinical studies.

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PC71

Psychoneuroimmunological mechanism of long-term *Preksha* Meditation

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Objective: Meditation is one of the important tools of psychoneuroimmunology (PNI) that helps in healing and sustenance of good health, also cure and protect from several psychosomatic disorders that result from mental stress. On this concept, the authors have previously provided the attention control mechanism of meditation using an innovative methodology. Presently, the authors have extended the previous attention control mechanism to a more psychoneuroimmunological approach of preksha meditation.

Methodology: Present scientific psychoneuroimmunological mechanism of *preksha* meditation has been framed using an innovative methodological approach which facilitated the real-time brain imaging (18FDG-PET as an imaging technique) during meditation with EEG recording. Furthermore, this

methodological approach allowed us to record electroencephalography (EEG) to affirm meditation objectively while easily avoiding the mutual interference between PET-CT and EEG electrodes.

Conclusion: The innovative methodology provides the backbone to frame the psychoneuroimmunological mechanism of preksha meditation. Therefore, present framework provides the scientific evidences to show prefrontal cortex (PFC) acts as a 'Connector Hub Region' where all the components of preksha meditation that include attention control, emotional regulation, and altered self-awareness function simultaneously to exert the positive benefits in the maintenance of neuropsychiatric illnesses. Also, it is expected that the present psychoneuroimmunological mechanism will provide a scientific platform for future clinical studies of *preksha* meditation.

Keywords: Meditation, psychoneuroimmunology, evidence-based research, stress, diseases, health.

Clinical Trial Registry: The study was registered at Clinical Trial Registry India (CTRI), CTRI/2009/091/000727.

Acknowledgements :- NA

PC72

A study of Psychiatric Co-morbidity in Thyroid patients using Global Mental Health Assessment Tool (GMHAT/PC)

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Introduction: Mental illness has often been associated with thyroid dysfunctions and if left untreated it can further aggravate the severity of underlying disease.

Objective: The study was planned to assess psychiatric co-morbidity in patients with thyroid disorders using GMHAT questionnaire.

Methods: The study was commenced after ethical clearance from IEC. All the patients >18 years of age, attending the endocrinology OPD were screened for inclusion in the study. Based on the diagnosis established by the Endocrinologist, the study subjects were divided into hyperthyroid (n= 40) and hypothyroid (n=70) groups. Age and sex matched controls (n=50) were also included. All the selected participants underwent psychiatric evaluation through structured interview using the Global Mental Health Assessment Tool, Primary Care Version (GMHAT/PC) questionnaire. The data were compiled and appropriate statistical tests were applied.

Results: As per the structured GMHAT interview, out of 70 hypothyroid patients, 31 were found to have psychiatric disorder. In the hyperthyroid and control groups, 2 participants each were diagnosed with psychiatric disorders. Psychiatric co-morbidities observed in the participants ranged

from anxiety, depression, mania to obsessive compulsive disorder (OCD). Significant association was observed within the three groups; X^2 (2, 161)=37, P<0.0001). Result implies that patients with hypothyroidism are 15 times more likely to have mental illness as compared to hyperthyroid (OR-15.1; 95% CI – 3.4-67.5) and 19 times more likely to have mental illness than normal individuals (OR-19.1; 95% CI – 4.3 - 84.7). These symptoms were observed in hypothyroid as early as 2 months of the disease onset.

Conclusion: Patients with hypothyroidism have high psychiatric comorbidity with an early onset. Medical experts should be aware of this fact and can be trained to identify mental illness using a standard and validated easy tool GMHAT/PC. It is recommended to safeguard the mental health of these patients and implement efficient holistic approaches at the earliest to improve their overall outcome and quality of life.

PC73

Effect of Resveratrol on Mechanical Nociception in Rotenone Induced Parkinson's Disease in Mice

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Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder characterized by early prominent death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and wide spread presence of alpha synuclein (aSyn). Dopamine deficiency in the basal ganglia leads to classical Parkinsonian motor symptoms viz, bradykinesia, tremor, rigidity and later postural instability. Parkinsonism is also associated with non-motor symptoms, which may precede motor symptoms by more than a decade. Resveratrol (3, 4', 5- trihydroxystilbene) is a nutraceutical that has recently attracted a lot of research attention due to its exciting pharmacological potentials. It is a phytoalexin found in many plants including grapes, peanuts. This study investigated the effect of resveratrol on mechanical nociception in rotenone induced Parkinson's disease in mice using Randall sellito analgesiometer. Forty eight (48) animals were randomly assigned into six (6) groups of eight (8) animals each. Group 1 (control group) was administered distilled water, group 2 was administered Dimethyl sulfoxide (DMSO) only. The group 3 were administered rotenone only while the other groups were administered rotenone and resveratrol at a dose of 10, 20, 40 mg/kg. The average mechanical threshold in Rotenone only group was (10.96 ± 1.64 g) increased compared to the control group (10.52 ± 0.35 g) but the increase is not significant ($p > 0.05$). The mechanical threshold for the Rotenone + RSV treated groups is as follows: Rotenone + RSV10 mg/kg (8.75 ± .49), Rotenone + RSV 20 mg/kg (7.97 ± 0.48 g) and Rotenone + RSV 40mg/kg (9.63 ± 0.60 g). The results suggest that resveratrol did not significantly influence mechanical nociception in rotenone model of parkinson's disease.

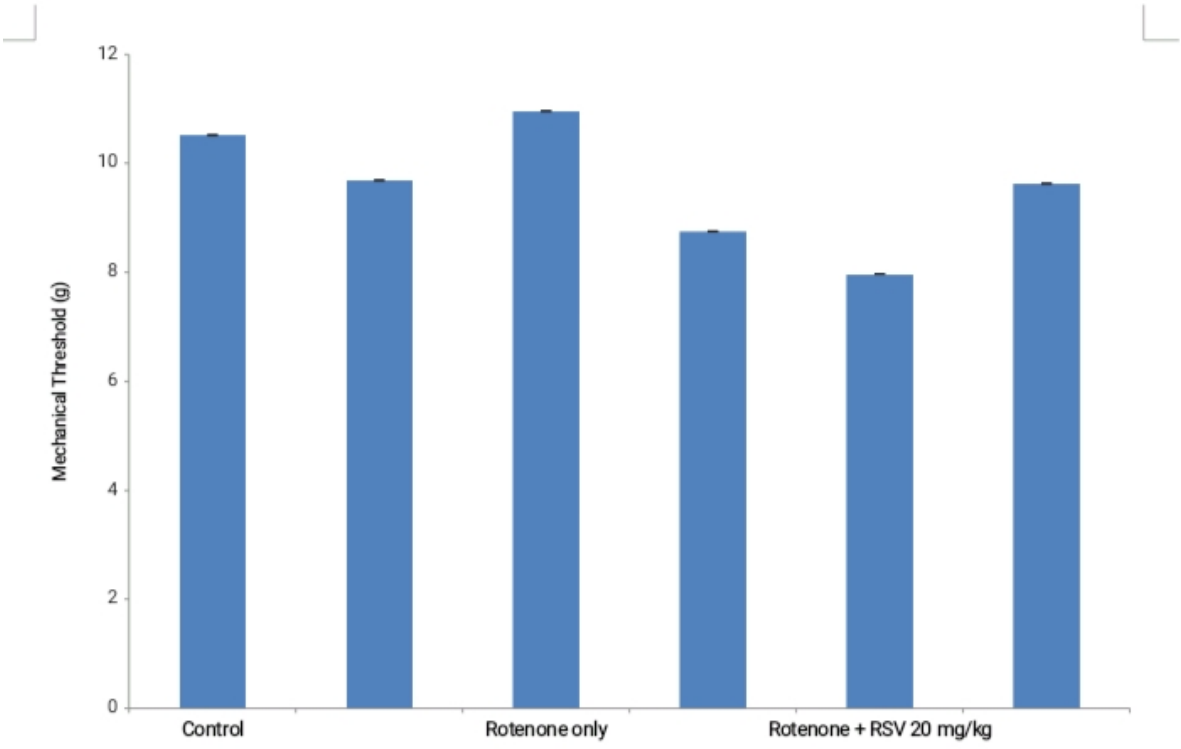

Figure 4.1: Effect of resveratrol on mechanical threshold in rotenone induced Parkinson's in mice treated with resveratrol. DMSO= Dimethyl Sulfoxide, RSV= Resveratrol (10, 20 , 40mg/kg).

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Changes in TRPV4, AQP1, and AQP4 in a Genetic Model of Hydrocephalus

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Hydrocephalus has an incidence of 1:1000 in the pediatric population. In hydrocephalus, cerebrospinal fluid (CSF) accumulates in the brain's ventricles causing ventriculomegaly. Hydrocephalus can affect various cell types in the brain, and this study focuses on astrocytes, ependymal and choroid plexus epithelial cells (CPe). The choroid plexus is a tight epithelial structure within the ventricular system, responsible for producing CSF. Ependymal cells serve an important barrier function and provide some CSF production and fluid/electrolyte maintenance. Astrocytes are supportive cells that serve in blood-brain barrier maintenance and brain fluid/electrolyte regulation. The CP, ependyma, and astrocytes contain channels and transporters that are important in regulating both CSF and brain interstitial fluid. These include transient receptor potential vanilloid 4 (TRPV4), aquaporin 1 (AQP1), and aquaporin 4 (AQP4). TRPV4 is a mechanosensitive channel and has been implicated in osmotic regulation. AQPs are a family of membrane proteins that facilitate water transport in various tissues. The CPe expresses AQP1 and TRPV4 which may be important in CSF production. Astrocytes express TRPV4 and AQP4 on their endfeet to regulate cell volume and vascular permeability. The ependyma mainly expresses apical AQP1 and basolateral AQP4. Previous studies done by the Blazer-Yost laboratory have shown increases in AQP1, but not TRPV4 mRNA of CPe from hydrocephalic animals compared to wildtype (1). This study further examined changes in localization and expression of TRPV4, AQP1, and AQP4 in a rodent model of hydrocephalus.

A single missense point mutation in the Transmembrane 67 (TMEM67) protein that causes a ciliopathy resulting in hydrocephalus and polycystic kidney disease (PKD) in our rodent model. This mutation is orthologous to the human Meckel Gruber syndrome type 3 (MKS3). The phenotypes seen in TMEM67 (-/-) (homozygous) animals are so severe that death will occur by post-natal day 21 (P21) (2). We have previously shown that treatment with TRPV4 antagonists ameliorate hydrocephalus in our genetic model of hydrocephalus (1). Using immunohistochemical techniques, TRPV4, AQP1, and AQP4 antibodies were used to examine localization in hydrocephalic animals at P15 (n=3). Changes of AQP4 and TRPV4 were observed in the cortex of homozygous animals. AQP4 appears to move from astrocyte endfeet in hydrocephalic animals and is increased at the brain-CSF barrier. TRPV4 appears to be increased throughout the cortex. qPCR was used to quantitate mRNA from the CPe and cortex. Preliminary qPCR showed increased TRPV4, AQP1, and AQP4 mRNA in the cortex and CPe of P15 hydrocephalic animals compared to wild-type (n=3). In summary, in a model of hydrocephalus, channels and transporters show changes in localization and transcription. These results provide further characterization of the role of several channels and transporters in the

pathophysiology of hydrocephalus. Futures studies will explore developmental changes in protein expression and membrane localization to compliment these studies. Examining channels and transporters can elucidate how brain fluid regulation may be altered in hydrocephalus and produce targets for pharmacological treatment in the future.

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SEX DIFFERENCES IN BRAIN ACTIVITY DURING VISUAL CHOICE REACTION TASK

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It is well known phenomenon in psychology that men and women usually make decision/choice using different mind strategies. The existence of such phenomen is obvious due to different ratio of determinant sex steroids that cause very powerful impact on brain development and functioning. However, the neurobiological aspects of men's and women's decision making strategies are still ambiguous and require more data. The knowledge of sex differences of brain activity potentially could provide better rehabilitation for men and women with various brain injuries.

Decision making implies execution of basic cognitive processes which are based on choice reaction. Thereby, in this study visual choice reaction task with 2 simple stimuli was used.

The aim of the study was to investigate whether there are any differences in brain activity and choice reaction time between male and female subjects during choice reaction task performance.

The study involved 30 male volunteers, right-handed, 23,2±3,1 y.o., and 25 female volunteers, right-handed, 18,4±0,6 y.o.. All participants were students of Taras Shevchenko National University of Kyiv and have no health complaints, reported brain injuries or psychiatric disorders.

In the study choice reaction time (CRT) of motor responses made by right (rhCRT) and left hands (lhCRT) were detected. Mean CRT was evaluated too. CRT data is presented below as Median [Lower Quartile (25%); Upper Quartile (75%)]. EEG was recorded during choice reaction task perfomence. EEG was done with 19 leads placed on the scalp according to the International 10-20 System. Localization and statistical analysis of 3D distribution of the generating electric neuronal activity were

performed applying the LORETA-software package v.20181107 [1,2]. Coherence analysis was performed for all possible coupled pairs of leads in delta (0,5-3,9 Hz), theta (4-7,9 Hz), alpha (8-12 Hz), beta-1 (14-19,9 Hz) and beta-2 (19-35 Hz) bands applying the Neuron-Spectrum software. Significant level of coherence value was established equal or greater than 0,7 [3].

There was no difference between results of male and female subjects in mean CRT (409 [392;430] ms vs. 420 [397; 460] ms (p=0,185)), rhCRT (402 [392; 435] ms vs. 413 [390;462] ms (p=0,473)) and lhCRT (413 [390;430] ms vs. 426 [409;476] ms (p=0,112)). Males demonstrated greater activation of the right hemisphere compared to females (p=0,013, threshold=0,487): BA 11, 17, 18, 19, 22, 23, 24, 25, 28, 30, 31, 32, 33, 34, 36, 37, 38, 39, 47. There are few left hemispheric brain zones that also were more active in males than in females: BA 11, 17, 18, 19, 24, 25, 28, 32 and 33. Coherence analysis revealed both males and females had distributed fronto-parieto-occipital networks in teta band within the left hemisphere. For males the same network in the right hemisphere was interconnected with lateral sites in fronto-temporal regions. Furthermore, females demonstrated no activation of right centro-parieto-occipital networks in alpha and beta-1 bands. Centro-parieto-occipital networks in beta-2 band were present bilaterally in females, while the activation of left fronto-parieto-occipital and right hemispheric centro-parieto-occipital networks was distinctive only for males. Also bilateral activation of centro-parieto-occipital networks in delta band was specific solely to females.

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