SA01

Contemporary Issues in an Ageing Society and Ageing Research

C. Stewart

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Healthy ageing is essential to prevent overburdening health care facilities and to capture the potential of an increasingly older population. Since 2001, commitments to achieving healthy ageing have featured heavily in government policy and directives. However, almost 20 years later, healthy ageing for all remains elusive. As a consequence, we have an urgent duty to reduce lifestyle-linked chronic ill health, if we are to enable healthy ageing for all. To deliver the goal of increasing healthy, active ageing, we must first understand what causes ageing, if we are to develop means to influence the processes. To achieve this goal, we need to study the ageing process in order to determine, manipulate and model functional, physiological, cellular and molecular adaptations with age. Using cellular (human skeletal muscle stem cells and C2C12 muscle cells), molecular, biochemical and human (age, exercise, disuse and cancer populations) intervention studies, it has been a career goal to develop useful, adoptable, translatable models to understand aspects of mal/adaptation with age. The purpose of this presentation will be to provide oversight of the work undertaken and career pathway followed to facilitate questions, challenge dogmas and enable collaborations in the highly important field of healthy ageing.

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

SA02

The Role of Epigenetics in Regulating Ageing Skeletal Muscle

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The deterioration of skeletal muscle into old age (sarcopenia) poses a significant risk to human health and well-being. Sarcopenia is not a uniform condition, with large inter-species variability in the intensity and progression of sarcopenia observed. With underlying genetics not able to adequately explain such variability in the condition, it leads one to suggest that environmental encounters, at least in part, regulate the sarcopenic milieu. In this regard, epigenetics, referring to gene expression changes not caused by underlying genetic variation, becomes a plausible candidate for the regulation of skeletal muscle during environmental insult. Indeed, recent work from has begun to characterise the role of epigenetic

modifications in mammalian skeletal muscle during periods of both anabolism and catabolism. For example, we have previously shown an important role for promotor associated DNA methylation of atro-genes during periods of acute muscular atrophy and recovery (Fisher et al., 2017) and global methylome changes during both acute and chronic resistance training induced muscular anabolism (Seaborne et al., 2018). Furthermore, classical research has shown the mammalian organism to be susceptible to early life stress encounters inducing adverse life-long phenotypic outcomes, referred to as developmental/foetal programming. Such a model has also been linked to epigenetics, where underlying modifications are maintained over time, leading to long-term regulation of the phenotype (Holland et al., 2016; Sharples, Stewart and Seaborne, 2016). We have recently shown a similar molecular memory mechanism in skeletal muscle (Seaborne et al., 2018). Epigenetic modifications may therefore act as the link between environmental insult and adverse transcriptomic and phenotypic outcome in skeletal muscle. We will therefore detail our current understanding of skeletal muscle epigenetics, the epigenetic aberrations associated with the sarcopenic niche and how exercise may alleviate these adverse outcomes.

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Holland, M. L., Lower, R., Caton, P. W., Gemma, C., Carbajosa, G., Danson, A. F., et al., (2016). Early-life nutrition modulates the epigenetic state of specific rDNA genetic variants in mice. *Science, 27,* 495-8.

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SA03

Circadian rhythms and molecular clock mechanisms: Working with time to develop new health strategies for humans

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Loss of muscle strength or weakness is associated with poor outcomes for aging and patients with a number of chronic diseases. In this session, I will present data to support the concept that one mechanism for weakness emerges from disruption of the circadian, or molecular clock mechanism in skeletal muscle. The molecular

clock is comprised of a core set of genes that function as a self-sustaining 24hr feedback loop. Beyond timekeeping, the molecular clock has a critical function in regulating a daily cell-specific transcriptional program. Studies from our lab, and others, have shown that targeted disruption of the molecular clock only in skeletal muscle is sufficient to induce muscle weakness defined by reduced force normalized for cross-sectional area. Our current work is focused on two areas: 1) We are working to define the skeletal muscle specific transcriptional landscape directed by the core clock factors, BMAL1:CLOCK. 2) We are using the transcriptomic analyses to guide our analysis of muscle structure and sarcomeric protein expression. To date, we have identified the muscle specific transcription factor, MYOD1 as key transcription factor linking the ubiquitous molecular clock to a daily muscle specific transcriptional program. We have also found that loss of clock function leads to altered expression of important sarcomeric genes that contribute to muscle structure. These results define a link between molecular clock disruption and muscle weakness and suggest a potential to target the muscle clock in conditions of weakness with aging and chronic diseases.

The work I will be presenting is the result of the hard work of a number of trainees and staff over many years. I would like to acknowledge the work of former PhD students Dr. Brian Hodge and Dr. Lance Riley, a current PhD student, Collin Douglas and my senior staff scientist, Dr. Xiping Zhang.

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SA04

Physiological and structural changes in skeletal muscle and nerve-muscle interactions: the effects of ROS and ageing

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With an ever growing population, and the fastest growing group being elderly, we need to understand and treat those factors which affect quality of life in later years. It is well established that there is an age-related decline in muscle mass and function with increasing age (termed sarcopenia) and this is associated with increased falls and hospital admissions.
There are many factors which have been associated with sarcopenia. Substantial alteration of motor neurons and changes in reactive oxygen species (ROS) with advancing age are widely described in rodent models. We have utilised several mouse models, deficient in the antioxidant enzyme superoxide dismutase 1 (SOD1), to examine to role of aberrant redox homeostasis on nerve-muscle

interactions. Whole body knock out of Sod1 in mice causes accelerated age-related muscle wasting, a decline in force production and alterations in neuromuscular junctions (NMJs).
The NMJ is composed of the pre-synaptic nerve terminal, the acetylcholine receptor clusters on the muscle and terminal Schwann cells that encase this region. It is a dynamic structure and multiple cycles of denervation and re-innervation throughout life are thought to result in structural changes which accumulate. When re-innervation fails the muscle fibre remains denervated and the incidence of denervated fibres is increased with advancing age which correlates with reduced muscle mass and function.
Using a surgical model of nerve transection we have shown that there is an increase in mitochondrial generation of hydrogen peroxide and other peroxides in denervated muscle fibres which also occurs in neighbouring fibres which have a "normal" NMJ structure. We speculate that the initial increase in peroxide production may stimulate adaptations to protect the muscle fibre. More chronic production of peroxides has also been shown to activate several degenerative processes preceding loss of muscle mass.
Recent *in vivo* imaging of HyPer2 transduced skeletal muscle from our lab has also drawn strong links between innervation status and peroxide production in nerve crush models Ongoing work seeks to understand the complex relationship between the redox status of motor nerves and skeletal muscle in the Sod1KO mouse model and in ageing of wild type mice together with the role that Schwann cells, which are responsible for NMJ stability, are playing in these situations.

The authors would like to thank BBSRC, MRC and NIH for supporting this work.

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SA05

DNA damage repair in vascular dysfunction

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Maintenance of DNA is an essential part of life sustainment in eukaryotic cells. Apart from being important for homeostasis, and its well-known role in cancer, accumulation of unrepaired DNA damage is a causative factor in organismal ageing. Ageing due to DNA damage is a process of progressive functional loss driven by changes in homeostatic mechanisms that are meant to protect against further accumulation of DNA damage, but that on the other hand result in the problems one encounters during the ageing process. In the past years our lab has shown that these problems include well-known features of vascular dysfunction the are found in aged organisms. With the use of mice that lack key DNA repair enzymes,

such as the endonuclease ERCC1, in all body cells, we have shown that defective DNA repair leads to a rapid development of increased blood pressure, decreased organ perfusion, decreased vasodilator responses, increased vascular stiffness, and increased vascular permeability. In animals lacking ERCC1 in endothelial cells or smooth muscle cells we demonstrate that the type of vascular dysfunction depends on the specific cell type in which DNA repair is ablated. Regarding signaling pathways we observed a decreased vasodilator function of the nitric oxide – cGMP axis. This is due to changes in eNOS, phosphodiesterase activity and oxidative stress. As a mediator of dysfunction we found that altered PDE1A and 1C is involved. Current studies with newly developed specific PDE1 inhibitors demonstrate that these drugs can restore vasodilator dysfunction as caused by DNA repair defectiveness, which might have implications for the treatment of vascular ageing.

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SA06

Lifestyle related diseases, aging and cardiovascular impairments

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A well-functioning cardiovascular system is key in keeping lifelong health. Impairments to the cardiovascular system, as observed with ageing and lifestyle related diseases, drastically shortens the healthy lifespan with great impact on quality of life.
Key components of the cardiovascular system are the heart, the larger conduit arteries, the smaller resistance arteries and the capillaries. The heart ensures a perfusion pressure sufficient to drive oxygenated blood to all regions of the body and especially the elastic and contractile properties of the heart are central elements of optimal cardiac function. The larger arteries serve as highways for blood delivery but importantly also as a pressure reservoir that lessens the workload of the heart. Thus, elasticity of the arterial wall is of outmost importance in absorbing the bulk of blood ejected from the left ventricle during systole and in gently propagating this pressurized blood down the arterial tree. The smaller arteries govern to which regions blood is being delivered and holds a key role in maintaining blood pressure within the optimal range. This function is mediated by a thick layer of smooth muscle cells arranged circumferentially giving the smaller arteries potential to control diameter within a very large range. The smallest vessels of the body, the capillaries are the site of delivery of oxygen and nutrients and removal of waste products. This exchange with the surrounding tissue is secured by the capillary wall structure, only one cell thick and with gaps, slits and transporters, allowing for diffusion and transport of smaller and larger molecules. Functional or

structural impairments at any level of this delivery chain from heart to capillary bed affects functionality of the cardiovascular system as they are all reliant on one another. Impairment introduced at one level by ageing or poor lifestyle may lead to conditions like hypertension that affects all levels and in turn can lead to even more severe conditions like heart failure and stroke.
Normal ageing has structural and functional impact on the heart and the vasculature that causes cardiovascular function to decrease as we age. These ageing induced changes include decreased elastic and contractile properties of the myocardium and the arterial wall, impaired control of vascular smooth muscle cells and thickening of the delicate capillary structure. Interestingly, lifestyle related diseases, like hypertension and diabetes mellitus, impose similar impairments to all components of the cardiovascular system, even at a very young age. Ultimately, this changes the health trajectory towards earlier morbidity and mortality. Fortunately, detrimental changes to the cardiovascular system can be prevented, stopped or even reversed by physical activity.
The focus of this talk will be on cellular mechanisms of ageing and lifestyle related diseases connected to impaired cardiovascular function and how these are affected by physical activity. Classic and novel data from invasive human studies will be presented in order to establish knowledge of how far this field of cardiovascular research has come. Furthermore, the talk will bring focus on future research strings that may have great potential in translating cellular mechanism into lifelong health strategies.

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SA07

Lifelong Aerobic Exercise, Exercise Factors, and Exosomes

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The long-term adaptations to aerobic exercise training (AET) are diverse, multisystemic, and include major health benefits and prolonged lifespan. Acute contractile activity stimulates the release of a myriad of bioactive factors, including cytokines, adipokines, and myokines (termed exercise factors or 'exerkines'), which are postulated to drive training adaptations by exerting autocrine, paracrine, and endocrine effects. Recently, extracellular vesicles (EVs) have been proposed to act as carriers of exerkines, thereby 'mediating' cell-to-cell communication and inter-organ crosstalk. *In vitro* and *in vivo* studies have suggested that exercise augments the biogenesis and release of several EV sub-populations, including exosomes and microvesicles (MVs), and that they may be associated with the

simultaneous release of classical myokines and/or cytokines (such as IL-6, IL-1, and TNFα). However, several inherent challenges to blood preparation procedures, EV isolation protocols, and classification of vesicle subpopulations, have delayed the advancement of our current understanding of exercise effects on the secretome. Independent variables, such as training status, gender, age, and disease also modulate EVs and exerkines (e.g., biogenesis, composition, secretion, and/or cargo), and must be considered when interpreting the growing body of knowledge. Because AET unequivocally decelerates biological aging, reduces all-cause mortality risk, and extends lifespan, academic and commercial interests are rapidly expanding across the globe, which necessitates methodological standardization and consistency between studies in order to generate high-quality, reproducible data.

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

Nilsson and Nederveen contruibuted equally to this work.

SA08

Sedentary behaviour: A behavioural target in the prevention and management of chronic disease

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Technological innovations and economic advances have led to increases in physical inactivity and sedentary behaviour (i.e., sitting). Evidence now indicates that it is not only necessary to be physically active at a moderate intensity for at least 150 minutes a week, but it is also important to limit the number of hours spent sitting. Over the past decade a wealth of epidemiological evidence has emerged suggesting that sedentary behaviour is associated with an increased risk of chronic disease (e.g., type 2 diabetes, cardiovascular disease, some cancers) and mortality. Acute experimental studies have shown that interrupting sitting with short (2-5mins) but frequent bouts of light intensity activity (standing, stepping and simple body weight exercises) throughout the day improves markers of cardiometabolic health. As such, interventions to reduce sitting time and break up prolonged sitting with light activity have been developed and evaluated, mainly in the workplace. This presentation will discuss this evidence, the strength of the evidence, how much sitting is too much, how much moderate activity is needed to eliminate the risk associated with high sitting time and how successful sitting reductions interventions have been.

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C01

Individual and Combined Genetic Variations are Associated with *in vivo* and *in vitro* Muscle Damage in Humans: a Genetic Approach to Elucidate the Mechanisms Underpinning the Response to Exercise-Induced Muscle Damage

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Introduction We aimed to test the hypothesis that a combination of genetic variations forming a polygenic profile could estimate high and low responders to exercise-induced muscle damage (EIMD).
Methods Sixty-five young female and male untrained Caucasians performed 120 maximal eccentric knee-extensions to induce EIMD. Maximal quadriceps strength, range of motion (ROM), muscle soreness and serum blood biomarkers were assessed before, directly after and 48 h after the EIMD intervention and participants were genotyped for 20 candidate single nucleotide polymorphisms (SNPs). Significant SNPs were further investigated regarding the muscle recovery following an artificial wound healing assay *in vitro.* SNPs that showed a gene-intervention interaction *in vivo*, were used to calculate a total genotype score in respect to the acute response following EIMD (TGS-A) and the cohort was then divided into a "preferential" (PG), "moderate" (MG), and "non-preferential" (NPG) genetic group.
Results Four SNPs, which showed an interaction/main effect with the EIMD-intervention *in vivo*, also demonstrated changes in muscle stem cell characteristics *in vitro*. Seven SNPs demonstrated significant interactions *in vivo*, and these candidate SNPs were used to compute the TGS-A. There was a main effect for isometric and isokinetic MVC torque regarding TGS-A (both $P<0.001$). Individuals of the NPG and MG group were consistently weaker compared to the PG group ($P=0.005$), and NPGs demonstrated higher muscle soreness ($P=0.003$) and decreased ROM ($P=0.006$) following the EIMD-intervention, respectively.
Conclusion Seven SNPs have been associated with EIMD and recovery. When SNPs were combined, NPG not only demonstrated poorer recovery following EIMD but this group was also generally weaker than PG, which could have clinical implications in terms of exercise prescription for reducing injury risk in those who are predisposed to greater muscle damage following strenuous exercise. Further, the associations between four of these seven SNPs with in vitro damage/regeneration suggest novel gene-cell-skeletal muscle mechanisms explaining the individual response to EIMD.

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C02

Single-Cell Transcriptomic Analysis Revealed molecular signature of Pnmt-derived Cardiomyocytes

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Rationale: Phenylethanolamine N-methyl transferase (PNMT) is the enzyme that catalyses the conversion of noradrenaline into adrenaline in endocrine cells. Our group previously reported a new type of cardiomyocytes that historically express PNMT during development stage. We call these cells as Pnmt-derived cardiomyocytes (PdCMs)[1]. PdCMs have unique left heart localisation in adult heart[1]. However, so far little is known about their molecular signature and their potential additional function in physiological or pathophysiological conditions comparing to conventional cardiomyocytes due to their neuronal origin.
Methods: single cardiac cells were isolated from either C57BL neonatal mouse hearts (aged 3 days, n=20) or adult (12 weeks old) YFP-ChR2/Cre-Pnmt mouse hearts for Single-cell RNA sequencing (scRNAseq). For neonatal cells, 10X Genomics scRNAseq was used. Single cell suspensions were converted to barcoded scRNA-seq libraries with the Chromium Single Cell 3′ kit with 10X Genomics platform, aiming for an estimated 10,000 cells per library. The libraries were sequenced using HiSeq400. Expression matrices were generated using CellRanger and analysed by Seurat package. Dimensionality reduction using principle component analysis was applied to identify major cell types and their subtypes. For adult myocytes, SORT- RNAseq was used.
Results: In neonatal heart samples, a total of 10550 transcripts were sequenced at single cell resolution. Major cell types were identified by leveraging single-cell transcriptomics analysis. We found Pnmt expressing cells are exclusively cardiomyocytes in neonatal heart. Rspo3 and Bmp2 are the most differentially expressed genes in Pnmt expressing cardiomyocytes compared to conventional cardiomyocytes. The clustering of Pnmt expressing cells are mainly presented in cardiac conduction system (CCS), left atrium and left ventricle. In adult mice, 192 YFP-Pnmt negative and 192 YFP-Pnmt positive cells were sequenced. Expression of Pnmt gene was significantly declined and even disappeared in most PdCMs. However, in both neonatal and adult $Pnmt^{+}$ cells, a high expression of exocytosis-related genes including CPLX2, SLC12A2, SLC6A4, SLC22A1, RAB31, RAB35, RAP2B, STX7, VAMP3, VAMP4 have been identified.
Conclusions: Single-cell RNA sequencing revealed molecular signature of Pnmt-derived myocytes. Their high expression of exocytosis-related gene expression and special localization suggest their potential role in localized endocrine signaling and regulation.

WANG, Y., LIN, W. K., CRAWFORD, W., NI, H., BOLTON, E. L., KHAN, H., SHANKS, J., BUB, G., WANG, X. & PATERSON, D. J. J. S. R. 2017. Optogenetic control of heart rhythm by selective stimulation of cardiomyocytes derived from Pnmt+ cells in murine heart. 7, 40687.

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

C03

A transcriptomic insight into the mechanism underlying the decrease in atrial $I_{Ca\text{-}L}$ in heart failure

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In heart failure (HF) impaired ventricular relaxation means the atrial component of ventricular filling becomes more important. However, the amplitude of the Ca^{2+} transient in the atria, which underlies contraction, is decreased in HF. We have shown that a decrease in atrial L-type Ca^{2+} current ($I_{Ca\text{-}L}$) in HF underlies the decrease in Ca^{2+} transient amplitude but the mechanism behind the decrease in $I_{Ca\text{-}L}$ in HF is not fully understood. Since transverse (t)-tubules, the deep invaginations of the cell membrane on which $I_{Ca\text{-}L}$ resides, are almost completely lost in HF we investigated if $I_{Ca\text{-}L}$ expression is decreased in the HF atria.

HF was induced in sheep by rapid ventricular pacing at 210 beats per minute for ~6 weeks. A pacing lead was implanted in the endocardial apex of the right ventricle using a minimally invasive transvenous approach under fluoroscopic guidance. Surgical plane anaesthesia was maintained under isoflurane (3-5%) mixed with of oxygen (4.5-6 $L.min^{-1}$). HF progression was monitored via echocardiography.

We have employed a transcriptomic based approach through RNA-sequencing and qPCR of atrial tissue in control and HF in order to determine the causes of reduced $I_{ca\text{-}L}$ in HF. RNA was isolated from time-matched control and HF tissue using a Trizol method and column-size isolation. Following quality control, samples (normalised to 1 μg), with an RNA integrity number of ≈8, were synthesised into a cDNA library by polyadenylation enrichment and sequenced on an Illumina HiSeq4000 at the Wellcome trust for Human Genetics, Oxford. Samples were pooled and run across three lanes at a length of 75 base pairs with paired-end reads. Subsequent BAM files were analysed through DESeq2 at QFAB.

Despite T-tubules loss in HF no change in the LTCC pore forming unit, CACNA1C, was detected through RNA-Seq or qPCR. Secondly, the associated subunits that form the LTCC together with CACNA1C were investigated. Expressional changes ($P<0.05$) were detected in three subunits, CACNB1, CACNA2D1 and CACNG6, none of which would be expected to decrease $I_{Ca\text{-}L}$. Finally, external modulators of $I_{Ca\text{-}L}$ were considered. Phosphodiesterases (PDEs) are known to modulate $I_{Ca\text{-}L}$ through

protein kinases A and G (PKA/G) and were identified as potential candidates via PANTHER. Six PDEs were found to change in HF; PDEs 3B,3A,8A,6D and 12 were upregulated whilst 1B is downregulated. Of these six PDE3B is thought the most likely to co-localise to the LTCC nanodomain at the cell membrane. Increased expression of PDE3B was confirmed by qPCR and thus may be responsible for the decrease in $I_{Ca\text{-}L}$ in HF.
In conclusion we show the decrease in atrial $I_{Ca\text{-}L}$ in HF is not brought about by a decrease in mRNA encoding the channel subunits. Our data suggests alterations in $I_{Ca\text{-}L}$ regulators such as PRKAR1A and an increase in PDEs may play a role in decreased $I_{Ca\text{-}L}$ function and warrant further investigation.

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C04

A proteomic insight into liver dysfunction and metabolism during obesity

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Obesity and insulin resistance are characterised by altered metabolism and function in various tissues. In the liver, obesity contributes to liver-related metabolic diseases including insulin resistance and non-alcoholic fatty liver disease (Kitade *et al.*, 2017). The ob/ob mouse, which produces a truncated inactive leptin protein, is widely used as a model of obesity-induced metabolic disease (Drel *et al.*, 2006). Whilst the physiological phenotype of ob/ob mice has been well characterised, the underlying alterations in protein content and function remain relatively understudied. Using a deep-proteomic approach, we have identified divergent proteins and pathways that may underpin the mechanistic basis of obesity-induced liver dysfunction.
Liver samples were harvested from four-month old ob/ob mice and lean littermates (n = 4 per group, C57BL/6J background) following a four-hour fast. Liver samples were lysed in a 4% SDS buffer and processed according to the MED-FASP protocol using Lys-C and trypsin. Peptides were separated on an Easy nano-flow HPLC system coupled to a LTQ Orbitrap mass spectrometer (HFX) via a nanoelectrospray source (Thermo Fisher Scientific). MS and MS/MS spectra were acquired in a data-dependent manner and analysed using MaxQuant software. Downstream data analysis was performed in Perseus software using label-free quantification (LFQ) intensities.
Proteomics analysis of liver from lean and ob/ob mice led to the quantification of 5551 proteins. 330 proteins were differentially regulated with obesity, of which 172 and 158 proteins were up- or downregulated, respectively, in ob/ob mice.

Gene Ontology annotations related to fatty acid metabolism were upregulated in the liver of ob/ob mice as well as the KEGG pathways PPAR signalling and peroxisome, indicating an adaptation to excess fatty acid availability. Furthermore, Gene Ontology cellular components related to the extracellular matrix were also upregulated in obese livers. Conversely, cellular component terms related to the endoplasmic reticulum were enriched in the downregulated proteins. The cytochrome p450 family, involved in the metabolism of arachidonic acid (a membrane phospholipid) and lipocalins, specifically the major urinary protein (MUP) isoforms, were also significantly downregulated in ob/ob liver.
Overall, we provide a deep-proteomic analysis of liver from ob/ob and wild-type mice, identifying known and emerging regulators of insulin sensitivity within the liver. Further analyses will provide additional mechanistic insight into the development of liver dysfunction during obesity, in particular into the dysregulation of the extracellular matrix.

Drel VR, Mashtalir N, Ilnytska O, Shin J, Li F, Lyzogubov VV & Obrosova IG. (2006). The leptin-deficient (ob/ob) mouse: a new animal model of peripheral neuropathy of type 2 diabetes and obesity. *Diabetes* 55, 3335-3343.

Kitade H, Chen G, Ni Y & Ota T. (2017). Nonalcoholic Fatty Liver Disease and Insulin Resistance: New Insights and Potential New Treatments. *Nutrients* 9, 387.

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

C05

Muscle adaptation induced by endurance activity involves changes to protein abundance that are regulated by protein degradation as well as protein synthesis.

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Muscle adapts to exercise through changes in the abundance of specific contractile and metabolic proteins. Exercise training also increases muscle protein turnover, but it is not known how synthesis and degradation contribute to exercise-induced changes in the abundance of individual proteins. We used stable isotope (deuterium oxide) labelling in combination with chronic low-frequency stimulation (CLFS) *in vivo* to investigate the synthesis, abundance and degradation of individual proteins during exercise-induced muscle adaptation.
Surgery was performed on 4 independent groups of rats (n = 3 in each) under aseptic conditions. Pre-operativeanalgesia 0.05 mg Buprenorphine/kg body mass was provided and anaesthesia was induced by 4 % isoflurane-O_2 adjusted to 1-2 % isoflurane during surgery. Unilateral CLFS (10 Hz, 24 h/d) and deuterium oxide were administered for 0 d, 10 d, 20 d or 30 d. Rats were humanely killed

and the extensor digitorium longus (EDL) was isolated from stimulated (Stim) and non-stimulated (Ctrl) legs. Proteomic analysis encompassed 30 myofibrillar and 47 soluble proteins. Protein fractional synthesis rate (FSR) was calculated from peptide mass spectrometry data. Absolute synthesis rates (ASR) were derived from FSR and abundance data and used to calculate protein degradation rates. Data are mean ± SD compared by within-animal paired t-test. CLFS tended (P = 0.145) to increase the synthesis of mixed myofibrillar proteins from Ctrl (FSR, 4.7 ± 0.3 %/d; ASR, 8.6 ± 0.3 pg/d) to Stim (FSR, 5 ± 0.1 %/d; ASR, 10.3 ± 1.6 pg/d). Whereas the synthesis of mixed soluble proteins increased (P = 0.001) from Ctrl (FSR, 5.2 ± 0.04%/d; ASR, 43.7 ± 0.1 pg/d) to Stim (FSR, 5.4 ± 0.4 %/d; ASR, 60 ± 0.7 pg/d).
Protein turnover responses differed on a protein-by-protein basis. The abundances of glycogen phosphorylase(PYGB), beta-enolase(ENOB) and troponin T, fast (TNNT3) significantly (P<0.05) decreased (-11 ± 3-fold) after CLFS. The decrease in abundance of ENOB was partially accounted for by a lesser synthesis in Stim muscle. The decrease in PYGB abundance was entirely accounted for by the decrement in its synthesis rate, whereas the abundance of TNNT3 decreased without a detectable change in synthesis. Myosin regulatory light chain 2 (MLRS) was resolved as 2 separate proteoforms (a and b). MLRSa was uniquely phosphorylated at S20 and significantly (P< 0.05) decreased (62 %) in abundance, whereas MLRSb tended (P = 0.100) to increase (16 %) after 30 days of CLFS. There was no difference in synthesis rate between Stim and Ctrl muscle of either MLRS proteoform. Therefore, we attribute the decrease in MLRSa abundance to proteoform-specific degradation. In conclusion, muscle adaptation in response to CLFS is underpinned by protein-specific changes in degradation as well as synthesis.

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

C06

MicroRNA control of the circadian rhythm in heart rate

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Background: There is a circadian rhythm in heart rate and conversely, bradyarrhythmias occur at night. Recent work from our lab has demonstrated heart rate rhythmicity to be underscored by a diurnal fluctuation in the key pacemaking channel HCN4, and its corresponding funny current I_f, likely driven by a local clock transcription factor BMAL1 (1). We have also previously shown that microRNAs (miRs) regulate sinus node pacemaking by direct negative regulation of *Hcn4* transcription (2). Here we tested whether rhythmic miR expression explains *Hcn4* and *Bmal1* rhythmicity in the sinus node.

Methods: Sinus node biopsies were collected from nocturnal adult male C57BL/6J mice at zeitgeber time (ZT) 0, ZT6, ZT12 and ZT18 (n=7/8). TaqMan Array MicroRNA A+B Cards were used to measure the expression of 750 miRs and 6 reference transcripts. JTK_Cycle (3) and sine wave fitting were used to identify rhythmic miRs (exhibiting a ~24 h periodicity) and *in silico* prediction algorithms RNA22, TargetScan and PITA applied to identify potential 3′ untranslated region (3′UTR) target sites. Functionality of predicted sites was tested by dual luciferase reporter gene assay; recombinant plasmids, in which a luciferase coding sequence was fused to (i) *Hcn4* 3′UTR and (ii) *Bmal1* 3′UTR, were co-transfected individually with (i) 19 and (ii) 11 miR mimics in H9C2 rat myoblast cells and compared to control plasmid. Firefly luciferase was measured and normalised to Renilla luciferase in 7 independent batches of cells.
Results: JTK_Cycle analysis identified 159 rhythmic microRNAs (Bonferroni adjusted $P<0.05$). Sine wave fitting was carried out on this dataset to identify false positives, after which 56 miRs were found to exhibit day-night rhythms in the sinus node. Of these miRs, 39 and 11, respectively, were expressed antiphase to *Hcn4* and *Bmal1*, indicating potential for negative regulation. Bioinformatics analyses identified 19 miRs with binding sites on the *Hcn4* 3′UTR whereas 11 miRs were predicted for *Bmal1* 3′UTR binding. Compared to control, 6/19 miRs predicted to target *Hcn4,* and 2/11 miRs predicted to target *Bmal1,* resulted in a significant reduction in reporter bioluminescence *vs.* control ($P<0.05$, unpaired t test). Compared to co-transfection with negative control *C.elegans* miR-67, miRs-146a-5p, -128-3p, -150-5p and -203-3p significantly reduced *Hcn4* 3′UTR activity ($P<0.05$, unpaired t test). Sine curve analysis of these miRs determined them to be expressed antiphase (peak ~ZT9) to *Hcn4* (peak ~ZT21).
Conclusions: This is the first report of rhythmic miR expression in the sinus node. miRs-146a-5p, -128-3p, -150-5p and -203-3p are novel regulators of *Hcn4* transcription and ongoing studies are examining their physiological relevance for the circadian rhythm in sinus node pacemaking. This could be the first step towards chronotherapeutic targets for the treatment of heart rhythm disturbances.

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

C07

3D Segmentation of the Cardiac Mitochondria and Nuclei from the Greenland Shark (*Somniosus microcephalus*) Insights Into Extreme Longevity

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The Greenland shark (Somniosus microcephalus) live up to 392 ± 120 years, making it the world's oldest-living vertebrate [1]. Because cardiovascular diseases are synonymous with age in humans, we aimed to understand how the heart of this vertebrate can beat since Shakespearian times without failing. Our objective was to elucidate morphological characteristics of organelles associated with natural aging, the mitochondria and the nuclei. Heart tissue samples from the compact region of the Greenland shark ventricle were collected from a ~200 year old female Greenland shark and processed for serial blockface scanning electron microscopy according to the Ellisman protocol [2]. Serial images were collected using Gatan 3View and analysed with IMOD. Heart tissue samples from female Greenland shark (aged 108-220 years-old) preserved in formalin were processed following immunohistochemistry procedures. Image analysis was performed using ImageJ. Approximately 1,200 mitochondria were reconstructed providing a mitochondrial volume density of 69% which is higher than that found in other polar fishes, and similar to that found in highly aerobic muscles such as billfish heater cells. The mitochondrial volume density observed in the Greenland shark may reflect aerobic need relative to its cold environment [3]. This high mitochondrial content could have happened through mitochondrial biogenesis through a molecular pathway contributing to longevity in a variety of species [4]. We observed mitochondrial syncitia which are clues for mitochondrial fusion. Mitochondrial morphology is shaped by mitochondrial dynamics, including mitochondrial fusion which is essential to maintain a normal cardiac function [5]. The shape of the cardiomyocyte nuclei and the heterochromatic structure further support a phenotype resilient to age. We conclude that the subcellular characteristics of the cardiac myocyte in the Greenland shark reflect a healthy and youthful phenotype. In the future, our dataset will be complemented with an increased sampling size, comparisons with juvenile Greenland shark cardiac myocytes and molecular assessments of mitochondrial dynamics.

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C08

The missing LINC to human healthy ageing: myonuclear architecture and mechanics in old age

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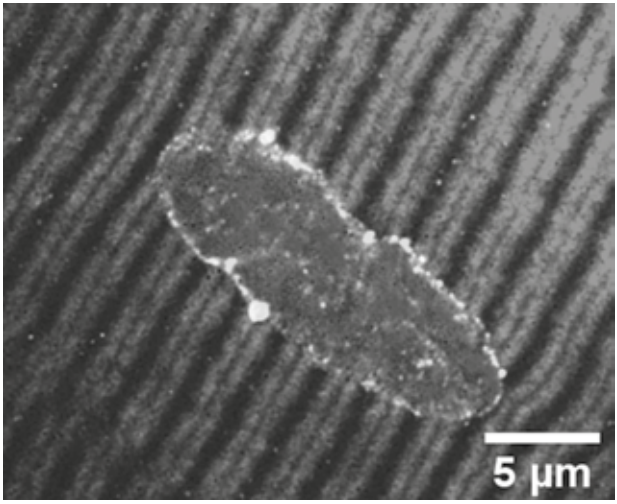
Age-related declines in cellular structure and function can be initiated or accelerated by physical inactivity. Ageing is associated with aberrant nuclear morphology, architecture and translation of forces to biochemical signals (mechanotransduction); this project focuses on whether, in skeletal muscle, these changes are influenced by physical activity status. Nuclear morphology and the distribution of nuclear envelope proteins important for nuclear architecture and mechanotransduction (SUN1 and LEM2) were studied in isolated human muscle fibres from individuals of different ages and physical activity statuses. So far, the data indicate that in humans, changes in myonuclear morphology occur in response to exercise regardless of age, indicated by a 25-30% reduction in myonuclear aspect ratio in young and older exercise-trained individuals compared to inactive young and older counterparts. No differences in the distribution of proteins SUN1 or LEM2 were revealed through super-resolution microscopy. Exercise therefore appears to influence nuclear morphology in a manner unaffected by age; alterations to nuclear envelope proteins other than SUN1 and LEM2 may be involved in this process. Future research will be focused on analysing myonuclear morphology in 3D, the distribution of nuclear envelope proteins Lamin A and Nesprin-1 and muscle-specific cytoskeletal protein Desmin. Whether alterations to myonuclear mechanics influence gene transcription will also be assessed through cell microharpooning and RNA sequencing.



Myonucleus of an isolated skeletal muscle fibre extracted from a vastus lateralis biopsy of a young inactive individual. Myonuclei in these individuals, and in older hip fracture patients (model of disuse), are elongated, which contrasts rounder myonuclei in active younger and older counterparts. Fibres stained to visualise Myosin heavy chain 7 (magenta), nuclear envelope/LINC complex protein SUN1 (green) and DNA (blue).

Professor Stephen Harridge, Professor Norman Lazarus, Dr Ross Pollock, Dr Jacob Ross, Tom Francis, Natasha Ranu, Abbi Hau, Ben Grimsdell, Dr Matthew Stroud & Dr Julien Ochala

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C09

The impact of replicative ageing and dietary flavonoids on C2C12 muscle cell mitochondrial function

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Dietary flavonoids possess distinct antioxidant and anti-inflammatory properties and may also regulate mitochondrial function. With advancing age and physical inactivity, skeletal muscle mitochondrial function is compromised. No studies have investigated the potential for flavonoids to modulate mitochondrial function in young and replicatively 'aged' C2C12 skeletal muscle cells. Therefore, the objective

of this study was to examine the impact of acute dietary flavonoid treatment on mitochondrial function in control (P5-8) and replicatively aged (P49-51) C2C12 myoblasts and myotubes. Undifferentiated myoblasts (0-24 h) and differentiated myotubes (96-120 h) were treated with flavonoids (Quercetin, Q; Epigallocatechin Gallate, EGCG; and (-)-Epicatechin, EPI) at four doses (0, 1, 5 and 10 μM). After 24 h treatment, mitochondrial bioenergetics were examined using an extracellular flux analyser (Seahorse XFe96). Mitochondrial and antioxidant related gene expression in young myoblasts was also determined by RT-PCR in response to Q, EGCG and EPI treatment (0, 5 and 10 μM) over 24 and 48 h. PCG1a gene expression was augmented 2.2- and 1.2-fold over control at 48 h by 5 and 10 μM EPI ($P = 0.011$) and EGCG ($P = 0.030$), respectively. Similarly, at 48 h, 10 μM EPI and 5 μM EGCG increased SOD2 expression by 1.8-fold ($P = 0.018$) and 2.2-fold ($P = 0.010$), respectively vs control. A significant main effect of age on basal respiration (BR), ATP production, proton leak (PL) and coupling efficiency (CE) was evident in myoblasts ($P < 0.05$), with aged cells demonstrating 35, 43, 27 and 6 % higher values over control, respectively. Compared to untreated myoblasts, BR was ~30% lower in EPI treated (1 and 5 μM) aged myoblasts ($P < 0.05$). EPI treatment (10 μM) also lowered PL in aged myoblasts vs. untreated control ($P = 0.020$). In myotubes, there was a significant main effect of age ($P < 0.005$) on maximal respiration, PL, ATP generation, spare respiratory capacity % and CE, with young controls exhibiting 190 % greater ($P < 0.001$), 38 % lower ($P = 0.090$), 20 % higher ($P = 0.534$), 62 % higher ($P < 0.001$) and 13 % higher ($P < 0.001$) values over aged controls, respectively. There was generally no significant effect of flavonoid treatment, although, EGCG (1 μM) lowered BR and PL in comparison to untreated aged myotubes ($P < 0.05$). In summary, we demonstrate flavonoids may upregulate the transcription of mitochondrial associated genes in myoblasts. Moreover, we show flavonoids may offer therapeutic potential in aged skeletal muscle cells via actions on mitochondrial bioenergetics. Our findings also suggest replicative ageing has divergent effects on mitochondrial function in myoblasts and myotubes. Whilst aged myoblasts show no evidence of mitochondrial deficiency over control cells, aged myotube bioenergetics seem impaired following differentiation vs unaged control myotubes.

We thank Dr. Johnathon Barlow from the Mitochondrial Profiling Centre at the University of Birmingham for his technical expertise and assistance in use of the Seahorse XFe96 Analyzer for measures of mitochondrial function.

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C10

Reduction in Multiple Cellular Mechanisms During Early Replicatively Aged C_2C_{12} Differentiation Results in Subsequent Lack of Myotube Formation

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Introduction: The ability to retain skeletal muscle mass and function declines with ageing (sarcopenia). Myoblast fusion is integral to the efficient regeneration and growth of muscle. Using a relevant model, we investigated mechanisms during early differentiation involved in preventing aged myoblast fusion. The hypotheses being challenged were: 1) during early differentiation, aged cells have impairments in cellular signalling and gene expression of markers of myoblast fusion, 2) aged cells have decreased abundance and turnover of specific proteins that are associated with inhibited cell fusion.
Methods: Control and replicatively aged (do not fuse but are not senescent) murine C_2C_{12} myoblasts were cultured for 96 h and myotube formation was assessed morphologically and biochemically (creatine kinase (CK)). During early myoblast differentiation (0-24 h) intracellular signalling (Akt, mTOR, ERK, p38) and gene expression (IGF-I, myogenin, ID3) were assessed. Differentiatingaged and control myoblasts were grown in the absence and presence of deuterium oxide (D_20). Samples were lysed at 0 h and 24 h and analysed using LC-MS/MS to measure changes in the protein abundance and to determine fractional synthesis rate (FSR) on a protein by protein basis. Experiments were repeated 3 times in duplicate. Data were analysed using one-way ANOVA.
Results: Control cells exhibited significant fusion ($P < 0.05$) with time, whereas aged myoblasts did not fuse and displayed significant reductions in CK (6-fold: $P < 0.05$) vs. control at 96 h. Akt and mTOR activation over 24 h were significantly decreased (8-fold and 3-fold respectively: both $P < 0.05$) vs. control. Significant suppression of myogenin, IGF-I (1000-fold and 10-fold; both $P < 0.05$) and a significant increase in ID3 expression (4-fold: $P < 0.05$) were observed at 24 h vs. control. Aged myoblasts had 33 ribosomal proteins with significantly lower ($P < 0.01$: FDR 10 %) abundance vs. control myoblasts. Ten metabolic enzymes with significantly greater ($P < 0.01$: FDR 10 %) abundance in aged myoblasts vs. control myoblasts. The average synthesis rate of proteins in aged myoblasts (0.47 ± 0.34 %/h) was significantly less (1.3-fold; $P < 0.05$) vs. controls (0.59 ± 0.28 %/h) and there were 157 individual proteins with relative differences in FSR.
Conclusion: Replicatively aged myoblasts failed to fuse, illustrated morphologically and confirmed biochemically. Suppressed levels of Akt and mTOR activation, together with reductions in myogenin and IGF-I and increases in ID3 may underpin this process. Aged myoblasts had reduced abundance of ribosomal proteins and altered energy demands, with reduced protein turnover compared to control.

Therefore, adaptations in multiple pathways during the initial 24 h of differentiation appear to underpin the inability of aged cells to fuse.

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C11

Adipose Tissue Remodelling in Response to Obesity, Exercise Training and Psychological Stress

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Pathological remodelling of adipose tissue in obesity; including the occurrence of enlarged adipocyte, is often as a result of leptin insensitivity, the mechanism of which is still poorly understood. Leptin is produced in proportion to fat mass to increase energy expenditure. However, the enlarged adipocyte displays an altered metabolic capacity and gene expression profile despite the elevated circulating leptin levels. We have studied the adipose tissue remodelling in Zucker (fa/fa) rats, proposed to be the most appropriate rodent model for the early onset of human obesity, due to a mutation in the long form of the leptin receptor. We determined the effects of obesity, exercise and/or psychological stress on visceral adipose tissue (VAT) phenotype in Zucker rats. Lean (LZR) and obese (OZR) Zucker rats (male, 8-9 weeks old, n=64) were divided into (1) Controls, (2) unpredictable chronic mild stress (UCMS), (3) treadmill exercise (Ex), or (4) UCMS+Ex, 8 rats per strain in each experimental group for 8 weeks (Brooks et al., 2018). VAT was sectioned and stained with haematoxylin and eosin (H&E) staining. Total RNA was extracted with TRIzol reagent as described (Toni et al., 2018). Values are means ± S.E.M., compared by ANOVA. To determine the adipose tissue phenotype, tissue cellularity and gene expression profile in VAT were analysed. Analysis of tissue cellularity using H&E staining showed that obesity increased adipocyte size in the VAT of the OZR compared with LZR (7728±286.7, 3089±160.3μm^2; $p< 0.001$; $n\geq4$). Neither exercise and/or psychological stress had any effect on the tissue cellularity. Leptin mRNA was quantified using quantitative polymerase chain reaction (qPCR). One of the features of OZR is the elevated circulating leptin levels compared with LZR. Contrary to the increased adipocyte size in OZR, obesity caused downregulation of leptin mRNA in OZR compared with LZR (1.2±0.2, 4.8±1.1; $p<0.05$; $n\geq6$). To further elucidate our findings, the mRNA of peroxisome proliferator-activated receptor-γ (PPAR-γ) was quantified using qPCR method. PPAR-γ is a transcription factor that positively regulate adipocyte differentiation and lipid storage. The data showed that obesity downregulated PPAR-γ mRNA when compared OZR to LZR (0.8±0.1, 1.8±0.3; $p<0.05$, $n\geq6$). However, the interventions of exercise, and/or psychological

stress did not affect the mRNA of leptin and PPAR-γ. Our study demonstrates that obesity increases adipocyte size but downregulated the gene expression of leptin. We demonstrate the impaired regulation of adipocyte differentiation in visceral adipose tissue of OZR group. However, exercise and/or psychological stress had no effect on tissue cellularity and, gene expression in both LZR and OZR. Further studies are needed to establish the extent to which leptin signalling links VAT to the development of metabolic and cardiovascular diseases.

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C12

Fibre and sex specific differences in mitochondrial content and subcellular distribution and morphology of lipid droplets in skeletal muscle biopsies obtained from lean, obese and type 2 diabetes patients

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Intramuscular triglycerides (IMTG) are stored in lipid droplets (LD) in skeletal muscle, and it is thought that fibre type distribution, subcellular location, size and number of LDs relate to insulin sensitivity (IS) more so than IMTG content, however this is yet to be investigated in human skeletal muscle. Oxidative capacity plays an important role in IS. In skeletal muscle LD are predominantly located adjacent to the mitochondria with greater abundance in the oxidative type I fibres. Reduced mitochondrial content may reduce oxidative capacity which hinders capacity to utilise IMTG as a fuel source.

The aim of the study was to identify fibre and sex specific differences in the content of both LD and mitochondria as well as the subcellular distribution and

morphology of LD in human skeletal muscle of lean, obese and type 2 diabetes (T2D) patients. Muscle biopsies were obtained from 48 male (n=24) and female (n=24) participants, categorised into groups based on metabolic health; lean (n=16), obese (n=16) and people with type 2 diabetes T2D (n=16). Cryosections (5µm) were stained using appropriate antibodies to identify fibre type and mitochondria. LD were stained using bodipy and cell membranes were labelled using WGA. Images were obtained using confocal immunofluorescence and widefield immunofluorescence microscopy, and analysed using Image Pro Plus. In both sexes, there was a hierarchical distribution (T1 > T2a >T2x) of both LD content (area fraction stained) and number ($P < 0.001$) and were located more in the central region than the peripheral region (5µm below cell membrane) of the cell ($P < 0.001$). Males had more LD content than females ($P<0.001$) in all fibre types due to both larger LDs ($P<0.001$) and a greater number of LDs ($P=0.001$). In type IIa fibres T2D have more LD than lean and obese participants which was driven primarily by an increased size of LD rather than increased number of LD ($P<0.05$). Mitochondrial density also followed a hierarchical distribution (T1 > T2) ($P<0.001$) in both males and females regardless of IS.

The study demonstrates that males have more LD than females which is contrary to previous literature which reports greater LD stores in females. However the females in the present study were postmenopausal therefore may have had impaired lipid metabolism due to the reduced oestrogen concentrations that occur at this time. The increased LD content in males compared to females alongside no difference in mitochondrial density may suggest that the LD-mitochondria interactions are coupled better in females leading to a greater oxidative capacity. The increased LD content specifically in type IIa fibres of T2D may suggest a dysregulation of LD turnover (synthesis and lipolysis) which is supported by the lower mitochondria content in these fibres.

More investigation is needed to understand whether the different LD-mitochondria ratio in males compared to females explains why women remain more IS than men for a given body mass.

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C13

Skeletal muscle weakness, atrophy, and abnormal blood flow response in an animal model of heart failure with preserved ejection (HFpEF)

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Heart failure (HF) induces skeletal muscle alterations that impair physical function and quality of life. However, most studies have been performed in HF with

a reduced ejection fraction (HFrEF) whereas skeletal muscle remodelling in HF with preserved ejection fraction (HFpEF) remains poorly explored. The present study, therefore, used an obese cardio-metabolic model to investigate functional, morphological, and vascular alterations that occur in the skeletal muscles of HFpEF rats. Methods: Lean (n=8) and obese (n=8) diabetic Zucker fatty/spontaneously hypertensive heart failure F1 hybrid (ZSF1) rats were compared at 20 weeks of age and cardio-metabolic function assessed (i.e. body weight, blood glucose levels and mean arterial pressure). Tibialis anterior (TA) fibre size (stained with haematoxylin and eosin) was evaluated alongside contractility measures for *in vitro* soleus function (specific force, shortening velocity and power) and *in situ* extensor digitorum longus (EDL) function (force and fatigability) following stimulation across a range of frequencies. Anaesthesia was induced with 4% isoflurane in 100% oxygen and maintained throughout experiments by constant jugular infusion (30-35mg kg^{-1} hr^{-1}) of alfaxalone (Jurox, Crawley, UK). Femoral artery blood flow at rest and during stimulation was quantified using Transonic (0.7PSB; Ithaca, NY, USA) perivascular flowprobes. Data were compared by unpaired Student *t* test and are presented as mean±SEM. Results: HFpEF rats were obese (424.50±11 *vs*. 549.50±11 g body weight; $P<0.05$), hyperglycaemic (8.50±0.80 *vs*. 16.80±0.50 $mmol.L^{-1}$; $P<0.05$) and hypertensive (151.34±3.14 *vs*. 177.44±4.39 mmHg; $P<0.05$) compared to lean controls. HFpEF rats developed ~40% fibre atrophy in the TA (3172±527 *vs*. 5667±332 μm^2; $P<0.05$). Despite no differences ($P>0.05$) in soleus specific forces or shortening velocities, maximal power was lower in HFpEF compared to controls by ~30% (0.95±0.04 *vs.* 1.33±0.11 W cm^{-2}; $P<0.05$). Compared to controls, both EDL twitch and maximal tetanic absolute forces were impaired in HFpEF by ~30% (57±5 *vs.* 42±4 g and 225±27 *vs.* 151±16 g, respectively; $P<0.05$), yet hindlimb blood flow was higher at rest (1.75±0.20 *vs.* 2.84±0.47 ml min^{-1}; $P<0.05$) but lower during contractions (5.44±0.70 *vs.* 3.74±47 ml min^{-1}; $P<0.05$). Conclusions: HFpEF induced skeletal muscle weakness, fibre atrophy and contractile dysfunction alongside impaired blood flow response during contractions. While the mechanisms underlying these deficits remain unclear, these data suggest that vascular, functional, and structural impairments of skeletal muscle contribute to life-limiting symptoms observed in patients with HFpEF.

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C14

Evaluation of the effects of WISP-1 on cardiac fibrosis mediated by cardiac fibroblasts via MMPs and TIMPs

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Wnt signalling plays a key role in the physiology of cardiac development and pathogenesis of cardiovascular disease. Cardiac fibrosis is characterised by the

net accumulation of extracellular matrix proteins in the heart, and has a detrimental effect on cardiac function. Wnt1-inducible signalling pathway protein-1 (WISP-1) is a crucial downstream growth factor of Wnt signalling which is involved in fibrotic remodelling. In order to investigate whether the deletion of WISP-1 attenuates angiotensin II (Ang II) induced cardiac fibrosis, male Apo-E$^{-/-}$ WISP-1$^{+/+}$ mice (single knockout mice, SKO mice, n=7) and male Apo-E$^{-/-}$ WISP-1$^{-/-}$ mice (double knockout mice, DKO mice, n=6) were infused with Ang II (1000 ng/kg/min) using mini-osmotic pumps for 4 weeks. Proteoglycan and collagen content in the heart sections of the mice were measured using alcian blue staining and immunohistochemical staining respectively. Human cardiac fibroblasts (HCFs) were used *in vitro* to demonstrate whether WISP-1 upregulates collagen synthesis. mRNA levels were analysed using quantitative PCR. Protein levels in cell lysates and concentrated conditioned media of cultured HCFs were assessed using Western blotting analysis. Values are presented as means ± SEM, compared by Mann-Whitney *U* test or Kruskal-Wallis *H* tests. Increased proteoglycan content (4.97±0.87 vs. 0.89±0.40, $p<0.05$) and collagen type 1 content (1.07±0.27 vs. 0.29±0.06, $p<0.05$) were detected in the SKO + Ang II mice compared to the SKO controls. However, WISP-1 deletion attenuated the profibrotic effect of Ang II (proteoglycan content: 2.56±1.27 (DKO + Ang II) vs. 4.97±0.87 (SKO + Ang II), $p<0.05$). Treatment of HCFs with WISP-1 did not alter the collagens expression in cell lysates, whereas an additional form of collagen with smaller molecular weight was observed in concentrated conditioned media due to collagens being cleaved. A broad spectrum MMP inhibitor abrogated the collagen cleavage induced by WISP-1. In addition, WISP-1 treatment significantly decreased TIMP-2 mRNA level (0.88±0.04 vs. 1.00±0.00, n=6) compared to the control group. In conclusion, WISP-1 is a pivotal mediator in Ang II induced cardiac fibrosis. These effects are dependent on the regulation of cardiac fibroblasts collagen processing, possibly via the mediation of expression and activity of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs).

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C15

A combination of major histocompatibility complex (MHC) I overexpression and Type I interferon (IFN) signature induces profound mitochondrial dysfunction in human skeletal muscle myoblasts

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Myositis is a group of rare autoimmune diseases with progressive skeletal muscle weakness and dysfunction. Morphological features of the disease include inflammatory infiltration (CD4+/CD8+ T-cells), the presence of circulating autoantibodies,

and interferons (IFN) upregulation in muscle cells (1). Specifically, patients with dermatomyositis (DM) have shown strong Type I IFN-alpha and -beta signature (2). Overexpression of major histocompatibility complex (MHC) I in muscle fibres is a key pathological feature, which is observed in the absence of inflammation or overt disease, while muscle weakness persists (3); this has been attributed to other non-immune mediated mechanisms, including mitochondrial abnormalities (4). Even though both IFN signature and MHC I have been identified to play an important role in DM, their downstream combinational effects remain to be revealed. The aim of this study was to examine the impact of MHC I overexpression in presence or absence of Type I IFNs, on mitochondrial function. Human skeletal muscle myoblasts were transfected with a mammalian HLA-A2/K^b overexpression vector, a MHC I isoform, and treated with Type I IFNs (100 ng/ml) for 18 hours. Mitochondrial and function, membrane potential and superoxide generation were assessed using seahorse extracellular flux analysis (n=4), JC-1 (n=6), and MitoSOX Red (n=8), respectively. Statistical analysis was performed by ANOVA with Dunnett's post-hoc test. MHC I overexpression results in decreased mitochondrial respiration, including maximal, basal, and ATP-linked respiration, alongside with a decline in overall reserve capacity of cells; which was significantly compounded in the presence of Type I IFNs. Specifically, MHC I overexpressed myoblasts treated with IFN-beta show significant decrease in maximal respiration (p=0.0223), ATP production (p=0.02), and proton leak (p=0.01). Myoblasts overexpressing MHC I and treated with IFN-alpha show significant reduction in maximal respiration (p=0.0403) and proton leak (p=0.01). The reduction in proton leak is further supported by mitochondrial membrane hyperpolarisation, which is significantly observed in presence of Type I IFNs (IFN-alpha, p=0.007; IFN-beta, p<0.001). Moreover, cells overexpressing MHC I induce significant increase in mitochondrial superoxide generation (p=0.0448). Data suggest that MHC I and Type I IFNs have a strong combinational effect on mitochondrial function, providing further insights into DM pathogenesis.

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

C16

The effects of age and acute aerobic and resistance exercise on circulating T lymphocyte vitamin D receptor (VDR) expression in healthy males.

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A low vitamin D status has been identified as an association risk factor in the etiology of numerous chronic diseases, with older adults identified as generally more deficient than younger populations. Exercise has been shown to act as a direct and indirect stimulus on key vitamin D metabolites, specifically the vitamin D receptor (VDR) (1, 2), however investigations are limited to murine model studies. The primary aim of the study was to determine whether a single bout of exercise upregulated VDR expression in circulating systemic T cells. Thirty-five recreationally active males were included in the study (means ± SD: age 44 ± 17 y, body mass 82.5 ± 11.4 kg, height 1.79 ± 0.08 m, BMI 25.7 ± 3.1 kg/m^2), separated into three age groups: 18-30 y (n=12), 31-45 y (n=11), and 60-75 y (n=12). Participants completed three trials: control (CON; 60 min rest), aerobic exercise (AE; 60 min cycling at 55% work load max) and resistance exercise (RE; repeated maximal voluntary isometric contractions), with intravenous blood samples collected pre- and post-CON/AE/RE (Pre, 0 h, 1 h, 3 h). T cells were isolated and analysed for cell-surface and intracellular T cell (CD3+, CD4+, and CD8+ T cell subsets) VDR expression via flow cytometry analysis. Values are means ± S.D., compared by ANOVA. Intracellular VDR expression (CD3+ T cells) was elevated following both AE ($P<0.001$) and RE ($P=0.001$) immediately upon cessation of the exercise, with AE promoting a greater increase compared to RE (1.5 fold vs 1.25 fold increase, respectively; $P=0.048$). VDR expression returned to baseline level after 3 h post-exercise. There was no difference between T cell subsets. The response was unaffected by age despite baseline VDR expression at rest declining with age (geomean: 882 ± 274 vs 796 ± 243 vs 594 ± 174; $P=0.015$). Moreover, there was no correlation between change in T cells and change in VDR expression in response to exercise. The results indicate that performing a single bout of exercise is an effective means to increase intracellular VDR expression in T cells. Interestingly, the upregulation in VDR protein expression may be independent of T cell mobilisation in response to exercise, and independent of age.

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

C17

Absence of an ageing-related increase in fibre type grouping in master athletes and non-athletes

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Abstract
Background
The ageing-related loss of muscle mass may be partly attributable to motor neuron loss and motor unit remodelling, where cycles of denervation and reinnervation may result in fibre type grouping in older muscles. The purpose of this study was to examine fibre type grouping in athletes and non-athletes of a wide age range (19-85 years) and to evaluate to which extent any observed grouping is explicable by the fibre type composition of the muscle. Since regular physical activity may

stimulate reinnervation, we hypothesised that fibre groups are larger in master athletes than in age-matched non-athletes.
Methods
Fibre type grouping was assessed in m. vastus lateralis biopsies from 22 young (19-27 years) and 35 healthy older (66-82 years) non-athletes, and 14 young (20-29 years), 51 middle-aged (38-65 years) and 31 older (66-85 years) athletes. An 'enclosed fibre' was any muscle fibre of a particular type surrounded by fibres of the same type only. A group was defined as a group of fibres with at least one enclosed fibre.
Results
Only type II fibre cross-sectional area (FCSA) showed an age-related decrement that was greater in athletes ($p < 0.001$) than in non-athletes ($p = 0.012$). There was no significant agerelated effect on group size or group number in athletes or non-athletes, and the observed grouping was similar to that expected from the fibre type composition.
Conclusions
At face value these observations do 1) neither show evidence for an age-related loss and remodelling of motor units nor that 2) improved reinnervation with regular physical activity, but 3) histological examination may not reveal the full extent of ageing-related motor unit remodelling.

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C18

Are older adults at greater risk of the adverse musculoskeletal consequences of physical inactivity?

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Introduction: Ageing and physical inactivity are independent risk factors for musculoskeletal decline. The aim of the current study was to assess the influence of age on the musculoskeletal changes incurred during short-term physical inactivity.
Methods: 26 young (28 ± 8 y) and 21 older (60 ± 6 y) habitually active (>10,000 steps per day) participants were recruited. Musculoskeletal assessments, including dual-energy x-ray absorptiometry to quantify lean mass and bone mineral density (BMD), magnetic resonance spectroscopy to quantify mitochondrial function and

intramuscular adipose tissue (IMAT) and a leg press protocol to assess muscle function/strength were performed at baseline, following 14 days of step reduction (~1,500 steps per day) and after 14 days of returning to habitual activity. Statistical analysis was performed using two-way repeated measures ANCOVA, and data are presented as mean±SD.
Results: Average daily step count reduced by 10752 ± 2943 steps/day in the young (83 ± 7%) and 10204 ± 2338 steps/day in the older group (84 ± 7%), with concomitant increases in sedentary time (P<0.001). The intervention induced significant losses in total body lean mass (Young 0.43±0.78kg vs. older 0.08±0.76kg; P=0.024;), lean leg mass (young 0.18±0.33kg vs. older 0.08±0.33kg; P=0.003) and muscle strength per unit of lean muscle mass (muscle quality) (young -0.33±0.92kg vs. older 0.33±0.51kg; P<0.001), which were not significantly different between age groups (P>0.05). Correspondingly, IMAT increased (young 0.67±2.84% vs. older 2.73±3.97%; P=0.04) with no difference between groups (P=0.27). Serum markers of bone resorption (CTX) were elevated (P=0.002) in response to the inactivity period to similar extents in both age groups (P=0.54). Cardiorespiratory fitness and mitochondrial function significantly declined during the inactivity period, indicating reductions in whole body (VO_2 peak: young 1.99±3.68ml.min.kg-1 vs. older 2.75±4.12ml.min.kg-1; P<0.001) and skeletal muscle oxidative capacity (rate constant: young -0.08 ± 0.39ks-1 vs. older 0.38±0.36ks-1; P=0.007). Importantly, these reductions were significantly greater in older adults (P>0.05).
Conclusion: Results suggest that even short-term modest reductions in physical inactivity induces substantial impairments in musculoskeletal decline, regardless of age. Reductions in oxidative capacity were more pronounced within older adults indicating an increased vulnerability to physical inactivity compared with young. This highlights the importance of maintaining levels of physical activity throughout the lifespan.

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C19

Comparison of two Home-Based HIIT protocols with '60HIIT' eliciting greater health benefits in VO_{2peak} compared to '30HIIT'.

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Currently 40% of the UK do not meet the physical activity guidelines with 'lack of time' the most commonly cited barrier to physical activity. High intensity interval training (HIIT) has been shown to be an effective time-efficient alternative to moderate intensity continuous training (MICT). However, public health researchers have criticised current HIIT research as exercise is often supervised and many protocols employ expensive equipment available only in laboratories or gym, creating

additional barriers to exercise. As such, HIIT protocols using simple body weight exercises that can be performed in one's home (home-based HIIT) are becoming more popular. However, the effect of interval duration has not been investigated using a home-based HIIT intervention. Therefore our aim was to investigate if two popular HIIT protocols (30 or 60 second interval durations (30HIIT; 60HIIT) induce similar improvements in aerobic capacity, arterial stiffness, and body composition when performed using simple body weight exercises.

Twenty-six, previously sedentary men (n=9) and women (n=17) were randomised to complete either 6 weeks of 30HIIT (n=15; 29±3 y, BMI 25±0.8 kg.m-2)) (4-8 30s intervals with 120s rest) or 60HIIT (n=11; 28±4 y, BMI 26±1.4 kg.m^{-2})) (6-10 60s intervals with 60s rest). Both training protocols used simple body weight exercises, and training sessions were completed without supervision in a place of the participants choosing, 3 times per week. Training adherence and intensity (Heart rate (HR)) were monitored using the Polar Beat mobile app. VO_{2peak}, body composition (Bio-impedance), and arterial stiffness (aortic pulse wave velocity (aPWV)) were assessed pre and post training.

VO_{2peak} increased post intervention in 60HIIT (33±7 to 35±7 ml.min^{-1}.kg^{-1}) ($P<0.05$) with no difference for 30HIIT (P=0.51). There was no significant difference in BMI (P=0.5), weight (P=0.3), and VAT (P=0.4) for 60HIIT. There was no significant difference for 30HIIT in BMI (P=0.052), weight (P=0.057), and VAT (P=0.062). There was no significant difference in arterial stiffness (aPWV) post intervention in 30HIIT (P=0.16) or 60HIIT (P=0.2).

6 weeks of 60HIIT induce improvements in aerobic capacity but not in body composition or arterial stiffness with 6 weeks of 30HIIT eliciting no changes. In both home-based HIIT protocols participants self-selected bodyweight exercise intensities, without researcher encouragement. Therefore, this data suggests that 60-second interval interventions could be used to improve health outcomes in a sedentary population in the real world, with 30-second interval interventions needing further research.

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C20

Can a Weekly Multi-Modal Exercise Class Preserve Motor and Non-Motor Function in Parkinson's?

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Introduction: Parkinson's Disease (PD) is a chronic neurodegenerative disease that can lead to disability and disengagement with active lifestyles. Single modality exercise is effective at improving and sustaining cognitive and/or physical function in people with Parkinson's (PwP) (1,2). However, multi-modal (MM) exercise (*e.g*. circuit training), including cognitive challenge, may be more beneficial. Only a few studies have assessed long-term effects of regular MM exercises in PD and there is a need to compare these results to non-exercise-attendees with PD (na-PD) and healthy older adults (HOA). We established a weekly community-based MM exercise session for PD (EX) to evaluate its effects on physical function, cognition and wellbeing outcomes over the period of 1, 2 and 3 years.
Methods: 25 participants (20 male, 5 female; age 64±8 years; Hoehn and Yahr (H&Y) scores≤III, indicating mild to moderate PD) attended a once-a-week MM group exercise session (60 minutes) for over one-year. A battery of health and functional assessments were completed at the start and every four months for one (n=25), two (=18) and three years (n=8). Additionally, a battery of cognitive function assessments plus Older People's Quality of Life Questionnaire (OPQOL-Brief) were measured. Results were compared to an aged-matched group of 20 HOA (8 male, 12 female; age 61±6 years) and 20 na-PD (12 male, 8 female; age 68±7 years; H&Y scores≤III) to evaluate the rate of functional and cognitive decline not influenced by the exercise session.
Results: At baseline, no health-related between-group differences were observed. EX scores for six-minute walking test (6MWT), timed up and go (TUG) and bilateral grip strength (GS) did not significantly decrease across 1, 2 or 3 years, and the number of 1-minute sit-to-stands (STS) increased during 1 year between baseline and after the first four months (from 21 to 23; P=0.001). Scores for Clock Drawing Test (CDT), Trail Making Test A (TMT-A) and B (TMT-B), and OPQOL-Brief did not significantly change across four different assessments (*i.e.* 1 year). Mini-Mental Parkinson's (MMP) increased between baseline and after 8 months (from 26.67 to 29.38; P=0.004). After 1 year of exercise, EX group's 6MWT scores were significantly lower than HOA (P<0.001) but TUG, STS and GS were not significantly different from HOA, while na-PD showed lower 6MWT, TUG and STS scores than HOA (P=0.000, 0.006 and 0.003, respectively).
Conclusion: A once-a-week MM exercise programme for PD showed an improvement in STS and MMP scores and no other significant changes (*i.e.* no decline) in health, cognition and physical function over 1, 2 and 3 years. Also, exercise reduced the difference in outcome scores between PwP and HOA. That functional and

cognitive performance were slightly increased or maintained is a positive outcome given the progressive nature of Parkinson's.

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PC01

Assessment of cervico-vaginal fluid protein concentration, protease activity, and its impact on tight junction integrity in a model of vaginal epithelium

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Every year, 60000 babies are born prematurely in the UK (7% of all births) [1]. Complications arising from preterm birth (PTB) significantly contribute to neonatal death. Greater understanding of the mechanisms leading to spontaneous PTB are needed to improve management strategies and pregnancy outcomes. Focusing on the involvement of the cervico-vaginal environment and risk of inflammation and infection related to spontaneous PTB, we have studied cervico-vaginal fluid (CVF) components and their impact on vaginal epithelium integrity. Proteases are proteolytic enzymes involved in peptide bond hydrolysis that can disrupt tight junctions via the PAR2 receptor [2]. An association between reproductive tract cell tight junctions and PTB has also been reported, as a decrease in claudins is linked with preterm and term cervical ripening [3].

Twenty-two pregnant women, between 14–23^{+6} weeks gestation, were recruited to a prospective cohort study (INSIGHT REC No. 13/LO/0393; written informed consent) and provided CVF via low vaginal swabs (LVS). Proteins were quantified from CVF using a microBCA Protein Assay as per protocol (ThermoFisher). Protease activity was measured using a fluorescent assay as per protocol (ThermoFisher). To mimic the acidic vaginal environment protein and protease assays were repeated on n=6 samples using a reproductive buffer (pH 4) (60 mM potassium phosphate and 20 mM sodium chloride) [4] instead of PBS (pH 7). Cultured vaginal epithelial cells (cell line VK2, passage 53-56; density $15x10^4$) were cultivated in 12 permeable Transwell inserts and monitored for eight days. Transepithelial electrical resistance (TEER) was measured using the EVOM2 voltohmmeter. Permeability assays were carried out 24 hours after the TEER measurement using a sodium fluoride dye.

Protein was quantified and detected in twenty-two CVF samples taken from LVS. Proteases were undetectable in neat CVF samples and in samples normalised to contain 10 μg/μL total protein. In six samples, lowering the pH from pH 7 to pH 4 reduced the protein concentration; this did not enhance any detection of protease activity. However, the addition of CVF (from n=9 different samples) to VK2 cells reduced the TEER and increased cell permeability.

We provide evidence that samples from LVS cannot be used to analyse CVF proteases. This may be due to the collection and/or processing technique, or because the low vagina does not contain pure CVF and is contaminated with other cells and debris. However, we do show that CVF from LVS impacts tight junction integrity as it lowers the TEER of cells and increases cell permeability. The presence of short chain fatty acids amongst other enzymes and proteins may be responsible for the reduction in tight junction integrity, but this requires further investigation.

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PC02

Relative Success Rates of Antiepileptic Drugs vs. Dietary Adherences vs. Surgical Procedures in Epileptic Seizures Frequency Reduction: a meta-analysis

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Background: There are several seizure treatment options available for patients who have been diagnosed with various forms of epilepsy. The being antiepileptic drugs, surgical procedures, and dietary changes. All three treatments focus on improving the quality of life by reducing the frequency of epileptic attacks suffered by patients, however, the different treatment types have been proven to prevent patient's seizures in varying ways (Somjen, 2004). While dozens of reports have been published on the relative success rates, with or without treatment, minimal research is available on relative success rates across various treatment types (Wheless et al., 2003). Motivation: The main purpose of this meta-analysis was to analyze different treatments of epilepsy and determine which treatment options are most successful at reducing the frequency of seizures. This was accomplished by conducting an inferential statistical analysis of variable correlation to overall health. Methods: Three treatments for epilepsy were analyzed, including: antiepileptic drugs, surgical procedures, and dietary changes, and for each relevant study found percent reduction rates in seizure frequency were compared and chi-squared tests and the t-test statistic for significance were conducted. As the basis for the study, literature published between 1996 and 2019 was analyzed. The primary search terms were "epilepsy" and "number of seizures". When searching for relevant articles, those that contained data subjects between the ages of 0-50 with a minimum

duration of three months were extracted. Results: Relative success of treatment was determined by extracting the percentage of seizure reduction relevant to different factors investigated from the studies found which overall shows a positive reduction in seizure frequency across treatment options and 67% of patients showed a mild to marked improvement in seizure frequency by use of antiepileptic drugs. Conclusion: In conclusion, controlled data from various collected observational studies suggest that management of electrochemical gradients via anticonvulsants, specialized diet, and surgical procedures all result in significant improvements in patients who have epilepsy. However, statistical analysis seem to indicate that medication and surgery are the superior treatments due to their relative success rates in lowering seizure frequency with treatment with antiepileptic drugs more practical and relatively more clinically successful at decreasing seizure frequency long-term.

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PC03

Salt sensitivity, baroreflex sensitivity and vascular resistance in a normotensive young-adult Nigerian Population

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Hypertension is a global public health challenge, and a high salt diet is its most important environmental risk factor (Meneton *et al.,* 2005). We have previously reported that about 60% hypertensive and 37% normotensive adult Nigerian

population are salt-sensitives (Elias *et al.,* 2014). However, salt-sensitivity increases with age (Weinberger, 2002), therefore this study was designed to evaluate salt-sensitivity and its relationship with baroreflex sensitivity (BRS) and systemic vascular resistance (SVR) in a normotensive young-adult Nigerian population. After an ethical approval from the Health and Research Ethics Committee (HREC) of the College of Medicine, University of Lagos and collection of both written and verbal consent, 24 (12 Males; 12 Females) young-adult [18-35: (26±1.0) years] normotensive [Blood Pressure (BP) < 140/90: 119±2/74±1 mm Hg] participants were salt loaded (200mmol/day x 5), after the collection of anthropometric [Weight (61.71±2.79 Kg) height (165.9±1.74 cm) and BMI (22.30±0.82 kg/m^2)] and basal parameters (BP, heart rate (HR), 12- hour urine, BRS, SVR via finger plethysmography and 5ml venous blood for serum measurement of Asymmetric Dimethyl Arginine (ADMA)). On day 6, which is the day 1 post salt-loading, all the above-listed parameters were taken again. Thereafter, collected data before and after salt-loading were analysed using paired t-test. Salt sensitivity was defined as post salt-loading MABP ≥3mm Hg. Differences in parameters between salt sensitive (SS) and salt resistant (SR) participants were analysed using student t-test while confidence interval was placed at $p \leq 0.05$. There was no significant difference between the pre and post salt values of MABP (89±1 vs. 88±1mm Hg); HR (73±2 vs. 70±3 beat/min); SVR (0.038±0.006 vs. 0.48±0.007) and Serum ADMA levels (91.07±4.67vs. 96.65±6.05 μg/ml) of the participants. However, about 29% (7/24) of the participants are salt sensitive. In the SS participants, post salt-loading values of MABP (84±3 vs. 89±2mm Hg($p=0.001$)); SVR (0.024±0.006 vs. 0.044±0.01 ($p=0.008$)) and serum ADMA concentration (80.57±5.51 vs. 104±6.73 μg/ml ($p=0.01$)) are significantly higher when compared to the pre salt values, while the BRS was blunted. These observations are not found in SR participant. Thus, findings from this study suggest that in SS normotensive young-adult Nigerians, salt loading blunted the BRS and impaired the SVR probably by increasing the concentration of ADMA – an endogenous inhibitor of nitric oxide production.

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PC04

NATURAL ENVIRONMENTS TO IMPROVE HEALTH AND WELLBEING

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As global populations continue to grow at rapid rates, governments across the globe are exhausting efforts to find appropriate health services alongside exploring opportunities to limit our impact on the natural environment. Health services are stretched beyond working limits, particularly in the Global North where many nations are facing ageing populations and similar obstacles. One suggested method to tackle these issues surrounds the implementation of radical Green Infrastructure (GI), using community gardens and care farms, particularly within deprived communities. This important development of GI establishes public connection to areas of environment. Greater facilitation of this can be provided through social prescriptions, where medical professionals advise holistic approaches for a variety of social, psychological and physical issues. Within the field, reports of accessibility to green spaces improves both mental and physical health, therefore implementation within the United Kingdom (UK) could provide lasting benefits for the National Health Service (NHS) and to general public health. Research in this field is relatively novel and tends to be based in Scandinavian countries or the United States – therefore informing the basis of this literature review whilst giving potential for further data collection. Our work sets out to use mixed-methodology cross-sectionally, adopting sciences across the breath of environmental and health spheres, therefore allowing comparison and disparities to be drawn from a quantitative and qualitative database. Ultimately, the research shows that care farms and community gardens can impact significantly across deprived areas, with more work needed to understand their longer-term impacts on communities. Therefore, our work is committed to understand how environmental stimuli effects human physiology across the life course.

The presenters would like to acknowledge the assistance provided by the supervisory team, consisting of Dr Michael Hardman, Dr Michelle Howarth and Professor Penny Cook.

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

PC06

EXPLOITING THE INHIBITION OF VASCULAR SMOOTH MUSCLE CELL PROLIFERATION AND INTIMAL THICKENING BY PRH/HHEX

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Objective: Human saphenous vein (HSV) is widely used for coronary artery bypass grafting in patients with ischemic heart disease1,2. However, 30-50% of venous grafts fail within 10 years3. Vein graft failure involves intimal thickening as a result of endothelial cell (EC) damage and inflammation which promotes vascular smooth muscle cell (VSMC) de-differentiation, migration and proliferation. Proline Rich Homeodomain (PRH) protein is a transcription factor required for growth and differentiation4,5. We investigated whether adenovirus-mediated delivery of non-phosphorylated PRH (PRH-CC) protein attenuates VSMC proliferation without detrimental effects on EC.

Approach and Results: PRH-CC overexpression in HSV-VSMCs significantly inhibited proliferation (27±0.1 vs. 36±0.2%), migration (27±9 vs. 356±33μm) and apoptosis (2.4±0.2 vs. 7.8±1.4%) compared to virus control. Western blotting and qPCR revealed that PRH-CC enhanced the expression of contractile markers (smoothelin and calponin) and enhanced collagen-gel contraction in HSV-VSMCs. Importantly, PRH-CC overexpression in HSV-ECs significantly reduced apoptosis (0.8±0.8 vs. 8±0.10%), without affecting proliferation (19±3 vs. 17±3%) and migration (154±57 vs. 190±86μm) compared to virus control. In HSV-ECs, PRH-CC significantly reduced TNF-α-induced VCAM-1 and ICAM-1 and monocyte adhesion (5.6±0.9 vs. 24±4 cells/field), and suppressed interleukin-6 (154±18 vs. 292±43pg/ml) and monocyte chemotactic factor-1 (111±48 vs. 1554±76pg/ml). Data were analysed with ANOVA, Student Newman Keuls post-hoc test, n=4, $p<0.05$.

Conclusion: We observed that PRH-CC attenuated VSMC proliferation, migration and apoptosis and enhanced VSMC differentiation, whilst promoting the endothelial repair and anti-inflammatory properties. These novel findings highlight the potential for PRH-CC to preserve the endothelial function and suppress VSMC synthetic activity and thereby reduce vein graft failure.

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PC07

Talking to adolescent girls to inform the development of a novel HIIT intervention

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BACKGROUND: 81% of adolescent girls in the UK are not currently meeting the daily physical activity (PA) guidelines[1] (60 minutes per day[2]) and are therefore at an increased risk of multiple health issues. Commonly cited barriers to PA include lack of time, perceived lack of competence and monotony[3]. High intensity interval training (HIIT) has been found to have equal benefits to traditional exercise in significantly less time and often in a more enjoyable format[4]. Numerous adolescent exercise interventions are created solely by exercise scientists, are not successful in long-term adherence and lack follow-up. Therefore, this study is critical to learning what protocol is preferred by adolescent girls, in order to inform the development of a HIIT intervention and optimise participant adherence. Therefore, this is the first study of a series which aims to develop and trial a home-based HIIT programme for inactive adolescent girls. This qualitative study has two main objectives, (1) obtain detailed feedback on a proposed HIIT programme and (2) to understand adolescents perceived facilitators and barriers towards PA. The results will inform the development of a HIIT protocol and the promotion strategy of the intervention.
METHODS: 45 inactive adolescent girls, recruited from 5 secondary schools and 2 youth clubs in Liverpool and Dublin, will participate in semi-structured focus groups, guided by the Youth Physical Activity Promotion model. Adolescents will also complete demographic questionnaires and self-reported PA questionnaires. Focus groups will be transcribed verbatim and analysed using thematic analysis to identify key themes.
RESULTS: As this study is still ongoing final results are not yet available but will be presented for the first time at the Future Physiology 2019 conference. Preliminary results show that choice in PA is a vital element of participation for adolescent girls, both inside and outside of school. A common theme which emerged in all focus groups was the girls self-perceived incompetence for PA in the presence of others

and the time barrier of homework, especially among older adolescent girls. The participants felt that home-based HIIT would be an exciting and acceptable form of exercise that they would adhere to, provided they had individualised regular support. They express the importance of having "normal" girls of varying shapes and sizes lead the instructional videos and that air-brushed fitness models with perceived unattainable physiques promoted by the media add to society's pressure of girls looking and behaving a certain way.
CONCLUSION: We hope that by listening to the feedback and opinions of adolescent girls we can develop a home-based HIIT intervention that is feasible, enjoyable and something that they can stick to for life, thereby decreasing the associated health risks of inactivity.

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PC08

Assessment of Cardiovascular and Pulmonary functions in Children with Juvenile Idiopathic Arthritis.

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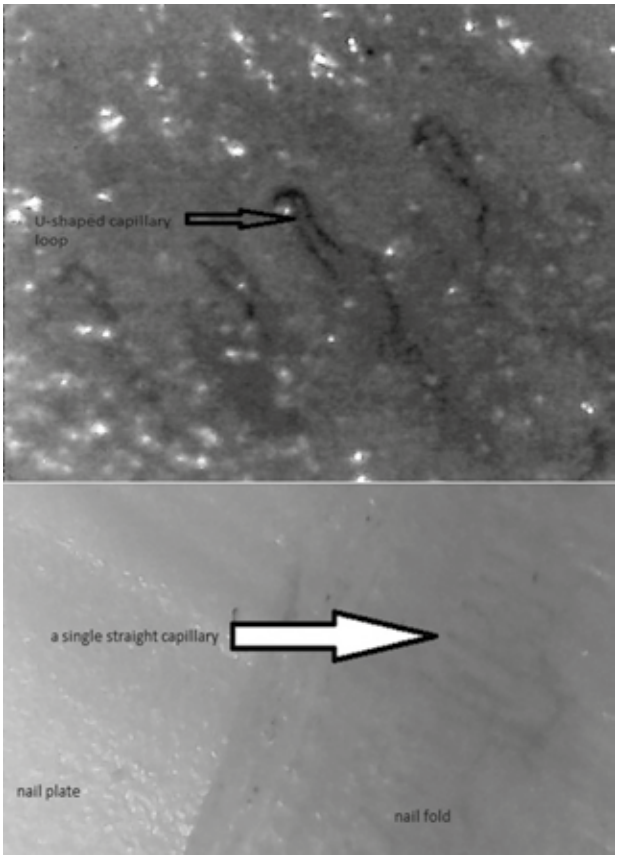
Juvenile Idiopathic Arthritis (JIA) is the most frequently occurring childhood rheumatological disorder and is quite prevalent in our population. It presents with joint involvement and is associated with multiple extra-articular features, one of which is premature development of atherosclerosis in cardiovascular system. This in turn causes raised levels of pro-inflammatory cytokines. Chronic immune activation by these cytokines plays a role in the development of restrictive spirometry

pattern. The purpose of this study was to assess the cardiovascular and pulmonary functional status in those suffering from JIA and to compare with normal children. It was a cross-sectional comparative study carried out at Children Hospital Lahore (Pakistan) after getting approval from the Institutional Ethical Committee. Fifty six children in the age range of 6 years to under 18 years were included; 28 children in group 1 (healthy controls) and 28 children in group 2 (diagnosed with JIA for at least 2 years). Heart rate (HR) and blood pressure (BP) was measured. Huntleigh Doppler ultrasound device (Mohamed et al., 2013) was used for Ankle Brachial Pressure Index (ABPI) measurement. Jingou Portable USB microscope for capillaroscopy and spirometry was done for pulmonary function assessment. Joint Juvenile Arthritis Disease Activity Score (JADAS-27) (Consolaro et al., 2014) was calculated for assessment of disease activity. *p* value <0.05 was considered statistically significant.
Values are median and inter-quartile range and frequencies, compared by Mann-Whitney U Test. HR (90, 88-96), systolic BP (108, 100-119) and mean arterial pressure (82, 76-86) was significantly high in JIA subjects than the controls (p=0.013, p=0.002 and p=0.017 respectively). There was a non-significant difference in the ABPI values and capillaroscopic findings of the two groups (p=0.198 and p=0.071). FVC 1.44(1.09-1.92), VC 1.50(1.13-2.00) and FEV1 1.42(1.05-1.84) were significantly lower in JIA group relative to the control group (p= 0.019, 0.045, 0.044 respectively). Pulmonary function tests revealed mild, moderate and moderately severe restriction in 10.71%, 21.43% and 3.57% respectively of group II participants. All controls showed normal spirometry pattern. There was a greater prevalence of stunting (height < -2 SD) and severe thinness (BMI < -3 SD) in JIA relative to the controls. In addition, 78.57% of JIA participants had anemia.
These data suggest that cardiovascular and pulmonary functions are deranged in JIA as demonstrated by the HR and BP findings. A greater prevalence of restrictive spirometry pattern in JIA. The growth and nutritional status of JIA participants is found to be poor as indicated by a greater prevalence of stunting, severe thinness and anemia.



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PC09

Artificial intelligence, Bioprinting and Nanotechnology: The role of future Physiologist.

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New statistics released by Allaboutcareers.com, a leading careers exploration website, reveal that 44% of undergraduates are unable to define the industry that they would like to work in once they graduate.
In the report, over 37,000 undergraduates and 1500 school and college students were asked about their career aspirations and 52% of school students agreed or strongly agreed with the statement, "I have no idea what I want to do with my career". These are worrying statistics that emphasise the need for high quality, free and easy to access careers information for young people which was a great step taken by the physiological society for organizing this conference themed "Future Physiology" which will increase undergraduate awareness with different careers that can be pursued as a physiologist.
From the very earliest moments in the modern history of the computer, scientists have dreamed of creating an 'electronic brain'. Of all the modern technological quests, this search to create artificially intelligent (AI) computer systems has been one of the most ambitious.
Computer scientists and healthcare professionals set about shaping a research program for a new discipline called Artificial Intelligence in Medicine (AIM). These researchers had a bold vision of the way AIM would revolutionise medicine, and push forward the frontiers of technology.
The role of physiologist in the advancement of AI in medicine cannot be overemphasized. As AI continues to advance, new analytical approaches, including those that go beyond data correlation are developed. Physiological genomic readouts in disease-relevant tissues, combined with advanced AI, can be a powerful approach for precision medicine for common diseases.
In another vein, nanotechnology is another exciting new area in science, with many possible applications in medicine. The application of nanotechnology to biosensor design and fabrication promises to revolutionize therapy at the molecular and cellular level which will be useful in physiological research.
Also, bioprinting, an emerging technology for constructing and fabricating artificial tissue and organ constructs. This technology surpasses the traditional scaffold fabrication approach in tissue engineering(TE) with potential as a future source for implants and full organ transplantation.
This paper overviews these three cogent and important technologies that needs the minds and hands of physiologist in there application in medical research. In addition, the current state of the art in bioprinting technology is reviewed with focus on the role of physiologist, describing the broad range of artificial intelligence

and the place of physiologist in the use of these technology in revolutionizing medical research, the application of nanotechnology in physiological research, and the future directions for next-generation bioprinting, nanotechnology and artificial intelligence.

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PC10

Role of HO-1 in melanoma resistance to BRAF inhibitor vemurafenib in MeOV-1 cells cultured under hyperoxia, physiological normoxia and hypoxia.

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The $BRAF^{V600}$ mutation is present in ~60% of melanomas and is translated into a continuous activated BRAF protein that dysregulates downstream mitogen-activated protein kinase (MAPK) signalling transduction, favouring melanoma proliferation, survival and progression [1,2]. The FDA approved the use of vemurafenib/PLX4032 specific inhibitor of $BRAF^{V600}$ protein, for melanoma patients. Even though the use of vemurafenib increases the survival rate of patients, unfortunately a relapse of disease often occurs [3,4].
It is widely known that heme oxygenase-1 (HO-1) and its metabolites are involved in cellular redox homeostasis and counteract the oxidative stress generated by different stimuli. Notably, high levels of HO-1 in different types of tumor enhances survival, aggressiveness and poor outcome [5]. In this study, we investigated the involvement of HO-1 in the resistance and the angiogenic potential of $BRAF^{V600E}$ primary melanoma cells to the treatment with PLX4032.
Primary melanoma cells (MeOV-1) were exposed to PLX4032 (10μM) for 24h and the involvement of HO-1 was studied by gene silencing. Experiments were conducted under standard cell culture conditions (air) and physiological and hypoxic oxygen levels (respectively 18kPa, 5kPa, 1kPa) in a Scitive workstation (Baker-Ruskin). Angiogenic potential was evaluated by seeding them on bovine aortic endothelium cells (BAEC) in Matrigel and treating them with conditioned medium derived from melanoma cells.
An MTT assay showed that cell viability was reduced by 40% in cells exposed to PLX4032, with no differences observed under different oxygen levels. Under 18 kPa O_2, PLX4032 increased HO-1 protein expression, whereas HO-1 induction was reduced at 5kPa and 1kPa O_2. Interestingly, PLX4032 treatment completely abrogated HIF-1α expression in cells under 18kPa and 5kPa O_2 but not under hypoxia. qPCR and immunoblotting analysis of Nrf2-dependent target genes shown that Nrf2 is not involved in the upregulation of HO-1.

When BAEC seeded on Matrigel were treated with medium derived from MeOV-1 cells exposed to 10μM PLX4032, tube formation, branching and density were increased: when BAEC were treated with conditioned medium from MeOV-1 cells silenced for HO-1 and treated with PLX4032 the ability to form tubes was reduced. In conclusion, downregulation of HO-1 may provide a promising target to increase the efficacy of vemurafenib on BRAFV600E melanoma cells.

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PC11

Comparing the effect of cultured Schwann cells and electrical stimulation in repairing crushed rat sciatic nerve

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Sciatic nerve injuries are very common lesions. Functional recovery with Schwan cells (SCs) through their migration, proliferation, expression of growth factors, and remyelination capacity is proved. Electrical stimulation (ES) applied after injury can also improve regeneration in crushed nerves. Aim: To assess the effect of combining SCs and ES in improving injured sciatic nerve. Rats were divided into five groups each containing 10 rats: (G1) was subjected to surgical procedure without sciatic nerve crush, followed by wound closure and post- surgical care. (G2) was subjected to sciatic crush nerve injury using standard hemostat. (G3), was subjected to sciatic nerve crush, then treated by ES for 30 minutes. (G4) SC treated group was subjected to sciatic nerve crush then treated with Schwann cells (3×10^5 cells/μl) in saline intra- lesion followed by wound closure. (G5) was subjected to sciatic nerve crush, and then treated with SC, then with ES. Induction of sciatic nerve injury, in vivo labeling with Brdu by donor rat intraperitoneal injection, Schwann cell preparation, application of electrical stimulation were followed by

oxidative stress markers assessment 48 hrs postoperative in all groups. Four weeks later, walking track analysis and calculation of sciatic function index (SFI), EMG, NCV were done. Sections were stained with H & E and Toulidine blue stain. Relative expression of BDNF was done by using (RT-PCR) technique. Results: MDA increased in injured group (10.76±0.05) compared to the normal group levels (3.87±0.03). Combination group anti-oxidant capacity values (1.76±0.02) were significant than those of ES (1.62±0.01) and SCs (1.61±0.01) groups with no significant difference between SCs and ES treated groups. Oxidative stress marker showed the least values in combination treatment group (4.39±0.01, P> 0.01). BDNF levels were increased significantly in all treated groups when compared to injured group. SFI means oscillate around -60. Combination treatment values showed significant improvement (-28.59±0.9) when compared to either ES (-39.12±1.2) or SCs (-31.45±1.2). ES treated group showed the least NCV (34.96±0.02) when compared to SCs (36.95±0.01)or combined treated groups values (36=98±0.01). EMG latencies in all treated groups decreased significantly when compared to injured group. Using Toludine blue stain, combination treated group showed marked preservation of the myelin sheath with mean optical density of (0.32 ± 0.003). Optical density of combination group showed statistically significant improvement when compared to ES and SCs groups (P value = 0.000). We concluded that combination of ES with SCs transplantation improve nerve regeneration, as shown by better results than sole treated groups in behavioral, electrophysiological, oxidative stress and BDNF results.

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PC12

Cardiotoxic effects of phenanthrene in zebrafish myocytes

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Phenanthrene (Phe) is a 3 ringed poly-aromatic hydrocarbon (PAH) that is an important component of water and air pollution. In air, Phe binds to the surface of fine particulate matter ($PM_{2.5}$) and in water, it is a major component of crude oil.

Previous work with marine fishes shows that Phe has direct negative impacts on the heart including proarrhythmic effects via inhibition of rapid repolarising potassium current (I_{Kr}) and L-type calcium current (I_{CaL}) (1, 2). The aim of this study was to further investigate the cardiotoxic effects of Phe using whole-cell patch-clamp in zebrafish (*D. rerio*) ventricular cardiomyocytes. We show significant cardiotoxic effects of Phe in zebrafish with different pharmacological potency and specificity compared to previous studies on marine fishes. Firstly, 3 μM Phe significantly inhibited isolated peak I_{Kr} tail currents by 50% (n=9; N=3; $p<0.05$, Wilcoxon t-test) with an IC_{50} value of 2.7 ± 0.1 μM and a Hill slope (*nH*) of 0.6 ± 0.09 (mean ± S.E.M; n=6-9; N=3) obtained from concentration-response relationship. Also, Phe increased the rate of decay, tau (τ), of peak I_{Kr} tail currents showing a significant reduction in mean τ_{fast} value in presence of 3 μM Phe. Surprisingly, when the effect of Phe was examined on action potential (AP) generated at 0.5 Hz through whole-cell patch current-clamp recording, it significantly shortened action potential duration at 90% (APD90) at both 3 μM (by 20%; n=4; N=2; $p<0.05$, paired t-test) and 10 μM Phe (by 41%; n=4; N=2; $p<0.05$, paired t-test); unlike previous studies in marine fishes that showed AP prolongation at 5 μM and 25 μM Phe. To understand and elucidate the differential effects of phenanthrene on AP and I_{Kr} currents, we further tested Phe's effects using an AP-like voltage-clamp protocol. Interestingly, 3 μM Phe exhibited only 25-30% inhibition of peak total outward current during the AP command. Furthermore, a shift of -6.2 ± 1.7 mV (mean ± S.E.M; n=4; N=3; $p<0.05$, paired t-test) in mean voltage at which current peaked during repolarization was observed. Lastly, the effect of Phe on L-type calcium channel current, I_{CaL} was assessed by selective recording, using a double-pulse protocol with potassium free solutions and in the presence of 0.5 μM TTX. 10 μM Phe exhibited significant (40%) inhibition of I_{CaL} currents (n=10; N=4; $p<0.05$, paired t-test) with no shift in the current-voltage relation. Zebrafish are used to model human cardiac electrophysiology. Thus our findings of significant cardiotoxic effects of Phe, a PAH found in air pollution, on zebrafish ventricular cardiomyocytes provide further evidence linking air pollution to cardiovascular toxicology.

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PC13

Older Adults Display Lower CD31$^+$ Circulating Angiogenic Cells, Which is Compounded by Low Cardiorespiratory Fitness

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CD31-expressing T-lymphocytes are highly vasculogenic T-lymphocyte subsets, with potential to stimulate angiogenesis and endothelial repair via paracrine means, including secretion of vascular endothelial growth factor (VEGF)[1]. Advancing age is associated with endothelial dysfunction, and subsequent cardiovascular disease risk[2]. Ageing results in altered T-lymphocyte composition due to infection history and antigen exposure[3], and thus may also affect the CD31$^+$ T-lymphocyte subset. A peripheral blood sample was taken from thirty-six young (18-30 years) and thirty-eight older (50-65 years) males and VO_2 max was measured using a graded exercise cycling test to exhaustion Peripheral blood mononuclear cells were isolated from peripheral blood samples, and incubated with monoclonal antibodies against CD3, CD31 and C-X-C Chemokine Receptor 4 (CXCR4). CD31$^+$ T-lymphocytes were quantified as CD3$^+$CD31$^+$ cells using flow cytometry. Cell surface expression of CXCR4 of these CD31$^+$ T-lymphocytes were also assessed using flow cytometry. Differences in CD31$^+$ T-lymphocytes between age groups were assessed using independent-samples t-test. Subsequent analyses were performed to assess effect of VO_2 max on these cells in the two age groups using factorial analysis of variance (ANOVA), using age group and VO_2 max tertiles as factors in the analyses. Older adults exhibited lower CD31$^+$ T-lymphocytes than younger adults (543 ± 229 cells/μL vs. 751± 236 cells/μL, p = 0.000), which is also reflected as lower proportion of CD3+ T-lymphocytes expressing CD31 (47.41 ± 8.99% vs. 61.67 ± 7.58%, p = 0.000). CXCR4 expression was significantly higher on CD31+ T-lymphocytes of older adults compared to younger adults (8.56 ± 1.18a.u vs. 12.91 ± 8.38a.u, p = 0.003). Young adults who displayed low VO2 max displayed lower proportion of T-lymphocytes expressing CD31 than those with high VO2 max (57.90 ± 5.15% vs. 65.06 ± 6.95%, p = 0.024), an observation observed in older adults (44.96 ± 7.86% vs. 53.85 ± 7.66%, p = 0.036). The number of CD31+ T-lymphocytes in the older high VO2 max group was still significantly lower than the low VO2 max young group (53.85 ± 7.66% vs. 57.90 ± 5.15%, p = 0.021). These data strongly indicate that advancing age results in lower number of angiogenic T-lymphocytes which may reflect endogenous vascular endothelial repair capacity, and thus elevating cardiovascular disease risk in older individuals. In addition, lower cardiorespiratory fitness in older people results in further reductions in CD31+ T-lymphocytes, suggesting that low cardiorespiratory fitness further exacerbates the age-related decline in these angiogenic cells.

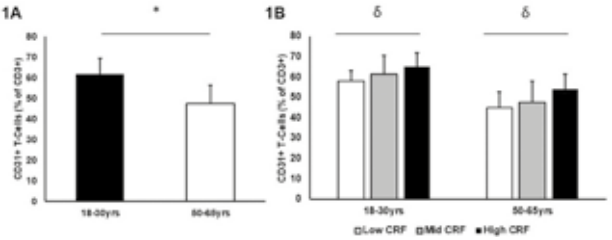

Figure 1. A) $CD31^+$ T-Lymphocytes in young and older adult men. B) Influence of cardiorespiratory fitness (CRF) on $CD31^+$ T-Lymphocytes in 18-30 and 50-65yr old men. *Values shown are mean ± SD.* * *$p < 0.005$ between young and older men,* δ *$p < 0.05$ between low and high CRF groups.*

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PC14

Pig and Sheep Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Have Enhanced ATP-Dependent Channel Gating

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Cystic fibrosis (CF) is a life-shortening genetic disorder resulting from loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (1). To understand the pathophysiology of CF and test new therapeutics, large animal models have been developed using pigs (2) and sheep (3). Because CFTR function and pharmacology vary across species (4), the aim of this study was to investigate the single-channel behaviour of pig CFTR with that of human and sheep CFTR (5). Using the patch-clamp technique, we studied CFTR Cl^- channels in excised inside-out membrane patches from CHO-K1 cells transiently transfected with CFTR constructs using a large Cl^- concentration gradient ($[Cl^-]_{int}$, 147 mM; $[Cl^-]_{ext}$, 10 mM) (5). Like other CFTR orthologues, pig CFTR formed Cl^- selective channels regulated by protein kinase A-dependent phosphorylation and intracellular ATP. However, when compared with human CFTR, pig and sheep CFTR differed in two important ways. First, the single-channel conductance of pig CFTR (9.7 ± 0.3 pS; n = 6) and sheep (9.9 ± 0.1 pS; n = 13) were greater than that of human CFTR (9.2 ± 0.1 pS; n = 5) (means ± SEM). Second, distinct differences were observed in the frequency and duration of channel openings. At 1 mM ATP, the long closures separating channel openings of pig CFTR (n = 6) were similar to those of human CFTR (n = 9), while those of sheep CFTR (n = 13) were 56% shorter. Moreover,

the duration of channel openings of pig CFTR (414%; n = 6) and sheep CFTR (174%; n = 13) were noticeably longer than those of human CFTR (n = 9). As a result, the open probability (P_o) of pig CFTR (0.68 ± 0.04; n = 12) and sheep CFTR (0.60 ± 0.02; n = 24) were greater than that of human CFTR (0.40 ± 0.02; n = 19). To explore how ATP gates CFTR, we examined the ATP dependence of P_o between 0.03 and 3 mM ATP. By fitting mean P_o data with the Michaelis-Menten functions, we determined the apparent affinity and efficacy of CFTR for ATP. When compared to human CFTR (K_D = 230 μM, $P_{o(max)}$ = 0.66, r^2 = 0.95; n = 4 - 16), ATP regulated pig CFTR (K_D = 32 μM, $P_{o(max)}$ = 0.81, r^2 = 0.82; n = 4) and sheep CFTR (K_D = 77 μM, $P_{o(max)}$ = 0.70, r^2 = 0.93; n = 5 - 13) with increased affinity and efficacy. Collectively, we conclude that: i) CFTR activity varies between species, decreasing in the rank order pig > sheep > human and ii) differences in current flow through open channels and ATP-dependent channel gating explain the variation in channel activity. In addition to assisting studies of CF animal models, these data provide insight into species-specific differences that will inform future analyses of CFTR structure and function with the potential to identify new CF therapeutics.

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

PC15

Cocoa-flavanol supplementation speeds pulmonary oxygen uptake kinetics in sedentary middle-aged adults

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Both ageing and physical inactivity are associated with impaired pulmonary oxygen uptake (VO_2) kinetics and ultimately, exercise tolerance. However, VO_2 kinetics are sensitive to exercise and nutrition intervention, and strategies that augment blood flow may speed VO_2 kinetics and enhance exercise tolerance in an ageing population. Cocoa flavanols (CF) are known to improve vascular endothelial function and augment nitric oxide (NO) production, but their potential to modulate VO_2 kinetics during exercise has not yet been studied. Therefore, the objective of this study was to test the hypothesis that, compared with placebo (PL), CF would speed VO_2 kinetics during moderate-intensity exercise and enhance exercise tolerance in sedentary middle-aged adults. Seventeen healthy, sedentary adults (11 male, 6 female) were assigned in a randomized, double-blind, crossover design to receive daily cocoa extract (400 mg flavanols, 11.6 mg caffeine, 90 mg theobromine) or PL (0 mg flavanols, 11.6 mg caffeine, 90 mg theobromine) supplementation for 7 days. Initially, maximal VO_2 ($VO_{2\,max}$) and the gas exchange threshold (GET) were determined. Subsequently, participants were familiarised with procedures and the time to exhaustion (TTE) trial. Following 7 days of PL/CF supplementation, participants completed a series of step-exercise tests: three 6-min bouts of constant-load cycling at 80% GET and one bout at 60%Δ to exhaustion. Pulmonary gas exchange, heart rate (HR), blood lactate and perceived exertion was measured throughout. Resting blood pressure was also recorded. After a one-week washout period, participants underwent identical tests on day 7 after a secondary PL/CF daily supplement cross over regime. No differences were found in resting systolic (PL: 128 ± 12 vs CF: 127 ± 12 mmHg) or diastolic (PL: 78 ± 7 vs CF: 78 ± 7 mmHg) blood pressure between PL and CF conditions. In response to moderate-intensity exercise, heart rate amplitude (*A*HR) and time constant (HRτ) were similar between PL and CF (*A*HR, PL: 31 ± 8 vs CF: 32 ± 8 b.min^{-1}, $P = 0.516$; HRτ, PL: 53 ± 22 vs CF: 47 ± 13 s, $P = 0.219$). Likewise, Δ blood lactate was similar between conditions for moderate- (PL: 1.2 ± 0.9 vs CF: 1.3 ± 0.8 mM, $P = 0.760$) and also severe-intensity exercise (PL: 7.4 ± 2.5 vs CF: 7.1 ± 2.8 mM, $P = 0.694$). Compared with PL, CF reduced the fundamental time-constant (τVO_2) during moderate-intensity exercise (PL: 35 ± 12 vs CF: 30 ± 7 s, $P = 0.047$). All other VO_2 parameters (VO_{2b}, AVO_2, $TDVO_2$, Gain and End-exercise VO_2) were similar during moderate-intensity exercise between PL and CF. No difference was observed in TTE between conditions (PL: 435 ± 58 vs CF: 424 ± 47 s, $P = 0.480$). Dietary supplementation with CF appears to speed VO_2

kinetics during moderate-intensity exercise but does not impact exercise tolerance in sedentary middle-aged adults.

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

PC16

Metallomic profiling of vascular cells in response to oxidative stress: Zn distribution under different ambient oxygen levels

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Hypoxia and oxidative stress are contributing factors in cardiovascular disease, and notably changes in zinc, iron and calcium homeostasis affect cellular responses to oxygen tension and oxidative stress. Dietary zinc intake correlates inversely with subclinical carotid atherosclerosis, and serum zinc levels are reduced in patients with heart failure or undergoing cardiac surgery. Although zinc is a redox-inert metal, physiological concentrations of zinc have anti-oxidant, anti-inflammatory and anti-proliferative properties, whilst zinc deficiency or overload generates oxidative stress. Zinc has also been shown to afford protection during ischaemia reperfusion injury. We have used ICP-MS (Inductively Coupled Plasma Mass Spectrometry) to map changes in total metal content in human coronary artery endothelial cells (HCAECs) cultured long-term (at least 5 days) under hyperoxia, physiological normoxia and hypoxia (18, 5 and 1 kPa O_2) and exposed to ischaemia reperfusion injury. Decreases in ^{66}Zn and increases in ^{56}Fe were measured as cells were adapted from 18 kPa to 1 kPa O_2 levels. In parallel experiments, mobilisation of intracellular free metals during exposure to *in vitro* ischaemia reperfusion injury was measured using metal specific fluorescent probes. Intracellular Fe^{2+} was unaffected by acute hypoxia but significantly increased upon re-oxygenation. Increased Fe^{2+} correlated with superoxide production and was abrogated by treatment with PEG-SOD but elevated by a SOD inhibitor (ammonium tetrathiomolybdate). We are currently employing Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) to further probe the spatial distribution of Zn, Fe, Ca, Cu and Mn in cells maintained under varying ambient O_2 conditions in a Scitive O_2-regulated workstation. Our findings highlight the importance of characterising metal fingerprints in cultured cells and tissues which will provide novel insights for potential therapeutic interventions to limit damage in ischaemia reperfusion injury.

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PC17

Cardioprotective and antioxidant effects of alpha lipoic acid in nicotine administered rats

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Nicotine has been implicated as the major player involved in smoking-induced toxicity. It is associated with an increased risk of cardiovascular diseases such as atherosclerotic vascular disease and hypertension. Alpha lipoic acid (ALA) is a unique and potent antioxidant that scavenges reactive oxygen species with the ability to regenerate other antioxidants as well. Given that oxidative stress plays a fundamental role in nicotine toxicity, the objective of this study was to evaluate whether dietary supplementation with ALA, could improve nicotine-induced cardiac toxicity. Blood pressure parameters, lipid profile, index of lipid peroxidation (MDA), antioxidant enzyme (GSH, SOD, Catalase) and a marker of inflammation (CRP) were to be estimated. Twenty-eight (28) male Sprague-Dawley rats (150-200g) were divided into 4 groups of 7 animals each. The animals were fed and treated for 4 consecutive weeks. Group 1 (control). Group 2 received Nicotine only. Group 3 received both Nicotine and ALA. Group 4 received Lipoic Acid only. Nicotine was administered intraperitoneally (0.5mg/kg) while Lipoic acid orally (200mg/kg). At the end of the experiment, the rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intraperitoneally at a dose of 5ml/kg body weight, blood pressure parameters were obtained by the cannulation of left carotid artery connected to a pressure transducer and a power lab system. Blood samples were also withdrawn for biochemical analysis. Data were presented as mean ± SEM and the level of significance was taken at $p<0.05$. Results showed SBP, DBP, MAP and RPP was elevated in the group which received only Nicotine (Group 2) however no change was observed in HR and PP. Elevated levels of CRP, MDA and reduced levels of antioxidant enzymes (GSH & SOD) was also observed. There was no change in catalase activity and lipid profile. Supplementation with ALA (group 3) reduced the elevated MDA levels and elevated SOD & Glutathione levels in the nicotine treated rats. This study reveals that ALA ameliorates nicotine-induced cardiovascular dysfunction through its antioxidant properties.

Keywords: Nicotine, Alpha lipoic acid, Cardiac function, antioxidant, Oxidative stress.

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PC18

Adaptation of bEnd.3 brain microvascular endothelial cells to physiological normoxia reduces superoxide production associated with reperfusion injury

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Treatments available for cerebral ischaemic stroke remain limited due to failures in clinical translation. To improve clinical translation, physiological oxygen encountered *in vivo* need to be considered in cell culture *in vitro*. Most *in vitro* studies are conducted under room air conditions (18 kPa O_2); however, as cells *in vivo* experience O_2 levels ranging from ~13 kPa to ~1 kPa, cells cultured under 18 kPa O_2 are hyperoxic. Using an O_2-sensitive probe (MitoXpress-INTRA, Agilent), we have established that intracellular O_2 in mouse brain endothelial cells, bEnd.3, was 3.6 kPa during long-term culture under 5 kPa O_2 in a Scitive O_2-regulated workstation (Baker-Ruskinn), recapitulating reported intracellular O_2 levels in the brain. We previously reported that long-term culture under 5 kPa O_2, results in a phenotype different to cultures under 18 kPa O_2, as evidenced by downregulation of specific Nrf2 target antioxidant genes. bEnd.3 cells (n=3-5) cultured either under 18 kPa O_2 (hyperoxia) or 5 kPa O_2 (physiological normoxia) were subjected to hypoxia (1 kPa O_2, 1 h) and reoxygenation (back to either 18 or 5 kPa O_2) to model *in vivo* ischaemic stroke. Reactive oxygen species (ROS) production was measured in real time in a CLARIOstar plate reader (BMG Labtech), using the chemiluminescent probe L-012 and mitochondria-specific ROS indicator, MitoSOX™ Red. Administration of a Nrf2 inducer, sulforaphane (2.5 μM), reduced the superoxide burst associated with reperfusion injury in cells cultured under 18 kPa O_2. Furthermore, administration of rotenone (1 μM) 5 min prior to reoxygenation significantly reduced superoxide production compared to vehicle, implicating complex I of the electron transport chain in oxidative stress. Adaptation of bEnd.3 cells to 5 kPa O_2 prevented superoxide production associated with ischaemia-reperfusion injury, suggesting that exaggerated response in cultures exposed to hyperoxia may potentially create misleading insights. Our findings highlight the importance of conducting vascular cell culture under physiological normoxia to recapitulate the redox phenotype of brain microvascular endothelial cells *in vivo*.

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PC19

Investigating the Impact of FKBPL (FK506 binding protein like) on Obesity-Driven Breast Cancer

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Obesity is a risk factor for the development and recurrence of breast cancer, and current treatments tend to be less effective in obese patients. Co-culture of breast cancer (BC) cell lines with adipocytes or adipocyte-conditioned medium (ACM) drives a pro-tumorigenic phenotype[1], however the molecular mechanisms underlying this effect are not fully known.

FKBPL is a divergent member of the immunophilin protein family with anti-cancer activity. An FKBPL derived therapeutic peptide, ALM201, has completed phase I clinical trial[2]. FKBPL and its drug peptide have been shown to inhibit angiogenesis and cancer stemness activity in vitro in BC cell lines[3]. FKBPL has also been shown to be protective in BC disease outcomes [4].

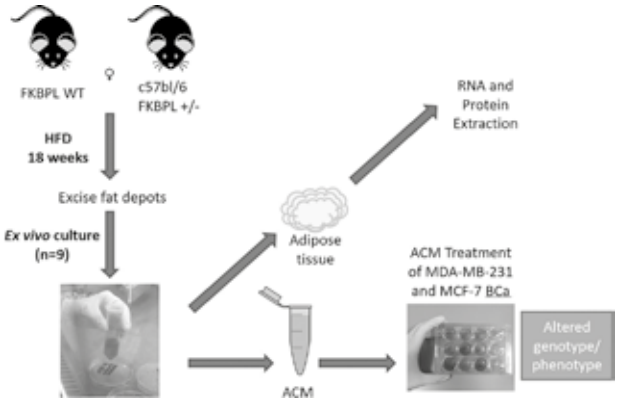
Like cancer, obesity is associated with dysregulated vasculature and inflammation. The adipocyte-cancer cell crosstalk leads to functional and phenotypical changes of both cell types. This microenvironment aids tumour onset and progression. Our group has shown that FKBPL deficient mice develop severe obesity. Thus, harnessing FKBPL could offer a novel approach to modulate obesity and protect against obesity-driven cancer.

This study investigates whether FKBPL has a role in modulating obesity-driven BC through treatment of BC cell lines with adipose-conditioned media (ACM) from herozyogous (Het) *Fkbpl*$^{+/-}$ and wild-type (WT) *Fkbpl*$^{+/+}$ C57bl/6 mice, and subsequent measurement of changes to cell viability/metabolic activity by MTT assay and gene expression profile changes using a QiagenRT2 Profiler qPCR gene array of 84 BC specific genes.

FKBPL mRNA levels, measured via qPCR, were lower in HET adipose tissue compared to WT (n=3, p=0.0506, student t-test). MDA-MB-231 cells were treated with Het ACM, WT ACM or serum-free basal medium for 48 hr to determine changes to cell viability/metabolic activity by MTT assay. Cells treated with FKBPL+/- ACM had higher cell viability/metabolic activity compared to the WT or serum-free basal medium treated cells (n=3, $p<0.01$, one way ANOVA with Tukey's multiple comparisons test).

MDA-MB-231 and MCF-7 were treated with WT versus Het ACM for 24hr (n=1), cDNA was synthesised and run on qPCR array plates. A 1.5-fold cut off was used for analysis purposes. 3 genes were altered in MCF-7 + Het ACM versus WT ACM (*ATM*:-1.5 downregulation, *BRCA2* 1.5 upregulation, *SERPINE1*: 1.98 upregulation). 2 genes were altered in the MDA-MB-231 + Het ACM versus WT ACM (*CDH1*: 2.0

upregulation and *FOXA1*: 25.2 upregulation). The large upregulation in FOXA1 is of note given that similar to FKBPL it is a prognostic marker of triple negative BC and associated with a less aggressive cancer phenotype[4].
While validation is required our results show that ACM from HFD *Fkbpl*$^{+/-}$ mice can stimulate higher cellular metabolic activity and differential gene expression compared to WT ACM.



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PC20

8 days supplementation with New Zealand blackcurrant extract improves free-living glycaemic control and insulin sensitivity in sedentary, overweight individuals

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Consumption of low-quality, high-energy diets in combination with a sedentary lifestyle have made obesity, metabolic syndrome and type 2 diabetes mellitus (T2DM) into worldwide epidemics. Obesity is characterised by visceral adiposity, dyslipidaemia, low-grade systemic inflammation and endothelial dysfunction which together contribute to the development of insulin resistance and progression to T2DM. Prolonged periods of postprandial hyperglycaemia are shown to be an independent risk factor for the development of T2DM and short-term anthocyanin supplementation has been shown to improve glycaemic control, however current evidence has only been conducted using laboratory-based control drinks (Torronen *et al.,* 2010; Willems *et al.,* 2017. In a double-blind, randomised, placebo-controlled design, 12 sedentary, overweight, office workers (6 male, 6 female, 28 ± 9 yr, BMI 29.9 ± 4.8), ingested 8 days of anthocyanin-rich New Zealand blackcurrant (NZBC) extract (600 $mg.d^{-1}$) or a visibly matched placebo before undertaking a 2 h oral glucose tolerance test (OGTT) where glucose and insulin concentrations were determined via intermittent blood sampling. Participants also wore a continuous glucose monitoring system (CGMS) before consuming a 24 h standardised diet under free-living conditions on day 7 of supplementation, whereby interstitial glucose excursions were determined. Values are means ± SD, compared by ANOVA. Fasting glucose (NZBC: 5.4 ± 0.7 vs. 5.3 ± 0.4 $mmol.L^{-1}$, control) and insulin (NZBC: 18.4 ± 7 vs. 20.3 ± 10.2 $uIU.ml^{-1}$, control) were similar between conditions. Following NZBC ingestion plasma glucose was lower at 45 (7.8 ± 2.1 vs. 8.9 ± 1.4 $mmol.L^{-1}$), 60 (7.4 ± 2.2 vs. 8.7 ± 1.9 $mmol.L^{-1}$) and 90 min (6.1 ± 1.7 vs. 6.8 ± 1.3 $mmol.L^{-1}$), with an 8% reduction in area under the curve (AUC) glucose (P=<0.05). There was no time effect for insulin (P=0.226), however NZBC $AUC_{insulin}$ was 14% lower (P=<0.05). Following NZBC supplementation, free-living $AUC_{glucose}$ was lower during breakfast (9%; P=<0.05) and lunch (8%; P=<0.05), with no difference at dinner (P=0.643). There was no difference in HOMA-IR (P=0.413), hepatic (P=0.430) or peripheral insulin resistance (P=0.426), however whole-body Matsuda insulin sensitivity index was improved by 22% following NZBC ingestion (P=<0.05). These findings suggest that short-term NZBC extract supplementation can enhance postprandial glucose and insulin responses to a glucose challenge and whole-body insulin sensitivity in overweight/obese individuals. Furthermore, NZBC supplementation is capable of improving free-living glycaemic responses under standardised dietary conditions and may be a viable strategy of improving insulin sensitivity.

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

PC21

Finger Millet Supplementation Alleviate Urea and Serum Electrolytes in Mercury Chloride Induced Toxicity in Wister Rats.

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Whole grain consumption is being regarded as a safe approach for management of metabolic disorders. Millets are also whole grains and their consumption has been shown to be inversely associated with metabolic diseases. Finger millet (FM), known as (*Eleusine coracana*) is an important food crop among Africans and Indians. It is regarded as "wonder grain/super cereal" due to health promoting effects. Heavy metals such as mercury, when exposed to, pose a great health problems. When absorbed, inhaled or ingested into the blood stream, they damage tissues like the kidney, brain and liver. Urea, creatinine, and serum electrolytes, (sodium, Na^+, potassium, K^+, chloride, Cl^- and bicarbonate, HCO_3^-) are very important parameters for maintaining the body's fluid balance. Their imbalance result in several disorders such as dehydration. This study was designed to investigate the effect of finger millet on urea and serum electrolytes in mercury chloride ($Hgcl_2$) induced toxicity in wistar rats. The rats were randomly divided into five groups of five rats each (n=5). Group 1 (control) were administered with distilled water (2.5ml/kg), Group 2 were given 6.23mg/kg (3.75%) of $Hgcl_2$ and 100mg/kg of finger millet, Group 3 were given same dose of $Hgcl_2$ and 50mg/kg of finger millet, Group 4 also received same dose of $Hgcl_2$ and 25mg/kg of finger millet while Group 5 received $Hgcl_2$ only, all treatments were administered via oral route. After two weeks of administration, rats were euthanized and blood samples were drawn from the heart by cardiac puncture and used to assay for serum urea, creatinine, sodium, potassium, chloride and bicarbonate. Analysis of variance (ANOVA) and Turkey's post hoc test were used to analyse the data obtained. The result showed a significant ($P<0.05$) decrease in serum creatinine from the group treated with 50mg/kg and 25mg/kg (63.08 ±3.22 and 62.12 ±4.08) when compared with the normal control (79.18 ±1.90) group. Also there was a significant ($P<0.05$) decrease in serum potassium level from the group treated with 50mg/kg (4.95 ±0.29) when

compared with the control (79.18 ±1.90) group. Furthermore there was no significant difference (P<0.05) in serum level of Urea, Sodium, Chloride and Bicarbonate when compared with the normal control group. The present study suggest that finger millet could be used as a supplement to improve the toxic effect induced by mercury chloride.

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PC22

Delving Deeper into the Coat of Cardiac Caveolae

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Caveolae, small ~80 nm membrane invaginations, regulate numerous membrane receptors and signalling pathways within cardiac myocytes. However much of the current knowledge of caveolae is based on experimental evidence from cell lines which do not contain the muscle-specific caveolar proteins (caveolin-3 and cavin-4). Here we examine the populations and protein composition of cardiac caveolae using quantitative protein analysis, protein coat isolation and super-resolution imaging.

Cardiac myocytes, isolated from male Wistar rats (230-250 g), were attached to coverslips for super-resolution imaging. Paraformaldehyde-fixed myocytes were labelled with caveolins-3 and cavin-1/cavin-4 antibodies, 10X Expansion Microscopy (ExM) was then performed to achieve ~25nm resolution [1]. In cell homogenates caveolar protein abundance was quantified using stable isotope dilution mass spectrometry with custom-made calibration standards [2]. HeLa cells and

cardiac myocytes were incubated with the cross-linker dithiobis(succinimidyl propionate) (DSP) for isolation of the caveolar coat complex (CCC) on a sucrose velocity gradient [3].

Caveolin-3 showed the highest expression within myocytes followed by cavin-1/4 (~60% of caveolin-3). Caveolin-1/2 and cavin-2/3 were detected at lower levels (<20% of caveolin-3)(n = 3 animals). The CCC isolated from HeLa cells was ~80S in size and contained the main ubiquitous isoforms (caveolin-1 and cavin-1)[3]. In myocytes, the ~80S complex contained only caveolin-1 and cavin-1/4 (n = 4 animals). Despite being the predominant isoform caveolin-3 was absent from these complexes in the cardiac cell. Indeed, caveolin-3 migration in the sucrose gradient did not change with DSP, suggesting that caveolin-3 does not integrate into the CCC in muscle cells as caveolin-1 does in non-muscle cells. Using ExM, caveolin-3 and cavin-1/cavin-4 can clearly be resolved within a single caveola with high levels of co-localisation. Taken together these data highlight distinct differences in caveolae between myocytes and non-muscle cells. Understanding the protein composition of the different caveolae is essential in understanding the multiple functions that caveolae perform.

Cavin-1 is lost from the CCC in response to mechanical stimuli (stretch/swelling) in non-cardiac cells and this may impact on caveolar shape and cell function. Whether the same occurs in the cardiac myocyte is not known, despite the key role that mechanotransduction plays in the heart. We are now looking at the composition of the CCC following hypo-osmotic swelling (cells) and stretch (whole heart) using both super-resolution imaging and sub-cellular fractionation.

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PC23

Can the health benefits of a walking-based exercise programme be enhanced by co-ingestion of a lipid lowering drug?

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Although insulin resistance in obesity and T2D is associated with IMTG accumulation in muscle, endurance training is associated with larger IMTG stores, but with high insulin sensitivity in a phenomenon termed the "athlete's paradox". Because endurance training increases muscle oxidative capacity and the utilisation of IMTG as a fuel during exercise, it is believed that the ability to utilise IMTG during exercise is mechanistically important to preserve insulin sensitivity alongside elevated IMTG storage. High rates of IMTG utilisation allows regular turnover if the IMTG pool, leading to reduced accumulation of lipid metabolites such as DAGs and ceramides. These lipid metabolites reduce the insulin-induced activation of IRS-1 and Akt, respectively. Thus, preventing activation of the insulin signalling cascade, GLUT4 translocation and glucose uptake into skeletal muscle.
Acute use of anti-lipolytic agents has shown to Improve insulin sensitivity and glucose control, but fail to maintain these beneficial effects long-term. Regular exercise training in lean, sedentary individuals is effective at increasing IMTG utilisation paired with improvements in insulin sensitivity. Low intensity brisk walking is well tolerated by overweight/obese individuals however walking interventions result in little or no improvements in insulin sensitivity. Since Acipimox ingestion enhances muscle glycogen utilisation, a combined intervention with regular brisk walking could effectively stimulate IMTG utilisation and enhance the benefits of a walking based exercise program in obese individuals.
Methods:
34 sedentary, overweight/obese people (aged 25-50 years, BMI >28 kg.m-2) with prediabetes will be recruited and split into two groups; exercise (EX), and exercise plus Acipimox ingestion (EX+ACIP). Participants attend 3 supervised walking exercise per week for 12 weeks. Body composition, maximal aerobic fitness ($VO_{2\ max}$), liver fat via MRI and skeletal muscle insulin sensitivity via hyperinsulinemic euglyceamic clamp will be measured pre- and post-exercise intervention.
Hypothesis and Implications:
It is hypothesised that an exercise programme of steady walking will have larger effects on insulin sensitivity and glycaemic control when combined with Acipimox prior to each exercise session in individuals with pre-diabetes.

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

PC24

In silico pharmacological assessment of Mibefradil in single detrusor smooth muscle cells towards understanding urinary bladder over activity

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BACKGROUND AND PURPOSE:
Enhanced spontaneous contraction of the detrusor smooth muscle (DSM) cells is associated with overactive bladder (OAB), a pathophysiological syndrome affecting millions of individuals socially [Young et al., 2008]. Due to the adverse side effects of conventional anticholinergic drugs, researchers are focusing on novel drug compounds with high specificity. Understanding the drug effects with respect to various ion channels offers additional possibilities for safety pharmacological assessment. Here we have implemented a novel computational approach to simulate the effects of the T-type Ca^{2+} channel blocker Mibefradil on the DSM cell action potential (AP) and subsequent DSM cell excitability.
METHODS:
The DSM cell model is described as an equivalent electrical circuit consisting of a membrane capacitance connected in parallel with a number of variable conductances representing two voltage-gated Ca^{2+} (T - type and L- type) channels, two voltage-gated potassium (Kv, KCNQ) channels, three calcium-dependent potassium (BK, IK and SK) channels, an ATP-dependent potassium channel and an inward rectifying cation channel [Mahapatra et al., 2018]. A drug model for Mibefradil was simulated by multiplying the maximal conductance of T-type Ca^{2+} channel with a scaling factor between 0 and 1 to mimic the drug concentration.
KEY RESULTS:
In this model, all ionic conductances were tuned to set the resting membrane potential (RMP) at - 50 mV. A synaptic input for 15 ms was injected to evoke the AP. The maximum conductance value of the T-type Ca^{2+} channel (g_{CaT}) was set to 0.0006 S/cm^2. Adding Mibefradil by 50% of its control value reduced the peak amplitude of AP and inward current. However, the addition of Mibefradil by 100% resulted in no AP and zero inward currents. Representative APs are shown in Fig 1 (A) with proper legends. The T- type Ca^{2+} conductance was varied by up to ± 50 % of the control value in discrete steps to study the RMP sensitivity analysis. The normalized changes in RMP of the AP are shown in Fig 1(B). Note that RMP varies at most by up to + 10% and -15%, indicating T- type Ca^{2+} conductance dependent DSM cell excitability. These results (Fig 1A and 1B) show that T-type Ca^{2+} channels play an important role in generating AP and deactivation of T-type Ca^{2+} channels decrease the excitability of DSM cell.
CONCLUSIONS AND IMPLICATIONS:
We investigated the ability of the Mibefradil to modulate DSM cell's AP using our computational model which provides a virtual electrophysiological workbench. This in silico assessment showed that inhibition of T-type Ca^{2+} channels hyperpolarized

the RMP, eliminated the AP and reduced DSM cell excitability. This study suggests that a compound, such as Mibefradil may form a part of a new pharmacological strategy in the treatment of OAB.

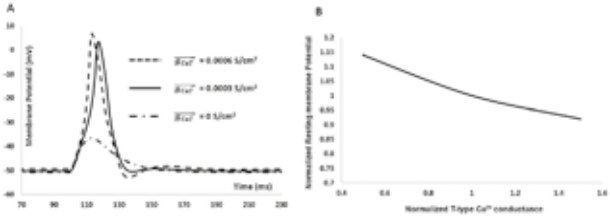

Fig 1: (A) Effects of Mibefradil on synaptic input based AP (B) RMP Sensitivity analysis with varying T- type Ca^{2+} conductance.

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PC25

Increased alternans susceptibility in heart failure is linked to action potential morphology

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New onset atrial fibrillation (AF) is increased ~2 fold in severe heart failure (HF). Alternans, a beat-to-beat oscillation in the atrial action potential (AP) and/or Ca transient, has been implicated in the promotion of AF. Atrial alternans aetiology is not fully understood and it remains unknown if susceptibility to alternans is increased in the HF atria.

Atrial contraction is driven by intracellular Ca cycling in response to membrane depolarisation. The cellular AP and Ca transient are directly linked via the L-type Ca current and the Na/Ca exchanger such that alteration in one can result in alternation of the other. Therefore, alternans often occurs in both the AP and Ca transient irrespective of causality. We investigated whether cellular alternans occurred more readily in HF and how remodelling of electrical or Ca handling parameters in HF exacerbated mechanisms driving alternans.

HF was induced in sheep by rapid ventricular pacing, via a pacemaker, with the pacing lead implanted in the endocardial apex of the right ventricle using a minimally invasive transvenous approach under fluoroscopic guidance. Surgical plane anaesthesia was maintained under isoflurane (3-5%) mixed with of oxygen (4.5-6 $L.min^{-1}$).
Left atrial myocytes were isolated from control and HF animals. Fluo-5F loaded atrial myocytes were incrementally paced at physiological rates (1-3Hz) under current clamp control. The lowest frequency at which alternans was detectable was deemed the alternans threshold. In HF vs. control a greater proportion of cells were seen to alternate (91% *vs.*72.4%) and the threshold for Ca^{2+} alternans (1.7 ± 0.1 Hz *vs.*2.2 ± 0.1 Hz) and AP alternans (1.6 ± 0.1 Hz *vs.* 2.2 ± 0.1 Hz) were decreased ($p<0.05$).
Increased alternans susceptibility in HF was associated with a longer AP duration at 1Hz (APD90; 414 ± 110 ms *vs*. 345 ± 120 ms; $p<0.05$). The longer HF AP was unable to rate adapt as effectively as the control AP, quantified by the steepness of a restitution curve through an S1S2 protocol (Gradient of curve; 1.3 ± 0.3 *vs*. 0.4 ± 0.1; $p<0.05$). We hypothesised that the long HF AP, that was less able to rate adapt, would be less likely to fully repolarise between stimulations suggesting alternans may arise via the Na current. Hyperpolarisation of the membrane potential to increase Na channel recovery from inactivation, increased repolarisation speed by ≈107 ms (APD85; 312 ± 38 ms *vs*. 205 ± 22 ms; $p<0.05$) and terminated alternans in every HF cell (n=4).
Our data shows that alternans occurs more readily in HF atrial cells, potentially providing a mechanism for increased prevalence of AF in HF.

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PC26

Effect of hyperglycaemia on peptide composition of *in vitro* airway surface liquid.

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The airway surface liquid (ASL), a protective layer secreted by the airway epithelium, represents the first line of defence against inhaled infectious material. It contains a complex array of proteins and peptides that aid the neutralisation and removal of inhaled microbes and toxicants. Diabetic hyperglycaemia has been associated with an increased susceptibility to acute lung infections. We hypothesised that diabetes does this by changing the state of the ASL proteome/peptidome. Therefore, we examined how hyperglycaemia changed the ASL peptide profile of

BMI-transformed normal human bronchial epithelial cells (HBEC), cell-lines Calu3 (submucosal adenocarcinoma) and H441 (Clara cell like adenocarcinoma). ASL was acquired by washing the apical surface of epithelial monolayers grown at air:liquid interface with PBS (100ml) at 0, 24 and 120 hours after exposure to hyperglycaemic (25mM glucose) or normoglycaemic (5mM glucose and 20mM mannitol) basolateral medium. The ASL peptides < 10KDa, were subsequently isolated and analysed using the Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. The resulting spectra of uncleaved peptides and their subsequent fragments were analysed using the pNovo sequencing tool. Our preliminary results identified 4765 unique peptides (NHBE-BMI1: 646, Calu3: 3150, H441: 1197). Of these, 37 peptides were common to all samples regardless of glycaemic state. Among these common peptides were several originating from histone and fibrinogen families, both of which are known to generate bioactive peptides. These common peptides were synthetically produced through SPOT synthesis and tested for antimicrobial activity. Histone H1.4 is a parent protein to several common peptides, our initial examination of its intracellular abundance showed a decrease in hyperglycaemic conditions. Alongside this an N-terminal H1.4 histone peptide has been identified through dot blotting in the ASL. Our aim is to test possible biologically relevant methods of activating these peptides to enable antimicrobial activity.

MRC London Intercollegiate Doctoral Training Partnership

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PC27

Calcium Signaling in the Endothelium and Control Over Vascular Tone in Health and Obesity

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Obesity has been a major public health problem worldwide and it has reached epidemic levels in the past few decades. It is also a significant contributor to cardiovascular disease, development of hypertension, and microvascular target organ damage. Although the underlying mechanism is unclear, endothelial dysfunction has been attributed to obesity which, in turn, can disrupt the vasodilation of the small arteries. The Endothelial Dependent Hyperpolarization (EDH) is the predominant vasodilatory mechanism in the small resistance arteries. EDH is tightly controlled by brief local Calcium (Ca^{2+}) signals: Ca^{2+} pulsars, which are IP_3-mediated, and Ca^{2+} sparklets that reflect Ca^{2+} entry through TRPV4 channels. Studying Ca^{2+} signals is critical in obesity- related research due to its direct impact on EDH and on endothelial health. However, no studies have been conducted to address endothelial local Ca^{2+} signals as related to obesity in humans. Therefore, this research is aimed at measuring the changes to endothelial Ca^{2+} signaling

(Ca^{2+} pulsars and Ca^{2+} sparklets) in human obesity and relate this to the vasodilatory capacity of the vascular endothelium. A rigorous approach and study protocol were developed to address the use of human arteries that were isolated from omental biopsies taken from healthy women undergoing elective cesarean sections at terms (37-42 weeks gestation). Isolated omental arteries were examined using high speed spinning disk confocal microscopy (Andor XD Revolution) for imaging endothelial Ca^{2+} events in an en-face configuration. Endothelial functional studies were carried out using four-channel wire myograph system (Danish MyoTech) to assess wire-induced stretch and agonist effects. Results have shown that in human endothelium, Bradykinin (30 nM) induced an increase in pulsars frequency (n=6). Pulsars frequency was unaffected by obesity (n=5). In regard to Ca^{2+} sparklets, imaging results have shown that TRPV4 Ca^{2+} sparklets could not be imaged by directly activating TRPV4 channels using GSK101 (100 nM) in the human endothelium. However, the myography studies have demonstrated that TRPV4 activator GSK101 (1-100 nM) dose dependently relax pre-constricted omental artery segments (n= 5). GSK101- induced relaxation was abolished by the large conductance potassium channels inhibitor Paxilline (10 μM) (n=6) in the Vascular Smooth Muscle Cell (VSMC). GSK101 is believed to have an agonistic effect on the TRPV4 channels in the VSMC and causes vasodilation of the artery. These findings serve as a fundamental basis for better understanding the mechanism of EDH in human arteries and represent a promising area for future research.

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PC28

Multi-state Modelling of AMPARs in plasticity events at a single synapse

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Multistate models (MSMs) provide an *in silico* representation of complex molecules, specifying all the possible functional states of a molecule in a computationally tractable manner. This is achieved through the use of rule-based languages that look for only those properties necessary for a reaction to proceed. We have built a MSM that explores the major components of the excitatory postsynapse, focussing on the properties and states of AMPA receptors (AMPARs), and how these may change in plasticity events.

Our understanding of how AMPARs are involved in plasticity, whilst rich in data and interpretation, lacks coherence. This is largely due to the necessarily piecemeal nature of experiments, which may only focus on a small part of the interactions AMPARs have within a dendritic spine. Our MSM avoids this issue by modelling all parts at once, based on experimentally-derived concentrations and kinetic parameters.

"Early" or "initial" plasticity is thought to be protein synthesis independent, and arise from the difference in kinase and phosphatase activity on AMPARS induced by changes in calcium concentrations. Different experimental induction protocols alter dendritic spine calcium levels in different ways over time, with some achieving stronger or more long-lasting plasticity. We took several protocols and assessed how the model responded to each. We show a broadly good fit with LTP and LTD protocols to existing data, and that in most instances the driving force lies in the balance of phosphatase and kinase activity.

Given the importance of enzymatic activity for successful plasticity, two commonly used sets of values for CaMKII binding to both calmodulin and PP1 were also assessed. The Li *et al*. (2008) values result in a slower reactivation of PP1 after LTP induction than those from the DOQCS database (Sivakumaram *et al*., 2003), and a smaller proportion of phosphorylated CaMKII for a given stimulus. The DOQCS values allow for a small chance that the potentiation seen can be maintained for over an hour by a modest random change in calcium concentrations post induction. No such capacity was seen when using the Li *et al*. vetalues.

Our MSM reinforces the importance of the kinase:phosphatase ratio when determining the direction and maintenance of plasticity, but also warns that some parameter sets may lead to different conclusions on the mechanisms at play.

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PC29

Effect of Ascorbic acid on fatigue of skeletal muscle fibers in long term cold exposed Sprague Dawley rats

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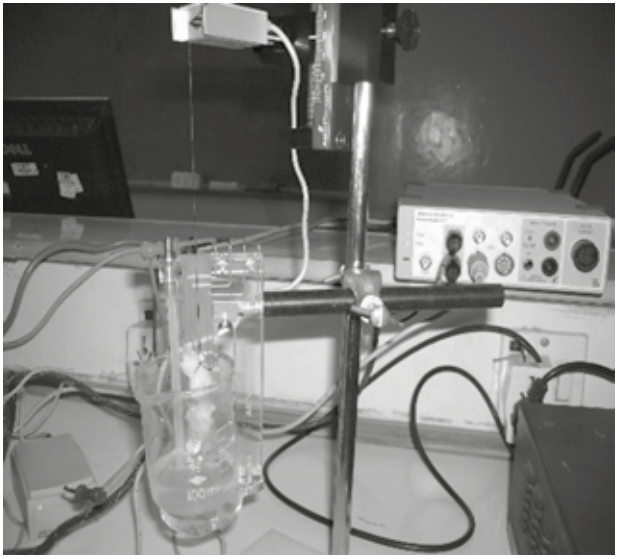
Background: On exposure to prolonged cold temperature, the body responds for effective heat production both by shivering and nonshivering thermogenesis. Cold exposure increases the production of reactive oxygen species which influence the sarcoplasmic reticulum Ca^{++} release from the skeletal muscles and affect their contractile properties. The role of ascorbic acid supplementation on force of contraction during fatigue of cold exposed skeletal muscles was evaluated in this study. *Method:* It was a randomized control trial. Nine weeks old healthy, male Sprague-Dawley rats, weighing 200±25 grams were included in this study. Female rats were not selected as their monthly cyclical changes affect the stress induced. Diseased rats or those which developed any disease during the study period were excluded from the study. After taking recommendation from the ethical committee of Riphah University, Pakistan, 90 healthy, male Sprague Dawley rats were randomly divided into three groups of control (I), cold exposed (II) and cold exposed along with ascorbic acid supplementation (III). Group II was given cold exposure by keeping their cages in ice-filled tubes for 1hr/day for one month. Group III was also exposed to cold along with ascorbic acid supplement as 500mg/L mixed in drinking water for one month. After the study period, the rats were ether anesthetized in glass jars. The extensor digitorum longus muscle was dissected out and force of contraction during fatigue in the skeletal muscle fibers was analyzed on computerized data acquisition system. Graphical events as well as calculations were obtained with the help of Lab Tutor of Power Lab. *Results:* The cold exposed group showed a significant decline in the force of contraction during fatigue of skeletal muscle fibers as compared to the control group. The third group showed less fatigability and a better force of contraction than the cold exposed group. Conclusions: Ascorbic acid increases the resistance to fatigue in the muscles exposed to chronic cold.

Key words
Ascorbic acid, cold stress, fatigue, skeletal muscles

Effect of Ascorbic acid on muscle fatigue

Time (min)	Control group (n=30) Force of fatigue (N)		Cold exposed group (n=30) Force of fatigue (N)		p-value
	Mean	Std. Deviation	Mean	Std. Deviation	
Fatigue At 0 min	0.253	0.001	0.246	0.002	0.000**
Fatigue At 1 min	0.159	0.001	0.133	0.002	0.000**
Fatigue At 2 min	0.129	0.001	0.161	0.003	0.000**
Fatigue At 3 min	0.106	0.001	0.118	0.002	0.000**
Fatigue At 4 min	0.083	0.002	0.094	0.002	0.000**
Fatigue At 5 min	0.048	0.001	0.075	0.002	0.000**

** P value < 0.01 is taken as highly significant



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PC30

Loss of Factor Inhibiting HIF1 (FIH1) is cardioprotective during ischaemia reperfusion injury in the heart.

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The transcription factor hypoxia inducible factor (HIF) plays a key role in orchestrating the cellular response to hypoxia. HIF activity is regulated by two families of hydroxylase enzymes, prolyl hydroxylase domains (PHD) and factor inhibiting HIF (FIH1). We have recently found that mice with a null mutation in the FIH1 gene ($FIH1^{-/-}$) are protected from the reduction in cardiac contractility seen in wild type (WT) control mice during chronic hypoxia. Here, we investigate the response of the $FIH1^{-/-}$ hearts to ischaemia/reperfusion (IR).

Hearts from 11 $FIH1^{-/-}$ mice and 10 WT litter-mate control mice (aged 10-12 months) were isolated and perfused in Langendorff mode with 11 mM glucose and 0.4 mM palmitate. Following 30 minutes baseline perfusion, ischaemia was induced by reduction of coronary flow rate to 0.5 ml/min/gww for 30 minutes, followed by 30 minutes reperfusion. Cardiac function was determined via water filled balloon inserted into the left ventricle of the heart, attached to a pressure transducer, expressed as rate pressure product (RPP, heart rate x left ventricular developed pressure). Rates of glycolytic flux were determined by ^3H glucose labelling and net lactate efflux determined in timed perfusate samples. A further group of WT and $FIH^{-/-}$ hearts (n = 7 & 5 respectively) were perfused with radiolabeled (9, 10^{-3} H) palmitate to determine fatty oxidation rates.

IR significantly impaired cardiac function in both groups. Recovery of function in $FIH1^{-/-}$ hearts was 1.4 fold greater than WT controls (proportion RPP recovery 78.2 ±4.90 % vs. 54.2 ±9.82 % (mean ±SEM, p = 0.0496 independent t. test)). Glycolytic flux was significantly greater in $FIH1^{-/-}$ hearts than WT during baseline (1.180 ± 0.06 vs. 0.908 ± 0.06; p = 0.0323) and reperfusion (0.832 ± 0.10 vs 0.528 ± 0.04; p = 0.0468), with no difference in glycolytic flux during ischaemia. Furthermore, the rate of net lactate efflux over the course of perfusion was greater from $FIH^{-/-}$ hearts than WT (two-way ANOVA, main effect of genotype p = 0.0268). There was no difference in the rate of fat oxidation between WT and $FIH^{-/-}$ hearts. In summary, the present data suggest that deletion of FIH1 is cardioprotective in the face of IR. FIH1 loss is associated with accelerated glycolytic flux and increased

net lactate efflux from the heart. These data are the first to suggest that modulation of FIH1 activity may provide an avenue for therapeutic intervention in the ischaemic heart.

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PC31

Fructokinase expression does not determine efficient fructose metabolism in the mouse sciatic nerve

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Dietary fructose intake has increased, with its link to diabetes there is a need to understand the metabolism of fructose by cells other than those of the liver and gut (Tappy & Rosset, 2019). Investigations into the central nervous system have shown many areas of the brain efficiently metabolise fructose and express key fructolytic enzymes e.g. fructokinase (Oppelt *et al.*, 2017). Specifically, fructose enabled sustained conduction of smaller diameter axons of the optic nerve due to fructokinase expression, in contrast to larger diameter axons which do not express fructokinase (Meakin *et al.*, 2007). This study aimed to investigate the link between fructokinase expression and differences in fibre subtype fructose metabolism of the peripheral sciatic nerve. Previously we have shown that only the C fibres, not A fibres, efficiently metabolise fructose (Rich & Brown, 2018).
All procedures were carried out in accordance with the Animals (Scientific Procedures) Act 1986, Schedule 1. Adult male CD-1 mice were euthanised by cervical dislocation and decapitated. Sciatic nerves (n=6) were dissected, placed in liquid nitrogen and cut into longitudinal and transverse sections for immunohistochemistry processing. Sections were co-stained with fluorescent antibodies against fructokinase and a protein specific to each cell type: neurofilament 200 (A fibres), peripherin (C fibres) and S100β (Schwann cells). Images were taken using confocal microscopy. Sciatic nerves were also placed in a perfusion chamber, superfused with aerated aCSF. The A fibre compound action potential was evoked with supramaximal stimuli at a baseline frequency of 1Hz, high frequency stimulation (HFS) was defined as 100Hz. The area under the normalised CAP amplitude vs. time (NCAP.mins) reflected axon conduction, expressed as mean SD (Rich & Brown, 2018).
Co-localisation of fructokinase with neurofilament revealed fructokinase expression by A fibres but not C fibres or Schwann cells. A fibre conduction was maintained when HFS was imposed during the supply of 20mM fructose (442 44.8 NCAP. mins n=4) whilst the addition of 200μM cinnamate (lactate transport blocker) or

reducing the concentration of fructose to 10mM prevented sustained conduction (133.2 18 NCAP.mins n=4 and 82.4 20.4 NCAP.mins n=4, respectively). This is in accord with our previous finding that A fibres indirectly benefit from fructose in the form of Schwann cell supplied lactate (Rich & Brown 2018).
Expression of fructokinase only by A fibres, despite little direct use of fructose, suggests fructokinase is not essential for efficient metabolism of fructose by the sciatic, in contrast to the optic nerve. Interestingly, maintenance of A fibre conduction requires Schwann cells to provide lactate, derived from fructose or glycogen, highlighting metabolic interactions between axons and glia of the peripheral nervous system.

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PC32

Development of sensor for determination of NO production from bladder urothelium to understand changes with ageing

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Bladder conditions affect up to a third of adults over 65, hence the urinary bladder is highly susceptible to ageing[1].
In the bladder, the urothelium is an important barrier that has been shown to be a key regulator of bladder function[2]. It is thought that alterations in urothelial signalling could cause age-related functional changes in the bladder[1,2]. Nitric oxide (NO) is one of the key signalling molecules within the urothelium, which is significantly difficult to measure[3,4]. This measurement is complex due to the rapid reaction of NO with a wide range of biomolecules and its very short half-life[5]. Therefore, this study is focused on the development of novel sensing tools which have the suitability for measuring NO production from the bladder urothelium in order to understand changes that occur with ageing.

Microelectrodes with electrochemical detection techniques have the required temporal and spatial resolution for monitoring NO production and metabolites such as nitrite in biological systems[5]. Varying compositions of conductive carbon composite microelectrodes containing 15% Multi Walled Carbon Nanotubes (MWCNTs) with varying quantities of Platinum black (0, 10, 20 and 30%) were fabricated. These sensors were tested for the measurement of different concentrations of nitrite (NO_2^-) using amperometry (Fig. 1).

Comparing their performance, the sensitivity data revealed that for the detection of nitrite the most appropriate sensor was that containing 10% Pt black, with it being $8.898\times10^{-11}\pm8.112\times10^{-12}$ A/μM for 0% Pt black, $1.775\times10^{-10}\pm8.732\times10^{-12}$ A/μM for 10% Pt black, $1.039\times10^{-10}\pm1.034\times10^{-11}$ A/μM for 20% Pt black, and $1.084\times10^{-10}\pm1.366\times10^{-11}$ A/μM for 30% Pt black (Fig. 1). The same procedure will be followed for the detection and measurement of NO in solution, in order to determine the required composition for microelectrodes to detect nitrergic signalling from bladder tissue *in situ*.

Measurements of young (3 months) and aged (24 months) bladder tissue will be performed using the electrodes containing the best composition for the detection of nitrergic species released by the urothelium. From the first studies conducted, the data proves that these sensors can detect both molecules in solution. The next steps in this study will investigate whether ageing causes any changes in nitrergic signalling in the bladder, which could lead to new therapies for patients suffering from bladder conditions.

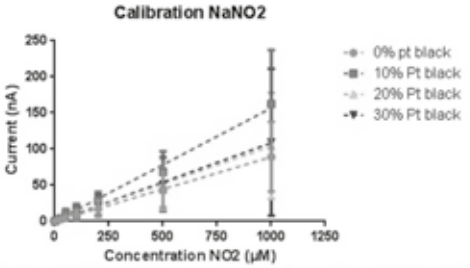

Figure 1. Calibration of $NaNO_2$ in solution using microelectrodes containing 0, 10, 20 and 30% Pt black.

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

PC33

A Combined Approach of Vitamin D Supplementation and a Physical Activity Intervention in an 'at risk' UAE cohort

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In the United Arab Emirates (UAE), low intake of vitamin D and inadequate exposure to sunlight have resulted in significant deficiencies in blood vitamin D concentrations, implicated in diabetes, cardiovascular disease, and cancers. There are notable associations between vitamin D deficiency and reduced exercise tolerance. The aim of this study was to determine how vitamin D status may be correlated with physical inactivity and other disease risk factors in a young UAE population. Upon ethical approval, 35 male and female students (average age of 20), participated in this study. Primary data was obtained on vitamin D status, cardiorespiratory fitness, body composition, and blood profiles. A cohort of the vitamin D deficient individuals were supplemented with vitamin D3 (1000IU). All students participated in an 8-week exercise intervention program, where they engaged in moderate/high intensity exercise at least 3 times a week for 60 minutes. Physiological and biochemical profiling was obtained post-intervention. Accordingly, students were grouped based on whether they were vitamin D deficient/insufficient and not supplemented (Group A, n=15), vitamin D deficient/insufficient and supplemented (Group B, n=18), or vitamin D sufficient and not supplemented (Group C, n=2). Results show that this cohort exhibited clear risk factors for CVD as well as distinct deficiencies/insufficiencies in blood vitamin D3 levels. The cardiorespiratory fitness of all groups was generally poor with average predicted VO2 max values of 28.3 ± 5 ml/kg/min. Nearly 38% of the overall cohort were overweight/obese (BMI ≥ 25), and 41% had systolic blood pressure values over 120 mmHg. The average vitamin D3 levels of Group A, B and C were 19, 13.1, and 34.8 ng/mL respectively. Post intervention, VO2 max of all groups increased to 31 ± 5.8 ml/kg/min showing a change from poor to average cardiorespiratory fitness, although there were no statistical differences between groups, pre-and post-intervention. High blood pressure also dropped from 41% of participants to 27%. There was a significant increase in vitamin D3 levels in group B upon supplementation ($p=0.001$). Although a larger sample set is needed, preliminary data demonstrates that an 8-week intervention can have a positive change on the cardiorespiratory health of this young population although increasing vitamin D levels does not appear to play a role in markedly enhancing their cardiorespiratory fitness. In conclusion, a combined approach of vitamin D supplementation and a physical activity intervention was used to investigate changes on CVD and diabetes risk factors after an 8-week intervention. Although improvements in cardiorespiratory fitness and health were notable,

vitamin D supplementation did not correlate with greater cardiorespiratory and physiologic changes in this subpopulation.

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PC34

Determination of the single channel conductance of *Cavia porcellus* epithelial sodium channels in the αβγ- and δβγ-subunit composition

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The epithelial sodium channel (ENaC) plays a crucial role in electrolyte homeostasis by mediating the transport of sodium ions across the apical membrane of epithelial cells. It is the only constitutively active member of the degenerin/ENaC protein family and is, consequently, stringently regulated. The canonical ENaC comprises three subunits (α, β and γ), but a fourth δ-subunit can replace α, forming functional δβγ-ENaCs which produce larger ion currents in heterologous expression systems (Wichmann *et al.* 2018). However, the physiological role of the δ-subunit is unknown, mainly since rats and mice – popular animal models in physiological research – lack a functional gene for this subunit. Therefore, in order to establish a suitable mammalian model in which the physiology of δ-ENaC could be investigated, *Cavia porcellus* (guinea pig) ENaC isoforms were heterologously expressed in *Xenopus laevis* oocytes and electrophysiologically characterized, using the Patch-Clamp technique. The specific aim of this project was to investigate whether enhanced ion currents generated by *C. porcellus* δβγ-ENaCs compared to αβγ-ENaCs are due to differences in single channel conductance.
cRNAs coding for *C. porcellus* αβγ- and δβγ- ENaCs were diluted in RNAse-free water to a final concentration of 20 ng/μl per subunit. *Xenopus* ovary lobes were purchased from the European Xenopus Resource Centre (EXRC). Stage V/VI oocytes were isolated from at least 3 different *Xenopus* ovaries, injected with 32.2 nl of cRNA and were incubated at 16°C in a low-sodium culture oocyte Ringer's solution (Wichmann *et al.* 2019). All procedures involving *Xenopus* ovaries and oocytes were approved by the Animal Welfare and Ethical Review Body of Newcastle University (project ID No: ID 630). Cell-attached Patch-Clamp recordings were performed on mechanically devitellinised oocytes 2-7 days after cRNA injection, as described by Wichmann *et al.* (2018). Current signals (pA) were obtained under different holding potentials (V_M 0 to -100 mV). For each recording (n), single channel amplitudes of at least 3 events were measured and the slope conductance was determined by linear regression. Values presented are means ± standard error of mean (S.E.M) and were analysed for significance by Student's unpaired t-test.

There was no significant difference between the slope conductance (G_{slope}) of the two ENaC isoforms (t= 0.59, d.f.= 10, p= 0.571). Specifically, αβγ-ENaC had a G_{slope} of 4.27 ±1.42 pS (n=6) and δβγ-ENaC a G_{slope} of 4.05 ± 3.45 pS (n=6).
In conclusion, there is no difference in the single channel conductance between *C. porcellus* αβγ- and δβγ-ENaCs. This suggests that channel open probability or membrane abundance likely account for increased transmembrane currents generated by δβγ-ENaCs compared to αβγ-ENaC in *C. porcellus*.

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PC35

Calmodulin-dependent kinase II inhibition augments SCN5A transcription in human ventricular cardiomyocytes

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Acquired arrhythmogenesis is a pressing global health issue[1], often precipitated by disturbances to the inactivation kinetics of $Na_v1.5$, the SCN5A-encoded pore-forming α subunit of the cardiac Na^+ channel. CaMKII has long been known to induce such perturbations via post-translational phosphorylation[2]. Yet, despite the well-established secondary role of CaMKII in modifying cardiac gene expression[3], less is known about whether excitation-transcription coupling extends to SCN5A. Indeed, in light of recent substantiation for both the rise in ROS-dependent CaMKII autonomy and the fall in SCN5A expression (regulated, in part, by the transcription factor GATA4) in structural heart disease, we aimed to investigate the relationship between CaMKII activity and SCN5A transcription. We thus applied the CaMK inhibitor KN93[4] to cultured human iPSC-derived ventricular cardiomyocytes, using its inactive analogue, KN92, and the $Ca_v1.2$-coding gene, CACNA1c, as negative and positive controls, respectively. Subsequent isolation of total cellular RNA and qRT-PCR assay enabled estimation of relative gene transcription. Unpaired, two-tailed t-tests comparing the fold changes (μ ± SEM, n= 3) in expression for CACNA1c (1.41 ± 0.03, $p < 0.01$) and SCN5A (1.56 ± 0.07, $p < 0.05$) averaged over the three cultures indicated a statistically significant increase in transcription 24 hrs following addition of either 10 or 20 mM KN93. Yet, by contrast to CACNA1c and SCN5A, the fold increase in GATA4 transcription was insignificant (1.28 ± 0.11, $p > 0.05$), as were the changes in expression, over the final two experiments,

of DNA- and RNA-binding proteins known to influence SCN5A transcription (CAPN1: 2.03 ± 0.47, $p > 0.05$), pre-$mRNA_{SCN5A}$ splicing (RBM25: 1.43 ± 0.04, $p > 0.05$), and $mRNA_{SCN5A}$ stability (HuR: 2.04 ± 0.30, $p > 0.05$). Over a series of three experiments, therefore, KN93 administration increased the expression of SCN5A by a similar magnitude to the positive CACNA1c control, indicating that, under normal physiological conditions, overactive CaMKII may depress SCN5A transcription in structural heart disease. However, importantly, KN93 is an inhibitor of other members of the CaMK family, including, most potently, CaMKI and CaMKIV, which have differential effects on the activities of the same transcription factors. As such, further investigations using an inhibitor that is specific to CaMKII are necessary to definitively infer the role of CaMKII from the effects of drug administration. Moreover, the lack of significant increases in transcription of the tested DNA- and RNA-binding proteins does not necessarily rule them out as mediators of the CaMKII-SCN5A relationship; Western Blot experiments are necessary to elucidate the much greater probability of their post-translational regulation[5] by CaMKII-mediated phosphorylation.

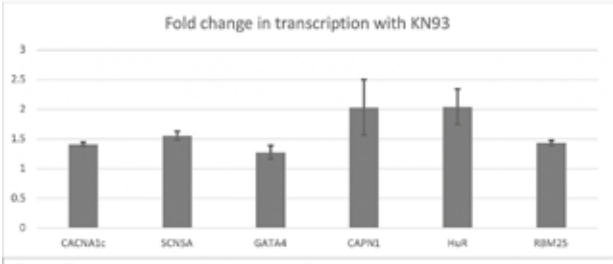

Figure 1. T-tests indicate that KN93 produces significant increases in the transcription of ion channel subunits CACNA1c and SCN5A, but not in that of the investigated DNA- and RNA-binding proteins.

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PC36

Developing a new web-based finger tapping test to measure distal bradykinesia in Parkinson's disease

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Background: Bradykinesia is the defining feature of Parkinson's disease (PD) and severity is typically assessed using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS). This rating scale is subject to inter- and intra-rater variability and does not provide information on the specific kinematics of motor impairment (1). As PD advances, patients frequently experience fluctuations in motor function as a consequence of treatment. Monitoring of motor complications remains reliant on history-taking, which is hindered by recall bias (2). In order to address these issues, more reliable and objective digital tests have been developed to quantify bradykinesia, including the BRadykinesia Akinesia INcoordination (BRAIN) test. The BRAIN test is an online tapping task which examines movement at the level of the elbow and shoulder, capturing proximal motor dysfunction (3) (see **Figure 1**). Here, a new Distal Bradykinesia Test (DBT) was developed to record motor impairment of the digits, aiming to provide a novel measure of distal bradykinesia (see **Figure 2**). Both tapping tests measure three parameters: kinesia score (KS) – the number of key taps over the duration of the test, akinesia time (AT) – the mean dwell time on each key in milliseconds and incoordination score (IS) – the variance of the time interval between key presses in milliseconds.
Methods: 10 PD patients with motor fluctuations were recruited from clinics at the Royal London Hospital. Home visits were carried out whereby patients performed the DBT in their 'on' and 'off' states. Paired t-tests were used to compare patients' 'on' and 'off' scores.
Results: The DBT differentiated between patients' 'on' and 'off' states using both KS ($p = 0.03$) and IS ($p = 0.04$). Whereas, the finger tapping sub-score of the gold standard MDS-UPDRS was unable to distinguish between states ($p = 0.14$). Furthermore, the KS parameter of the DBT showed a significant inverse correlation with total MDS-UPDRS part III (motor) scores in the 'off' state; a decreasing number of key taps was associated with an increase in UPDRS-III scores (Pearson's $r = -0.73$, $p = 0.04$). Similarly, the correlation between the percentage change of KS scores from 'off' state to 'on' state against the percentage change of UPDRS-III scores also showed a strong inverse correlation (Pearson's $r = -0.85$, $p = 0.01$).
Conclusion: Preliminary evidence was obtained to show that the new DBT may be a reliable method for differentiating between when patients are 'on' and 'off' medication and monitoring motor fluctuations. The DBT was able to detect subtle changes in motor function, which were not reflected in scores derived from the

MDS-UPDRS. The new DBT offers several practical advantages over current rating scales and has the potential to serve as a supplementary clinical tool in longitudinal monitoring of PD motor complications.

Figure 1: Illustration of the BRAIN test measuring proximal bradykinesia. Alternate tapping of the 's' and ';' keys with one index finger, as fast and as accurately as possible, for 30 seconds.

Figure 2: Illustration of the new DBT measuring distal bradykinesia. Occupying the left/ right arrow key and simultaneously, repeatedly tapping the down arrow key, for 20 seconds.

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PC37

Modulation of voltage-gated calcium channels via protofibrillar amyloid-β

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The amyloid-cascade hypothesis proposes that the main protagonist in Alzheimer's disease is oligomeric amyloid-β (Aβ). Protofibrillar $A\beta_{1-42}$ induces cytotoxicity through various signalling cascades, including calcium dysregulation via modulation of neuronal voltage-gated calcium channels (VGCCs). The aim of this work was to examine the role of protofibrillar $A\beta_{1-42}$ in modulating VGCCs present in SH-SY5Y cells. Within undifferentiated cells, Cav1.2, Cav1.3, Cav2.2 and Cav3.1 were detected at gene level (qPCR). Upon retinoic acid (10μM; 3 days) differentiation of SH-SY5Y cells, a reduction in Cav3.1 was observed. No gene expression of Cav1.1, Cav1.4, Cav2.1, Cav2.3, Cav3.2 and Cav3.3 was detected in SH-SY5Y of either differentiation states. Prior to experimental use, $A\beta_{1-42}$ aggregation status was verified via transmission electron microscopy. A time-dependent (0-48h) formation of $A\beta_{1-42}$ protofibrils (>100nm in length) was observed using NH_4OH pre-treatment. Protofibrillar $A\beta_{1-42}$ (24h) induced neurotoxicity (XTT assays) in undifferentiated SH-SY5Y cells, in a concentration-dependent manner. A significant reduction in viability was observed with 3μM (21.2±6.1%; n=11; P<0.05) and 10μM $A\beta_{1-42}$ (23.9±6.8%; n=8; P<0.01) treatment. In contrast, lower concentrations of 1nM (1.8±6.8%; n=8; P=1.0), 100nM (3.7±5.5%; n=16; P=0.9) and 1μM $A\beta_{1-42}$ (2.7±5.4%; n=17; P=1.0) did not induce cytotoxicity. In parallel, PCR gene expression analysis revealed that treatment with 100nM $A\beta_{1-42}$ induced a significant reduction in Cav1.3 expression (0.81±0.04; n=5; 24h; P<0.01) within differentiated cells. Additionally, following 1μM $A\beta_{1-42}$ treatment, an increase in Cav1.2 expression (1.8±0.15; n=3; 24h; P<0.05) was also seen. No significant change in expression was observed within undifferentiated SH-SY5Y cells treated with either 100nM or 1μM $A\beta_{1-42}$ treatment (24h). These data demonstrate differential $A\beta_{1-42}$ concentration-dependent modulation of VGCCs selectively within differentiated SH-SY5Y cells. Whole-cell patch clamp studies using Cav2.2 ($\alpha_{1B(CaV2.2)}/\beta_{1b}/\alpha 2\delta$) stably-transfected HEK293 cells revealed changes in the voltage-dependence of activation (slope factor k) (-7.4±3.1mV; n=15; P<0.05) with 100nM $A\beta_{1-42}$ treatment. Current amplitude and other biophysical properties (Gmax, Rp and Vhalf), as calculated by Boltzmann function fitting, did not significantly alter. Collectively, our work demonstrates that protofibrillar $A\beta_{1-42}$ can induce neurotoxicity, and modulate VGCCs through changes at both the molecular and functional level. Our work will aid the understanding of amyloid pathology, and highlight the potential to target VGCCs to suppress Aβ disease mediated pathology.

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PC38

Acetate Weakens the Vaginal Epithelial Mucosal Barrier in a Multilayer Vaginal Epithelial Cell Culture Model

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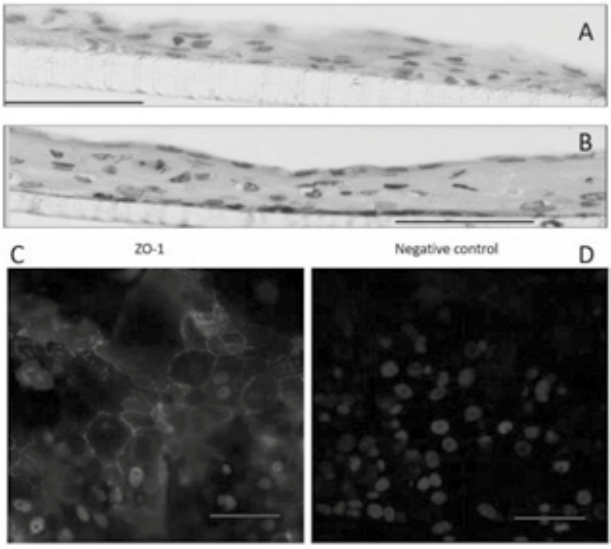
Preterm birth is the leading cause of death in children under the age of five, and spontaneous preterm birth (sPTB) is often associated with infection and inflammation. A shift in the vaginal microbiota from lactic acid producing bacteria to a rise in anaerobic bacteria is associated with an increased risk of sPTB. This shift changes the metabolite profile in the vaginal epithelium, leading to a loss of lactic acid and the production of short chain fatty acids (SCFAs) [1]. The mechanism by which this shift in metabolites affects the vaginal epithelium is not well understood. Consequently, the aims of this study were to characterise a physiologically relevant *in vitro* vaginal epithelial model to study the interactions between SCFAs and the vaginal epithelium and understand the effects that the vaginal microbiota has on the mucosal layer and the production of elafin, an antimicrobial peptide previously found to be reduced in women with Bacterial Vaginosis [2].

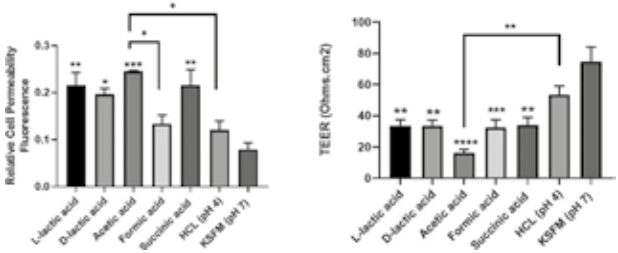
VK2 cells were grown on transwell inserts at an air-liquid interface and trans-epithelial electrical resistance (TEER) measurements were taken. Cells were fixed for histological and immunofluorescence staining prior to confocal imaging. Cells were treated with SCFAs and resulting supernatant was collected for ELISA. Paracellular permeability, measured as relative fluorescence, and TEER was measured. Values reported are means ± S.E.M, compared by ANOVA.

Histological staining confirmed the formation of multilayers and production of glycogen (n=3). TEER values increased from day 2 vs. day 10 from 12.76 ± 1.351 $\Omega.cm^2$ to 37.96 ± 4.015 $\Omega.cm^2$ ($p = 0.0043$, n=4). Additionally, the formation of tight junctions, represented by ZO-1 localisation on the cell membranes was observed (n=3). Acetate caused the most pronounced increase in cell permeability compared with other SCFAs, and increased cell permeability compared with Keratinocyte Serum Free Medium (KSFM) (0.0794 ± 0.1412 vs. 0.2453 ± 0.0017; $p = 0.0008$, n=3) and compared to HCl (0.1210 ± 0.0184 vs. 0.2453 ± 0.0017; $p = 0.0101$, n=3). Furthermore, TEER measurements were reduced upon treatment with acetate when compared to both KSFM (74.84 ± 9.257 $\Omega.cm^2$ vs. 16.32 ± 2.206 $\Omega.cm^2$; $p < 0.0001$, n=3) and HCl (53.46 ± 5.625 $\Omega.cm^2$ vs 16.32 ± 2.206 $\Omega.cm^2$; $p = 0.0029$, n=3). Moreover, acetate treatment decreased elafin production from 0.4487 ± 0.0688 μg/ml in the KSFM group to 0.3666 ± 0.0495 μg/ml (n=3).

In conclusion, we have characterised a multilayer vaginal epithelial model and shown that acetate causes an increase in cell permeability and reduction in cell layer integrity and elafin production. This work presents a potential mechanism of how a shift in the vaginal microbiota from lactic acid producing bacteria to anaerobic bacteria could affect the epithelial mucosal barrier and increase susceptibility to ascending intrauterine infection and sPTB.



Representative images (400 x) of 5 micron sections of day 10 cultures, showing formation of multilayers through H&E staining (A) and production of glycogen through PAS staining (B, n=3). (C) Representative confocal microscopy images of the tight junction protein zonula occludens 1 (ZO-1) fluorescently labelled with AlexaFluor 488 (green) and DAPI (blue) on day 10 (n=3) and (D) showing negative controls.



VK2 cells were treated with 0.3 % w/w of acid for 24 hours on day 10 in culture. (LEFT) cell permeability was assessed by measuring the relative transport of sodium fluorescein in the basolateral chamber 2 hours after treatment in apical chamber (n=3). (RIGHT) TEER measurements were taken upon completion of the permeability assay (n=3). All data expressed as means ± S.E.M. and asterisks represent comparison to control (KSFM).

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Tommy's Charity for funding study

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PC39

Renoprotective effect of Curcumin in L-NAME induced hypertensive rats

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Background: Hypertension may be induced by inhibition of nitric oxide synthesis with L-NAME which also has a role in oxidative stress. Curcumin has been shown strong antioxidant property. So, we aim to explore the preventive role of curcumin on renal dysfunction secondary to hypertension.
Material & Methods: Twenty-four adult male Albino rats divided in four groups: Normal (N) group; Curcumin (C) group; received curcumin (100 mg/kg/day) by oral gavage for 10 weeks. Hypertensive (H) group; received L-NAME (40 mg/kg/day) in their drinking water for 4 weeks. Hypertensive- curcumin (HC) group; received L-NAME and curcumin. Non-invasive arterial blood pressure was evaluated for 4 weeks consecutively then rats were sacrificed the end of experiment to evaluate the parameters of oxidative stress (catalase, lipid peroxidase, reduced glutathione and superoxide dismutase), renal function and structure and apoptotic markers (Bcl-2 and caspase-3). AT1R expression and renal mtDNA integrity were assessed.
Results: There was a significant increase in MABP in the hypertensive group in comparison to normal and curcumin groups all over the study duration. The curcumin treated group show a significant decrease in MABP beginning from the second week in comparison to the hypertensive group (Figure 1)
In L-NAME-treated (hypertensive) rats, plasma nitrate/nitrite concentrations were significantly reduced when compared to those of normal and curcumin groups. Treatment of L-NAME rats with curcumin significantly restored the plasma nitrate/nitrite levels.
Compared to the normal and curcumin groups, serum creatinine and BUN were significantly higher in the hypertensive group ($p < 0.05$), while the treated group (cur+hypertensive) was lower than the hypertensive group ($p < 0.05$).
Treatment with curcumin significantly improve both creatinine clearance and urinary albumin level.
The expression of Bcl2 in the hypertensive group was significantly decreased, while the expression of caspase-3 in the hypertensive group was significantly increased when compared to the normal, curcumin and treated groups. There

was significantly higher expression of the AT1R in the hypertensive group ($p < 0.05$), while the treated group (cur + hypertensive) showed lower expression of the receptor than the hypertensive group ($p < 0.05$).
In the normal and curcumin groups, the intact form of mtDNA was electrophoresed as a major band of approximately 16.5 kb (lanes N, C). On the other hand, hypertension markedly reduced the amounts of intact mtDNA in the renal tissue (lane H). However, mtDNA from treated group (lane HC) was electrophoresed in its intact form.
Conclusion: Curcumin improved the blood pressure elevation, renal dysfunction. These improvements mediated through anti-oxidant capabilities and downregulation of AT1R favoring reduced apoptosis and preserved mitochondrial DNA.

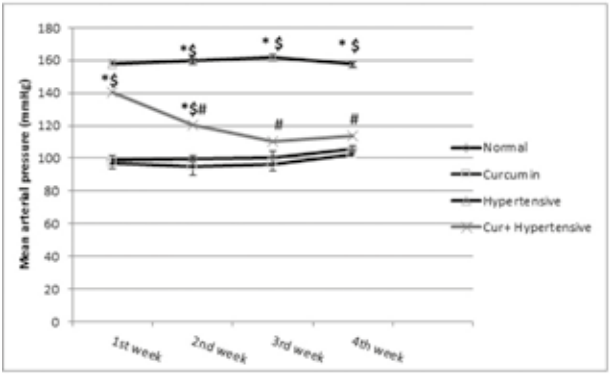

Figure (1): Mean Arterial blood pressure (MABP) in all studied groups. All data are expressed as mean± SEM and analyzed using one-way ANOVA. * Significant in comparison to normal group. $ Significant in comparison to Curcumin group. # Significant in comparison to Hypertensive group.

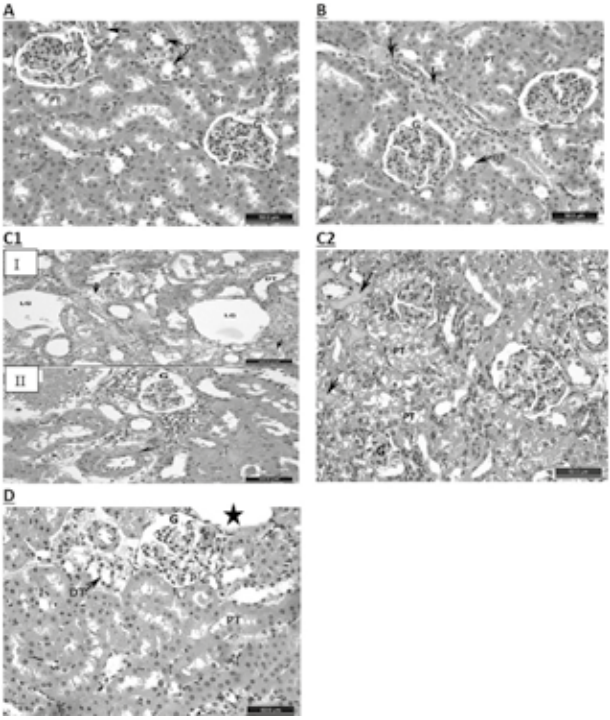

Figure (2): Photomicrographs of renal cortex of all study groups: (A) normal group, (B) curcumin group, (C) hypertensive group and (D) cur+ hypertensive group. A & B show renal corpuscles with normal glomerular (G) blood capillaries covered by nuclei of podocytes and mesangial cells and surrounded with urinary space. Proximal convoluted (PT) and the distal convoluted tubules (DT) show normal appearance

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PC40

The effects of adropin on viability of lipopolysaccharide-treated 3T3-L1 cells

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Over-accumulation of fat leads to obesity, one of the major public health problems. Obesity is a high-risk factor of obesity-associated disorders, e.g. insulin resistance, cardiovascular disease, dyslipidaemia. Obesity is accompanied by

low-grade inflammation (1, 2). Inflammation promotes synthesising and releasing proinflammatory adipokines, and cytokines from adipocytes, e.g. leptin, IL-6, TNFα (3). Inflammation may be induced during treatment preadipocytes with lipopolysaccharides (LPS).

Adropin, peptide encoded by Energy Homeostasis Associated gene, is involved in lipid metabolism and body weight gain. Adropin deficiency in mice leads to increased adiposity and dyslipidaemia (4). There is negative correlation between adropin plasma level and BMI in human (5). The role of adropin in adipose tissue inflammation was unknown.

The aim of the study was to evaluate the effects of adropin on proliferation, viability, and cell death of LPS-treated preadipocytes.

Murine 3T3-L1 cell line as a model of fat precursor cells treated with LPS (from bacteria E.coli) or adropin was used. Preadipocyte proliferation was studied using BrdU assay. Viability of preadipocytes was assessed by MTT assay. Cell death was determined by Cell Death ELISA kit. Statistical analysis was performed using ANOVA followed by the Bonferroni post hoc test. Data are shown as mean ± SEM.

Preadipocyte proliferation increased after adropin 100 nM and LPS 500 ng/ml treatment for 24h (0.16±0.02, 0.15±0.01 vs. 0.08±0.01 OD 450-690, $p<0.05$). Adropin failed to affect the effects observed after treatment with LPS.

LPS treatment (250, 500 ng/ml) increased viability of 3T3-L1 preadipocytes after 24h of incubation (0.84±0.02, 0.86±0.02 vs. 0.71±0.17 OD 570–650, $p<0.05$). Viability also increased after treatment with both adropin 100 nM and LPS 500 ng/ml (0.80±0.01, $p<0.05$). Moreover, viability of preadipocytes increased after 48h of incubation with 100 nM adropin, LPS 500 and 1000 ng/ml (0.69±0.02, 0.79±0.04, 0.70±0.02 vs. 0.57±0.02, $p<0.05$). Viability was also greater after incubation with both LPS 500 or 1000 ng/ml and adropin 100 nM (0.84±0.02, 0.74±0.03 vs. 0.57±0.02, $p<0.05$).

Cell death was decreased by 100 nM adropin (0.82±0.02 vs. 1.18±0.01), and LPS (100 and 500 ng/ml) (0.71±0.01, 0.66±0.06 vs. 1.18±0.01 OD 405–490, $p<0.05$) treatment after 24h of incubation. Similar effect was observed after incubation both with adropin 100 nM and LPS 100 or 500 ng/ml (0.85±0.04, 0.67±0.04 vs. 1.18±0.01, $p<0.05$). Adropin 100 nM (1.15±0.04) and LPS 500 ng/ml (1.08±0.06 vs. 1.41±0.07, $p<0.05$) were also effective after 48h.

Obtained data suggest that short-term induced inflammatory may enhance proliferation, and viability, and prevent preadipocyte cell death. Adropin failed to modify the effects observed after LPS treatment. Further studies are required to determine the role of adropin in adipose tissue inflammation.

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PC41

The role of physical activity on metabolic and cardiovascular health during pregnancy

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Obesity and the metabolic syndrome (MetS) are associated with an increased risk of cardiovascular disease (CVD). Data suggest that MetS may predict CVD risk better than body mass index (BMI). As well as increased CVD risk, obesity during pregnancy is associated with adverse outcomes for maternal and foetal health, including an increased risk of caesarean birth and gestational diabetes (Marchi et al., 2015). Furthermore, it remains unknown whether low levels of physical activity (PA), which are common during gestation, have an effect on these outcomes. This study aims to explore the role of PA on metabolic health during pregnancy and the associated impact on CVD risk factors. Pregnant women (n=100) will be recruited during the first or early second trimester and complete; a blood test, submaximal cardiorespiratory fitness, macrovascular function, anthropometric and habitual PA assessment. These physiological measures will be repeated at trimesters 2 and 3. The women will be phenotyped according to BMI (non-obese <30 vs obese ≥ 30 kg/m^2) and MetS status according to the International Diabetes Federation criteria. Specifically subgroups will consist of; (i) metabolically unhealthy obese, (ii) metabolically unhealthy normal weight, (iii) metabolically healthy obese, (iv) metabolically healthy normal weight, which highlight individuals most at risk of disease. Postpartum visits (at 0-4 weeks and 6 months) will consist of maternal qualitative and physiological and neonatal macrovascular structural assessments. Physiological and habitual PA variables will be compared between phenotypes. It is hypothesised that metabolically unhealthy women will confer greater CVD risk factors and display low levels of PA compared to metabolically healthy subgroups.

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PC42

Optical measurement of neural plasticity in fear-related neurons

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Fear is an emotional experience deeply ingrained in evolution to promote survival and enable avoidance of potentially harmful circumstances. However, fear may become maladaptive and give rise to anxiety and trauma-related disorders, such as post-traumatic stress disorder. Fear extinction is a plastic mechanism, which represents the decline in fear responses towards a stimulus that was previously eliciting fear. Extinction is essential for understanding fear-related disorders as it is the process which exposure therapy, the main therapeutic approach to combat post-traumatic stress disorder, is based on. Previously, it was shown that a specific population of neurons form basolateral amygdala, which express thymus cell antigen 1 (Thy1), encode fear extinction. However, the plasticity of these neurons has not been investigated yet. We are investigating the neural plasticity of extinction neurons by selectively recording from Thy1 neurons expressing a genetically encoded calcium indicator, GCaMP6. Ex vivo 2-photon calcium recordings were performed following paired-pulse stimulation in basolateral amygdala and hippocampal CA1 slices (n=5). Preliminary control experiments exhibited increased synaptic evoked activity in Thy1 neurons following administration of forskolin, an adenylyl cyclase activator, which was subsequently blocked by application of NBQX, an AMPA receptor antagonist. These experiments enabled visualization of plasticity at different sites including somatic, dendritic, as well as synaptic,

in Thy1-expressing neurons. A better understanding of how fear extinction is encoded and processed will ultimately lead to novel-biologically driven approaches for treatment and prevention of fear-related disorders.

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PC43

Investigation of lung pathogens growth patterns using Artificial Sputum Medium (ASM)

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High blood glucose has been linked with increased glucose concentration in the airway surface liquid. This has been shown to be associated with an increased risk of developing a pulmonary infection in patients in intensive care unit and in patients who have diabetes and chronic lung disease [1].

Contrary to popular belief that lungs are sterile, it was recently discovered that lungs are inhabited by resident bacteria. Several chronic lung conditions, such as cystic fibrosis (CF) and chronic obstructive pulmonary disease, have been shown to induce changes to the lung bacteria.

To investigate the effects of hyperglycaemia on the pathogens residing in the lungs of CF patients, we have used Artificial Sputum Medium (ASM) [2]. ASM was designed to imitate sputum found in CF patients. *Staphylococcus aureus* (ATCC29213) and *Pseudomonas aeruginosa* (PAO1) were used for experimental design as they are some of the most common CF pathogens.

S. aureus and *P. aeruginosa* were grown in the ASM at 0mM and 8mM glucose. Approximately $3x10^7$ bacteria were added to a 50ml tube. Bacteria were then grown at 37°C with constant shaking.

Growth curves were constructed by quantifying CFUs using serial dilutions. Interestingly when performing colony counts for the CFU calculations, it was impossible to count individual colonies of *P. aeruginosa*. When *P. aeruginosa* was grown in both ASM and Mueller-Hinton (MH) medium in parallel, colonies from MH medium formed as expected, but formed a single spread super-colony when plated from ASM. As colony counting was not possible, bioluminescent *P. aeruginosa* (H174) was used to construct the growth curve instead. With this strain, production of light was used as an indicator of viable, metabolically active cells.

In the presence of 8mM glucose, *S. aureus* demonstrated increased growth rate and increased peak bacterial population when compared to no glucose.

Glucose did not have the same effect on the growth of *P. aeruginosa* as it did on the *S. aureus*. In presence of glucose, *P. aeruginosa* grew slower than in the glucose-free environment. Despite the slower growth rate, the peak population and total bacterial population were higher in the presence of 8mM glucose.
When *P. aeruginosa was* placed into the ASM the population decreased by 90% within the first ten hours. Afterwards it increased by 100-fold from the original population. The decrease in growth was observed repeatedly with varying initial populations of *P. aeruginosa.*
In conclusion, ASM mimics the nutrients available in the lungs and allows the studyof lung pathogen growth patterns in the presence of glucose. We speculate that initially *P. aeruginosa* utilises mucin and amino acids from ASM for growth and begins to use glucose when other nutrient sources are depleted. The possibility of co-culturing of multiple pathogens could unveil complex growth interactions between bacterial species residing within the lungs.

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PC44

Effect of cold exposure on dietary nitrate metabolism and blood pressure following the acute ingestion of nitrate-rich beetroot juice

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Background: Dietary supplementation with inorganic nitrate (NO_3^-) has been reported to lower blood pressure (BP) in healthy adults. This effect has been attributed to an increase in circulating plasma nitrite (NO_2^-) and subsequent nitric oxide (NO) synthase-independent NO generation. Cold exposure increases vascular resistance and BP, effects which are partly mediated by an attenuation in NO synthase-derived NO. Since cold exposure also elevates salivary flow rate, which would be expected to increase NO_3^- secretion by the salivary glands for reduction to NO_2^- by oral bacteria, this study tested the hypothesis that acute dietary NO_3^- supplementation would increase salivary and plasma [NO_2^-] and lower BP to a greater extent in cold compared to normothermic conditions.
Methods: Twelve healthy males volunteered to participate in this study. Participants reported to the laboratory on four occasions for assessment of oral and mean skin temperature, salivary flow rate, salivary and plasma [NO_3^-] and [NO_2^-], BP and resting skin perfusion. These measurements were completed in an environmental chamber at baseline, with the temperature fixed at 28°C. Subsequently, participants ingested 140 mL of concentrated NO_3^--rich (BR; 8.4 mmol NO_3^-) or

NO_3^--depleted (PL) beetroot juice. Measurements were then repeated over 3 h at either 28°C (normothermia) or 20°C (cold). The four experimental trials, BR and PL ingestion in normothermic (BR-Norm and PL-Norm) and cold (BR-Cold and PL-Cold) conditions were administered in a double-blind, repeated-measures, cross-over experimental design. Data were analysed using ANOVAs and paired t-tests. Values are presented as means ± SD.
Results: Oral and mean skin temperature were lower throughout the cold conditions compared to the normothermic trials ($P<0.05$). Salivary flow rate was greatest in the BR-Cold and PL-Cold conditions ($P<0.05$). Salivary and plasma [NO_2^-] were greater post BR ingestion in the BR-Cold compared to the BR-Norm condition ($P\leq0.06$). Systolic BP was lower at 3 h in BR-Norm (113 ± 10 mmHg) compared to PL-Norm (117 ± 6 mmHg; $P<0.05$). Systolic BP increased above baseline in the cold conditions, with the mean increase being lower in BR-Cold (3 ± 6 mmHg) compared to PL-Cold (7 ± 7 mmHg; $P<0.05$). There was no difference in skin perfusion between BR-Cold and PL-Cold ($P>0.05$).
Conclusions: These results suggest that consumption of NO_3^--rich beetroot juice is more effective at increasing salivary and plasma [NO_2^-] and lowering systolic blood pressure in cold compared to normothermic conditions. Importantly, NO_3^--rich beetroot juice does not alter skin perfusion or compromise thermoregulation in the cold. These findings might have implications for attenuating the cardiovascular strain that accompanies cold exposure.

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PC45

Investigating the effects of two weeks of 5:2 intermittent energy restriction or continuous energy restriction on basal and postprandial metabolism in normal-weight, young participants

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Intermittent energy restriction (IER) works as an alternative for weight loss considering the difficulty in long-term adherence to continuous energy restriction (CER). To investigate the effects of 2-weeks of 5 days normal diet, 2 days restricted diet (5:2 IER) compared to CER on basal and postprandial metabolism, a two-week randomized parallel trial was conducted in 16 healthy normal-weight participants (aged 20-35). Participants were randomly assigned to either the CER of 20% restriction below estimated requirements 7 days/week or the 5:2 IER, with 70% restriction delivered for two non-consecutive days/week with no restriction on the other 5 days/week. Weight, anthropometry, resting energy expenditure, diabetes and cardiovascular disease risk markers, appetite regulation 3 hours after the test drink and energy intake in an *ad libitum* test meal were assessed pre- and post- intervention.

Weight loss was similar among participants in both groups, mean ± SEM body weight for 5:2 IER fell from 63.1 ± 4.9 to 60.9 ± 4.7 kg vs. 66.1 ± 4.0 to 63.9 ± 3.9 kg for CER ($p = 0.61$). Both groups experienced comparable reductions in waist and hip circumferences. There was a significant treatment-by-time interaction between 5:2 IER and CER diets for fasting blood glucose ($p < 0.05$), which were significantly lower after the 5:2 IER diet than after the CER diet ($p < 0.01$). The fasting blood glucose concentrations fell significantly after the 5:2 IER diet ($p < 0.01$), from 4.4 ± 0.1 to 4.1 ± 0.1 mmol/L, compared to no changes after the CER diet. There was a significant interaction between the 5:2 IER and CER diets for resting heart rate ($p < 0.05$), which fell with the 5:2 IER diet from 68 ± 2 to 62 ± 2 BPM, compared to no changes in the CER diet. There were significant interactions between diet patterns for fasting subjective hunger, satiety, fullness, desire to eat and prospective food consumption ratings ($p < 0.001$, $p < 0.01$, $p < 0.01$, $p < 0.05$ and $p < 0.001$, respectively). After the 5:2 IER diet, the fasting ratings of subjective hunger, desire to eat and prospective food consumption were observed to decrease, and subjective satiety and fullness were observed to increase. Opposite responses were found for all fasting ratings after the CER diet. The responses for hunger and satiety for the 1-h postprandial *ad libitum* test meal period showed significant interactions between the diets ($p < 0.05$ for both).

These findings suggest that 5:2 IER would be as effective as CER in decreasing body weight and may also lead to a similar or better improvement of metabolic disease risk markers, and to changes in subjective appetite and satiety ratings which may help to reduce the desire to eat. 5:2 IER may offer a suitable approach to weight reduction for individuals who find CER difficult to follow.

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PC46

EFFECT OF ORAL ADMINISTRATION OF LAURIC ACID ON BLOOD GLUCOSE AND SOME PHYSIOLOGICAL PARAMETERS IN HIGH FAT DIET/STREPTOZOTOCIN-INDUCED TYPE 2 DIABETIC MALE WISTAR RATS

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Diabetes mellitus is a term that describes a metabolic syndrome of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrates, fats and proteins metabolisms resulting from defects in insulin secretion, insulin action or both. The aim of the study was to evaluate the antidiabetic potential of lauric acid in high fat diet/streptozotocin-induced type 2 diabetic male wistar rats. Type 2 diabetes was induced by a high-fat diet (HFD) along with a low dose of

streptozotocin (STZ) (30 mg/kg). Thirty Five Male Wistar rats were used in the study of which twenty five of them were diabetic. They were divided into seven groups comprising five animals each. Animals in Group 1 (Normal control) received 1 ml/kg Distilled water and Group 2 (Diabetic control) received 0.5 ml of tween 80 while those in Groups 3 (Normoglycemic) received 125 mg/kg Lauric acid, group 4, 5, 6 and 7 were administered 125, 250, 500 and 100 mg/kg body weight of lauric acid and metformin respectively orally once daily for a period of three weeks. The results showed that lauric acid at all doses significantly ($P < 0.05$) decreased the fasting blood glucose level after three weeks of treatment. The serum levels of TC, TG and LDL-c in diabetic treated rats were significantly ($P < 0.05$) reduced as compared to the diabetic control (untreated) group. While the result on high-density lipoprotein cholesterol showed a significant increase ($P< 0.05$) as compared to the diabetic control (untreated) group. The atherogenic risk predictor indices (CRR, AC and AIP) were significantly ($P < 0.05$) decreased when compared with diabetic control (untreated) group. Serum malondialdehyde (MDA) levels were significantly ($P < 0.05$) reduced (1.32±0.04, 1.40±0.04 and 1.42 ± 0.06 nmol/l) respectively when compared with the diabetic control (untreated) group with a value of (2.25±0.10 nmol/l), while there was up-regulated activities of serum endogenous antioxidant enzymes: SOD (1.97±0.08, 2.02±0.16, 1.98±0.12 IU/L), CAT (44.5±0.64, 43.2±0.85, 43.7±0.85 IU/L) as compared with diabetic control (untreated) (1.35±0.02 and 34.0±0.91 IU/L) respectively. Subsequent histomorphological evaluation also showed necrosis and vacuolization of islet β-cells to be reasonably reduced in the diabetic treated rats. In conclusion, the data from this study suggest that lauric acid is a potential candidate for the development of an effective drug for the management of T2D.

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PC47

Feasibility and reliability of ultrasonography for the estimation of diaphragm power output in response to volitional and non-volitional perturbations in humans

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Introduction
Human diaphragm muscle exhibits similar force-velocity-power characteristics as limb locomotor muscles when assessed *in situ* and *in vitro*. *In vivo*, however, only force – in the form of transdiaphragmatic pressure (Pdi) – can be accurately quantified. Thus, unanswered questions remain regarding *in vivo* power output of the human diaphragm. Subcostal ultrasonography offers a direct measure of diaphragm displacement velocity that may be used for the estimation of *in vivo* power output. Our aim was to establish the feasibility and within-day reliability of diaphragm displacement velocity and estimated power output in response to volitional and non-volitional perturbations.
Methods
We studied diaphragm displacement velocity in 10 healthy adults (5 women; mean ± SD age 22 ± 2 y) in response to maximal inspiratory sniffs and unilateral magnetic stimulation of the right phrenic nerve. Phrenic nerve stimulation was performed as five single (1 Hz) and paired (10, 50 and 100 Hz) twitches, immediately followed by five sniffs. After a standardised rest of 20 min, the protocol was repeated. A low-frequency (1.5-4.0 MHz), phased array ultrasound probe was positioned subcostally on the right mid-clavicular line. Using anatomic M-mode (AM-mode), displacement velocity was calculated as diaphragm displacement divided by displacement time. Diaphragm power output was calculated as the product of Pdi swing and displacement velocity. Within-day reliability was assessed with intra-class correlation for absolute agreement (k = 3; 2-way mixed effects) with 95% confidence interval (CI).
Results
Diaphragm displacement velocity was measured successfully in 95% (range 83-100%) of ultrasound images. Displacement velocity ranged from 9.4 ± 4.5 (sniffs) to 21.9 ± 6.7 (100 Hz) cm s^{-1} (mean ± SD; with a strong correlation with rate of pressure development ($r = 0.985$, $p < 0.001$). The within-day reliability was moderate to excellent for all stimulation frequencies; ranging from 0.808 [95% CI 0.325-0.958, $p = 0.008$] for sniffs to 0.943 [0.746-0.988, $p < 0.001$] for 100 Hz paired twitch. Estimated diaphragm power output ranged from 130 ± 59 at 10 Hz to 916 ± 444 cmH_2O $cm^{-1}s^{-1}$ for sniffs, and demonstrated a within-day reliability ranging from 0.674 [0.069-0.943, $p = 0.002$] for 100 Hz paired twitch to 0.889 [0.475-0.980, $p = 0.002$] for sniffs.

Conclusion
Ultrasound-derived diaphragm displacement velocity is quantifiable in response to volitional and evoked diaphragm contractions, and demonstrates a moderate to excellent within-day reliability. The measure may be used in the estimation of *in vivo* diaphragm power output and furthermore offers a potential new insight into the *in vivo* force-velocity-power characteristics of the human diaphragm muscle.

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PC48

No benefit of periodised low carbohydrate training for increasing fibre type-specific mitochondrial content and capillarisation in elite triathletes

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Purpose: Training with low carbohydrate (CHO) availability is suggested to enhance the adaptation to exercise, but no studies have investigated this within an elite population. Therefore, this study aimed to determine whether periodic CHO restricted training in elite athletes would increase fibre type-specific mitochondrial content and capillarisation. Methods: In a previous study (Gejl et al. 2017), 19 male elite triathletes (VO_{2max} 65.0 $mL.kg^{-1}.min^{-1}$) took part in 4 weeks of training supplemented with either low CHO (three days per week) (LOW, n = 12) or energy-matched CHO enriched training (HIGH, n = 7). The CHO manipulation days consisted of a morning high intensity interval cycling session to deplete muscle glycogen followed by 7 h of recovery in which subjects consumed energy-matched diets containing either high (6 g CHO.kg bm^{-1}) or low CHO (1 g CHO.kg bm^{-1}). Subjects then completed a 2 h moderate-intensity cycle either with low or high CHO availability. Muscle biopsies obtained pre and post-training were used to determine fibre type-specific mitochondrial content (COXIV) and capillarisation using immunofluorescence microscopy. Statistical significance was set at 0.05 confidence level and parameters were assessed using two-way mixed design ANOVAs. Results. Training increased COXIV expression in both type I (LOW +22%, HIGH +20%) and type II (LOW +22%, HIGH +21%; $P < 0.001$), with no difference between groups. Training also increased capillary density (LOW +9%, HIGH +14%; $P = 0.001$) with no difference between groups. Capillary-fibre perimeter exchange ratio (CFPER) also significantly increased with training in both type I (LOW +14%, HIGH +14%) and type II (LOW +9%, HIGH +11%; $P = 0.006$) with no difference between groups. Conclusion. These results demonstrate for the first time that periodically training with low CHO availability has no additional benefits to fibre type-specific training adaptations in elite triathletes. Interestingly though, the

training intervention induced significant increases to both mitochondrial content and capillarisation even within highly-trained endurance athletes. Future studies should aim to determine whether exercising with low CHO availability can enhance the adaptive response to training and expand health-span in older less trained populations.

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

PC49

Ultrasonography for the assessment of diaphragm kinetics during reflexively driven hyperpnoea in humans

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The activity and movement of the human diaphragm muscle is coordinated for both ventilatory and postural tasks. Thus, dynamic exercise-induced hyperpnoea might elicit larger pressure and kinetic responses than static CO_2-induced hyperpnoea. Subcostal ultrasonography offers a direct measure of diaphragm kinetics in the form of craniocaudal displacement and displacement velocity. Our primary aim was to investigate the feasibility of diaphragm ultrasonography for the quantification of diaphragm kinetics during CO_2-induced hyperpnoea and ramp cycle exercise.

Methods: Ten healthy participants (5 women; mean ± SD age 22 ± 2 years) underwent modified Read CO_2 rebreathing method (3.1 ± 1.0 min) and maximal ramp exercise on a recumbent cycle ergometer (8.0 ± 2.1 min). Initially, the closed-circuit rebreathing bag contained 5% CO_2 and 95% O_2, and the test was terminated at $PETCO_2$ of 55 mmHg. A low-frequency (2.4 - 5.0 MHz), curve-linear ultrasound probe (Vivid 7, GE Health), positioned subcostally on the right mid-clavicular line, provided 15 s cine-loops twice each minute. These cine-loops were time-matched offline with breath-by-breath pressure and ventilatory responses. Diaphragm displacement was measured with anatomic M-mode, and displacement velocity was calculated by dividing inspiratory diaphragm displacement by inspiratory displacement time.

Results: Diaphragm displacement and displacement velocity were quantifiable in 94% (range 72-100%) and 97% (84-100%) of the ultrasound-derived cine-loops during exercise and CO_2 rebreathing, respectively. Feasibility was independent of test mode, minute ventilation (V_E) and tidal volume. V_E increased similarly between

the two tests (p = 0.920). When matched for similar V_E, Pdi was significantly higher during exercise than CO_2 rebreathing (p = 0.023); increasing from 12.0 ± 1.5 to 18.9 ± 3.0 cmH_2O (exercise) and from 10.0 ± 2.6 cmH_2O to 15.8 ± 2.6 cmH_2O (CO_2 rebreathing). Although diaphragm displacement was similar for the two tests at rest (18.6 ± 7.6 mm; p = 0.327), the diaphragm showed significantly larger (p = 0.015) displacement during CO_2 rebreathing (34.10 ± 1.07 mm) than during exercise (27.87 ± 2.90 mm). During both tests, displacement velocity increased as a function of inspiratory flow (CO_2 rebreathing: r = 0.959; exercise: r = 0.982), and the displacement velocity did not differ significantly between the test modes (p = 0.371).
Conclusion: Kinetic responses of the human diaphragm can be quantified during reflexively driven hyperpnoea. When matched for similar V_E, the diaphragm muscle generates higher pressures during exercise, but moves significantly less. Diaphragm displacement may be constrained during exercise due to the quasi-isometric contraction required for simultaneous postural support and meeting ventilatory demands.

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

PC50

The Impact of a Fluid Restriction and Exercise-Induced Dehydration Intervention on the Power to Mass Ratio of Hill Climb Cyclists

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Introduction: Exercise-induced dehydration (EID) is associated with impairments in aerobic and anaerobic exercise performance primarily due to disturbance of the physiological function of the cardiovascular and central nervous systems and metabolic pathways. Regardless, dehydration is often used to manipulate mass in sports with strict mass categories to be adhered to, such as boxing. Although the impact of dehydration has been assessed in endurance exercise, little is known about the potential for performance enhancement over short durations, particularly when body mass is a key determinant of performance. One such example is UK hill climb cycling where races are ~60-300s in duration. *Aims and Hypothesis:* This study aimed to reduce body mass via a fluid restriction (FR) and EID intervention and monitor the effects on the power output normalised to body mass during short duration, maximal exercise such as experienced by hill climb cyclists. It was hypothesised that despite dehydration causing a decrease in raw power output (W), the drop in body mass would be sufficient to result in a significantly increased normalised power (W/kg). *Methods:* 10 well-trained cyclists (Mean±SD: 36±16 years, 77±13kg, 179±10cm and VO_{2max} 44±3ml/kg/min)

performed a short, 180s maximal cycling exercise test (SMET) on two separate days in either a euhydrated (EUH) or dehydrated (DEH) condition. Dehydration was induced via overnight FR and EID wearing an insulated bodysuit. The order of testing was randomised. Power output (W) was recorded during the SMET and normalised to body mass (W/kg) and reported at 5, 30, 60, 90, 120, 150 and 180s. Paired samples t-tests were used to compare conditions. *Results:* Mean body mass decreased by 2.02±1.28% following dehydration. Although raw power fell at all time points except 5 and 30s, the reductions in body mass resulted in small but significant increases in normalised power at 5 and 30s, no change at 60, 90, 120 or 150s and a significant reduction in normalised power at 180s (Table 1). *Discussion:* Despite dehydration reducing raw power output overall, the fall in body mass resulted in no significant detriment to normalised power up to 150s of exercise. Beyond this timepoint, the physiological effects of dehydration overwhelmed the ergogenic effects of decreased body mass and led to a drop in normalised power. In conclusion dehydration may be a viable performance enhancement strategy for short events less than 150s in duration when normalised power is a key determinant of performance.

Table 1

	5s Power (W/kg)	30s Power (W/kg)	60s Power (W/kg)	90s Power (W/kg)	120s Power (W/kg)	150s Power (W/kg)	180s Power (W/kg)
Euhydrated	7.48 ± 2.28	6.28 ± 1.21	5.52 ± 0.92	4.90 ± 0.86	4.53 ± 0.81	4.29 ± 0.79	4.14 ± 0.80*
Dehydrated	7.91 ± 2.41*	6.55 ± 1.47*	5.59 ± 1.03	4.91 ± 0.91	4.49 ± 0.82	4.23 ± 0.77	4.07 ± 0.77

Power output (W/kg) over various timepoints in a euhydrated and dehydrated condition expressed as Mean±SD.

* indicates a significantly higher ($p<0.05$) value.

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PC51

Lack of habituation of cardiovascular responses to mental stress in Black men and women: women are more predisposed to stress induced hypertension

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INTRODUCTION.
There has been evidence of higher rates of hypertension in US black women compared with men and stress was implicated (1,2). Exaggerated pressor responses to environmental stressors and endothelial dysfunction have been implicated in hypertension (3,4). Habituation of forearm vasodilation but not pressor response to mental stress predict hypertension (5). We tested whether or not BA men and women differ in responses to mental stress.

METHODS. Study 1: In 16 men and 12 women BAs (18-26 years), we recorded mean arterial pressure (MABP) and heart rate (HR) by Finapres and forearm blood flow (FBF) by venous occlusion plethysmography following arterial occlusion for 2min (reactive hyperaemia) and 5 sound stress stimuli (S1-S5; 100dB, 2KHz, for 30s each at 5-10min intervals).
Study 2: In 7 men and 7 women responses to 5 sound stress stimuli repeated on 3 alternate days.
In both studies, digital and forearm cutaneous blood flow (DCRCF,FCRCF) recorded by Laser fluximetry and vascular conductances were calculated as blood flow/ MABP. Changes from baseline were used for analysis.
RESULTS.
Study1: Resting systolic, but not diastolic pressure was higher in men than women (114.7±3.5 vs 102.1±2.1*mmHg, *: $p<0.05$ and 68.0±1.5 vs 66.1±1.9mmHg respectively). Reactive hyperaemia was similar : peak change in FVC: +0.37±0.03 vs +0.35±0.04 conductance units(CU). Further, before S1, MAP was higher in men than women (89.7±3.3 vs 77.8±4.4*mmHg). In men, S1-S5 had little effect on MAP: -2.36±2.0 vs +1.2±2.1mmHg in S1 and S5 respectively, whereas in women, MAP progressively increased from +6.0±1.5* in S1 to +13.4±3.4 §mmHg in S5 (§: S1 vs S5: $p<0.05$). Further, S1-S5 evoked forearm vasodilatation in men but vasoconstriction in women (Figure1).
Study 2: Resting systolic was higher in men than women (115.7±3.3 vs 100.6±4.5*mmHg, * $p=0.02$). Before S1, MAP was similar, $p>0.05$. On the first day, sound elicited net vasodilation in men, whereas women showed net vasoconstriction with augmented pressor responses. On day 2, forearm vasoconstriction occurred in both men and women, with sustained pressor responses.
On day 3, forearm vasodilation occurred in men but vasoconstriction in women with sensitization of the pressor responses (Figure2).
CONCLUSION. One session of repeated mental stress failed to elicit habituation in men and women. BA women showed exaggerated pressor responses. In response to repetition over 3 days, BA men showed habituation of forearm vasodilation on day 2 followed by recovery, but the pressor responses did not habituate. On the other hand, BA women lacked forearm vasodilation suggesting stress induced endothelial dysfunction and sensitization of pressor responses. These changes provide insight into the mechanism of increased stress related hypertension in BA women.

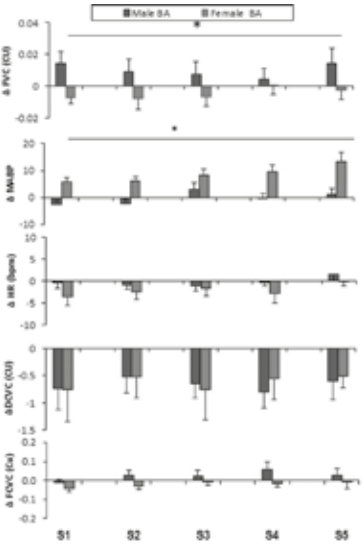

Figure 1: Mean changes from baseline values of FVC, MAP, HR DCVC and FCVC in one session of sound repetition in BA men and women. Values are mean ± SEM. * p<0.05, Men vs Women

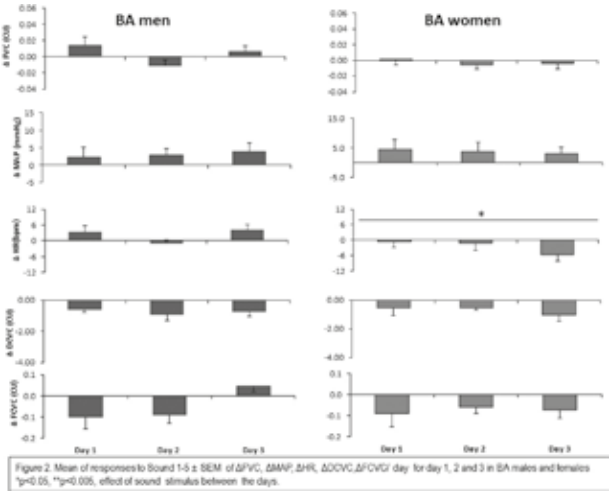

Figure 2. Mean of responses to Bound 1-5 ± SEM of ΔFVC, ΔMAP, ΔHR, ΔDCVC,ΔFCVC/ day for day 1, 2 and 3 in BA males and females *p<0.05, **p<0.005, effect of sound stimulus between the days.

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Racial disparities in hypertension prevalence, awareness, and management

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University of Ibdan, Tertiary Education Trust fund (TETFUND), Study particpants

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

PC52

Copper toxicity increases erythrocyte energy metabolism and glutathione in male Wistar rats

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Copper is a micronutrient vital to several cellular energy metabolic processes and drives erythropoiesis. However, it affects cellular biological activities and causes oxidative damage when in excess. This study investigated the effects of copper toxicity on erythrocyte energy metabolism in male Wistar rats. Ten Wistar rats (150-170g) were randomly divided into 2 groups: control (given 0.1ml distilled water) and copper toxic (given 100mg/kg copper sulphate). Rats were orally treated for 30 days. Blood, collected retro-orbitally after sodium thiopentone anaesthesia (50mg/kg i.p.) into fluoride oxalate and EDTA bottles, was subjected to blood lactate assay and extraction of red blood cell respectively. Red blood cell nitric oxide (RBC NO), glutathione (RBC GSH), adenosine triphosphate (RBC ATP) levels, RBC hexokinase, glucose-6-phosphate (RBC G6P), glucose-6-phosphate dehydrogenase (RBC G6PDH), and lactate dehydrogenase (RBC LDH) activity was estimated spectrophotometrically. Values (Mean±SEM, n=5) were compared using Student's unpaired T-test at $p<0.05$. Copper toxicity significantly increased RBC hexokinase (23.41±2.80μM), G6P (0.48±0.03μM), G6PDH (71.03±4.76nmol/min/ml) activities, ATP (624.70±57.36μmol/gHb) and GSH (3.08±0.37μM) level compared to control (15.28±1.37μM, 0.35±0.02μM, 54.41±3.01nmol/min/ml, 330.30±49.58 μmol/gHb, and 2.05±0.14μM respectively, $p<0.05$). Also, RBC LDH activity (145.00±19.88mU/ml), NO (3.45±0.25μM) and blood lactate (31.64±0.91mg/dl) level were lowered significantly compared to control (467.90±94.23mU/ml, 4.48±0.18μM and 36.12±1.06 mg/dl respectively, $p<0.05$). This study shows that copper toxicity increases erythrocyte glycolytic rate and

glutathione production. This increase could be connected to a compensatory mechanism for cellular hypoxia and increased free radical generation.

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PC53

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 of 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 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 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 compared to 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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PC54

Peripheral Capillary Oxygen Saturation, Mean Arterial Pressure and Pulse Rate Assessment in Young Shisha Smokers at Kano State, Nigeria.

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Emerging evidences have shown that shisha (water pipe tobacco) smoking is associated with higher exposure to carbon monoxide and nicotine than cigarette.[1] Despite these evidences, the prevalence and popularity of shisha smoking are rapidly increasing among youth globally including developing countries like Nigeria.[1,2] Basic assessment of peripheral capillary oxygen saturation (SPO2), mean arterial pressure (MAP) and pulse rate in young chronic smokers is therefore necessary. The study was conducted at Nassarawa local government area of Kano state, Nigeria. 103 young (15 – 30 years) shisha smokers who have been smoking for 3 years or more with at least 1 session of smoking per day and with BMI of 19 – 24 kg/m2 were recruited and classified as group 1 (study group). Likewise, 100 young nonsmokers matched for age, BMI and living in the same environment with group 1 members were recruited as controls (group 2). Participants with known history of cigarette smoking, cardiovascular and respiratory diseases were excluded. Ethical clearance and approval with reference number MOH/Off/797/T.I/527 was obtained from Kano state Ministry of Health prior to the commencement of the study. SPO2 and pulse rate of the participants were assessed at rest and values were taken from middle finger using pulse oximeter. Blood pressure measurement was done at the same time using sphygmomanometer and MAP was estimated as MAP = Diastolic pressure + 1/3 pulse pressure. Values were expressed as Mean ± S.E.M and Data were analyzed with IBM SPSS Statistics for Windows version 23.0. Results were compared between the groups using independent sample t test. P values < 0.05 were considered statistically significant. SPO2 was found to be significantly lower ($P < 0.05$) among group 1 members (91.98 ± 0.42 %) compared to group 2

(97.94 ± 0.18%). The MAP and pulse rate obtained from group 1 (100.15 ± 0.78 mmHg and 91.32 ± 0.83b/min) were significantly higher ($P < 0.05$) compared to group 2 with MAP and pulse rate of (92.44 ± 0.79 mmHg and 79.19 ± 1.18b/min respectively). The study implies that shisha smoking is associated with risk of hypoxia, hypertension and tachycardia by causing decrease in SPO2, increase in MAP and increase in pulse rate.

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PC55

The BTBR mouse model of ASD displays altered motility and nerve sensitivity in the gut.

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterised by deficits in social communication and repetitive behaviour. The BTBR T+tf/J strain is a well-characterised mouse model of ASD which has genetic alterations also found in human ASD. Previous studies have shown that ASD may be associated with functional bowel disease and a reduction in myenteric neurons [1,2]. The aim of this study was to investigate the function of the enteric nervous system (ENS) and the extrinsic afferent innervation of the gut in the BTBR model of ASD.

Peristaltic motor complexes were measured using an isovolumetric Trendelenburg organ bath in which intraluminal pressure was recorded at rest. To assess the extrinsic innervation of the gut, afferent nerve firing from isolated segments of the jejunum were measured using electrophysiological multi-unit recording of mesenteric nerve bundles in response to mechanical and chemical stimuli. Afferent responses to application of the TRPV1 agonist capsaicin (1μM), or an inflammatory soup composed of; bradykinin, serotonin, histamine and prostaglandin E_2 (5μM final concentration) was recorded. Data are presented as mean ± SEM and significance was confirmed with t-test or two-way ANOVA as appropriate.

Tissues from C57 control mice exhibited a regular and consistent pattern of peristaltic motility with a mean contraction amplitude of 49.2 ± 4.356 mmHg and an average contraction duration of 44.5 ± 4.581 seconds (n = 6). Conversely, tissues from BTBR mice (n=6) had disordered motility with irregular, inconsistent

contractions. Both peak contraction amplitude and contraction duration were significantly decreased (P<0.05, independent t-test).
Ramp distension of the jejunum evoked a graded increase in afferent nerve firing due to the activation of mechanosensitive nerves. However, the magnitude of the afferent response was significantly reduced in preparations from BTBR mice (P<0.005, 2 way ANOVA). Peak afferent nerve firing at 50 mmHg was 92.68±12.80 imp/s^{-1} in recordings from C57 mice (n = 10) and 76.49 ±15.44 imp/s^{-1} in tissues from the BTBR mice (n = 9). Moreover, BTBR afferents showed a significantly prolonged response to capsaicin compared to C57 afferents. An increase in the time taken to for the fibres to desensitize was also observed (P=<0.0001, BTBR n = 5; C57 n = 5). Preparations from BTBR mice (n = 5) also exhibited an increase in the afferent response to the inflammatory soup (P<0.05).
These results suggest that the BTBR model of ASD exhibits significant deficits in gut motility and alterations in jejunal extrinsic afferent nerve firing in response to mechanical and chemical stimuli. These data may suggest that both extrinsic and intrinsic innervation of the GI tract is altered in the BTBR mouse model of ASD. Further studies are now warranted to investigate the mechanisms by which ASD effects visceral sensitivity.

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PC56

Cerebral Blood Flow Velocity during Aortic Arch Repair in Neonates

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Introduction: Neonates requiring aortic arch repair are unable to maintain adequate oxygenation levels and require surgical intervention. A high percentage of survivors exhibit signs of neurological deficit possibly due to inadequate cerebral blood flow during surgery. Middle cerebral artery velocity (MCAv) using transcranial Doppler (TCD) provides information about perfusion. Our aim was to continuously monitor MCAv during aortic arch repair.
Methods: MCAv was monitored in five neonates (age 19±6 days, body mass 3.6±0.6 kg) undergoing surgery on the aortic arch, alongside near infrared spectroscopy (NIRS), blood pH, pO^2, pCO^2, HCO^3, lactate, Hb, Htc (%) and temperature (core and rectal).

Using general linear models, MCAv was compared between initial sedation, cardiopulmonary bypass (CBP), cooling at 30, 25, 20, the lowest temperature, during selective cerebral perfusion, whole body perfusion, during rewarming at temperatures of 25, 30, 36, once off cardiopulmonary bypass and after surgery. MCAv was obtained for 'healthy' neonates (aged 2±1 days, body mass 3.5±0.6 kg) and used as a reference point.
Results: During and following surgery MCAv was lower when compared to healthy neonates expect for during cooling. MCAv and NIRS changed during surgery (p=0.06). MCAv was 9.65, 14.9 and 13.1cm.$^{s-1}$ higher during cooling when at 30, 25 and lowest temperature, respectively when compared to CBP (p=0.03). Once off CBP, MCAv returned to pre surgery values.
Conclusion: Our data suggests that MCAv as a marker of cerebral perfusion is lower during cardiopulmonary bypass and higher during cooling. These changes could provide clinicians with information on how to optimise cerebrovascular health.

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PC57

Primary human myogenic cells obtained from old hip fracture patients display evidence of an elevated senescent phenotype compared to young donors.

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Ageing is associated with a decline in skeletal muscle mass and function, partially attributed to an impaired ability of muscle to repair and regenerate following mechanical stress. This decline has been associated, in part, with decreased muscle stem cell function (Sousa-Victor *et al.*, 2015). A potential mechanism underpinning this is cellular senescence, by which cells undergo proliferative arrest and release senescence-associated secretory phenotype (SASP) factors (Campisi, 2013). However, it is unclear whether this mechanism is a prominent issue affecting skeletal muscle (myogenic) stem cells. Thereby, the present study aimed to compare markers of cellular senescence in human primary myogenic cell populations that

had been obtained from older hip fracture (HF) patients (n=6, 70+ yrs) with those obtained from young (YO) healthy male donors (n=6, 22±1 yrs) at early passage in cell culture. Samples were obtained from the vastus lateralis either during surgery (HF) or using the Bergström needle technique with applied suction following local anesthesia (2% lidocaine, YO). Samples were purified using $CD56^{+ve}$ magnetic activated cell sorting (Agley *et al.,* 2015). Due to slow HF cell expansion rate, an average of Day 30 in culture had to be used to compare YO and HF data. All samples were examined for myogenic purity (% Desmin), senescence associated-β-galactosidase (SA-β-gal), p16, and γH2AX protein expression using immunocytochemistry. mRNA expression of a small panel of known SASP factors were investigated using qRT-PCR. Unpaired T-tests tested for statistical significance between HF and YO samples ($p<0.05$). Desmin-positive cells from HF patients displayed elevated SA-β-gal expression compared to YO (n=3, 81 ± 2.6% vs. n=5, 28 ± 2.3% positive cells, $p<0.001$), and increased γH2AX expression (integrated density n=6, 95380 ± 11148 vs. n=6, 55709 ± 8543, $p<0.05$). SASP factor mRNA expression are given as fold change compared to YO, early passage (Day 10 in culture) populations. HF cells displayed increased fold change expression of several SASP Factors compared to YO: CXCL5 (n=3, 13.77 ± 15.31 vs. n=6, 1.62 ± 1.53, $p<0.05$), IL-8 (n=3, 20.38 ± 18.19 vs n=6, 1.39 ± 1.41, $p<0.05$), and IGFBP-3 (n=3, 10.81 ± 6.29 vs. n=6, 1.75 ± 2.15, $p<0.01$). Despite senescence markers having to be investigated at relatively late passage in YO to align with the slowly expanding HF cell populations, significant differences were observed between the young and older populations when exposed to similar times in culture. However, as these data were obtained from older HF patients, as opposed to healthy older individuals, it is not possible to conclude if the increases seen in senescent marker expression can solely be attributed to an inherent ageing process.

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In this project it should be noted that there was an equal contribution from first and second authors Sophie Mathewson and Thomas Francis.

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PC58

A high-PRotein Mediterranean diet and resistance Exercise for cardiac rehabilitation (PRiME): a feasibility study and pilot randomised controlled trial.

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Cardiac rehabilitation (CR) patients present characteristics that exacerbate their cardiometabolic (CM) risk, such as higher frailty and anabolic resistance (1). There also seems to be a link between higher mortality and low body mass index (BMI) in this population, known as the "obesity paradox" (2), which may be primarily due to low lean body mass (LBM), and in some cases sarcopenia, a progressive loss of LBM associated with aging (3). Patients presenting simultaneously low LBM and abdominal obesity (sarcopenic obesity, SO) have increased CM risk. Current CR practice is primarily focused on aerobic exercise, combined with dietary and lifestyle advice to improve cardiovascular fitness. We hypothesize that increasing relative LBM is an appropriate target in CR patients, achieved through a combination of resistance training and higher protein intake in order to overcome anabolic resistance (5). In addition, Mediterranean-style dietary patterns are effective for the primary and secondary prevention of cardiovascular disease (4). Therefore, we propose a pilot study to investigate the prevalence of SO in CR patients, and to assess the feasibility and efficacy of an intervention based on a high-protein, Mediterranean-style diet combined with resistance training to improve LBM and reduce CM risk. Firstly, a cross-sectional study will analyse body composition and estimate the prevalence of SO in CR patients. Secondly, a pilot randomised controlled trial (12 weeks) will be performed with CR-SO patients assigned to four groups: 1) control group receiving standard CR, 2) resistance training group, 3) high-protein diet group, and 4) combined resistance training and diet group. Primary outcomes will be determinants of the feasibility of the protocol for a larger, fully powered study. These will include standard deviations of secondary outcome measures (selected biomarkers of CM risk), willingness of participants to be randomised, number of eligible participants, follow-up rates, acceptability of dietary and training protocols, adherence and compliance rates, time and finances needed to implement the intervention. Secondary outcomes will be changes in LBM and body fat assessed by dual-X-ray densitometry (DXA) and bio-impedance, muscle strength indicated by hand grip, and changes in CM risk markers (*e.g.*, blood lipids, cholesterol subfractions, fasting glucose and insulin, and HbA1c). The results of this study have the potential to inform current CR practice

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

PC59

Can handgrip exercise protect the vessels from ischaemic injury?

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The cardioprotective benefits of exercise are partly explained via long term improvements in cardiovascular risk factors and/or physiological remodelling of (coronary) arteries. In animal models a single bout of running can offer immediate cardioprotection (Hoshida *et al., 2002*). Ischaemic preconditioning (IPC) using a blood pressure cuff to cause a cyclical bout of non-lethal ischemia also offers cardioprotection against ischaemic injury which is evident when IPC is performed locally (at site of injury) or remotely (site distant to injury e.g. forearm) (Kharbanda *et al.,* 2002). Our aim was to compare handgrip exercise and forearm IPC on the ability to attenuate local and remote endothelial ischaemia reperfusion (IR)-injury, a surrogate for coronary artery IR-injury in humans.

Following ethical approval, six healthy males (age 24±4 years; BMI 24.6±1.73) attended the laboratory on 3 occasions. During each visit bilateral brachial artery flow-mediated dilation [(FMD) Thijssen *et al*., 2019) was examined at rest, immediately following an intervention and following 15-minute upper-arm occlusion (220 mm Hg) with 15-minute reperfusion to induce a temporary endothelial IR-injury. Prior to the IR-injury, participants performed either 4x5 minutes 50% maximal voluntary contraction unilateral handgrip exercise (which mimicked the blood flow pattern evident during 4x5 minutes of IPC in pilot data collection), were administered unilateral IPC (220 mm Hg, 4x5 minutes) or rested in the supine

position (control) in a randomised and counterbalanced order. Data were analysed using repeated measures general linear models and reported as change from rest with 95% confidence intervals.
The change in FMD from rest to immediately following the intervention in the local arm was similar with handgrip exercise, IPC and control, 3.05% (1.05, 5.05), 1.80% (0.78, 2.81) and 0.75 % (-1.47, 2.97) respectively (P = 0.19). A similar change was evident in the remote arm (P = 0.60). Both handgrip exercise (1.02% [-0.26, 2.29]) and IPC (0.43% [-3.36, 4.22]) displayed an attenuation in the decline in FMD following an IR-injury compared to control (-0.89% [-3.26, 1.48] in the local arm. In the remote arm, handgrip exercise, IPC and control were -2.2% (-5.4, 1.0), -1.4 (-3.87, 1.07) and -2.3 (-5.8, 1.01%), respectively following an IR-injury.
Both handgrip exercise and IPC can attenuate brachial artery endothelial IR injury. If these benefits of handgrip exercise translate to remote areas, like with IPC, this may have clinical benefit when implementing prior to planned ischemia reperfusion injuries (e.g. cardiac surgery) or in individuals who are at increased risk of an myocardial infarction.

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

PC60

The relationship between training variables and sleep quality amongst mixed martial arts competitors

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Sleep quality appears to be an important variable for recovery and training adaptation of competitive athletes, with reduced sleep quality having negative effects on performance and wellness. Overtraining (OT) caused by an imbalance between training load and recovery has been suggested to reduce sleep quality(1). Little is known about the relationship between training and sleep quality amongst mixed martial arts (MMA) competitors. As a multi-discipline combat sport, training

volume and intensity is thought to be high in this population, though this has not been adequately quantified in the literature. The aim of this study was to measure the training load of MMA participants and assess the affects of these loads on sleep quality. n = 6 human MMA participants (age = 20.5±3.6; mass = 71.3±4.4 kg; stature = 169.8±8.9 cm) took part in this study with institutional ethical approval for 8 consecutive weeks. Participant's daily mean training load (sessional rating of perceived exertion, strain and monotony) was recorded after every training session(2). At the end of each day participants recorded soreness using a 10cm visual analogue 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(3). Sleep quality was recorded via Pittsburgh Sleep Quality Index (PSQI) at the start of each week(4). Relationships between variables were assessed using Bayesian Kendall's Tau coefficient (BF_{10}). To determine which model of variables most likely affect PSQI, Bayesian multiple regression (BF_{10}) was performed. Moderate or better correlations with moderate or better BF_{10} are reported(5). All analyses were completed in JASP 0.10.2. The following variables were found to be moderately correlated: daily mean load-lower torso (T = .304, BF_{10} = 34); strain-lower torso (T = .305, BF_{10} = 36); strain-legs (T = .316, BF_{10} = 50); fatigue score-upper torso (T = .331, BF_{10} = 81); fatigue score-PSQI (T = .315, BF_{10} = 49); lower torso-PSQI (T = .326, BF_{10} = 69). Bayesian multiple regression found the strongest model to predict PSQI to be strain + fatigue score (adjusted R^2 = .471, BF_{10} = 67), which provided the following predictive equation: predicted PSQI = 1.532 + ($8.279e^{-4}$*strain) + (0.131*fatigue score). Increased strain caused by an unbalanced training load in MMA is related to increased soreness which leads to increased feelings of fatigue. Soreness appears to mainly affect the lower torso and legs. These issues appear to combine to reduce sleep quality amongst this population. This in turn may negatively affect the recovery of MMA competitors and contribute to the potential onset of OT. These findings may be used to assist coaches plan appropriate recovery after high load sessions to prevent OT and optimise training adaptation.

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PC61

The protective effects of GLP-1 and liraglutide on insulin resistant 3D kidney spheroids.

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Increased plasma levels of non-esterified fatty acids (NEFAs) through their over-consumption in the diet, link obesity with the onset of type 2 diabetes mellitus (T2D). Increased NEFA concentration is implicated in the development of insulin resistance (IR) in insulin sensitive tissues. Patients with T2D have shown impaired cardiac remodeling following an ischemic event, where hypoxia-inducible factor1 alpha (HIF-1α) activation is diminished due to the presence of increased long chain fatty acids. Glucagon-like peptide-1 (GLP-1) receptor agonist; GLP-1 (7-36) and incretin mimetic; liraglutide, have been implicated in their ability to reverse or diminish the effect of IR in multiple organ types by increasing cellular viability. We aim to induce IR in human embryonic kidney cells (HEK293T) using the three most abundant NEFAs present in T2D patients; oleate, stearate and palmitate. Furthermore, we aim to distinguish the protective effects of GLP-1 and liraglutide, in multiple organ types that are affected by IR under an ischemic event. Traditional *in vitro* 2D monolayer studies will be further compared to 3D spheroid studies, to demonstrate physiological relevance in 3D models over traditional methodology. Statistical significance was calculated using a one-way ANOVA and post-hoc Tukey HSD test ($p \leq 0.05$).

Palmitate has been shown to induce IR in multiple cell lines however, to ensure a more physiologically relevant profile of NEFAs, a relevant ratio of 4:4:1 was used combining palmitate, oleate and stearate (POS), respectively. A viability assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) indicated that 500 μM palmitate concentration in normoxic and hypoxic conditions diminished cellular viability significantly ($p \leq 0.05$), where POS revealed similar results ($p \leq 0.05$) (n=6). Western blot analysis showed that POS and palmitate at 500 μM induced IR in HEK293T cells compared to controls, decreasing levels of insulin stimulated p-Akt (ser473) alongside additional markers of IR (n=4). 3D spheroids of HEK293Ts (100 cells) were used to better stimulate the *in vivo* microenvironment. MitoTracker red CMXRos, Caspase 3/7 reagent and DAPI were used to determine spheroid viability, mitochondrial protection and apoptosis. Fluorescence studies showed that at 500 μM POS, cellular viability was diminished. However upon treatment with GLP-1 and liraglutide, this was attenuated significantly ($p \leq 0.05$)(n=4).

In conclusion, the presence of NEFAs palmitate, oleate and stearate, in HEK293T kidney cells decreased cellular viability and induced IR. GLP-1 and liraglutide indicated an ability to diminish these effects by increasing cellular viability. The data observed in monolayer studies, appeared to be replicated within 3D spheroid models. However further work is essential to characterize this and to determine the mode of action of GLP-1 in protection.

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PC62

Exercising control over signs and symptoms of stress, anxiety and depression

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The WHO places depression as the leading cause of global mental and disability. Improving prevention and suboptimal patient treatment are essential to limit the personal/societal burden and reduce escalating healthcare costs. Regular exercise consistently prevents, protects and increases resilience against the development of stress-related responses in depression, whereas sedentary behaviour heightens symptoms. The beneficial impact of exercise on brain function and behaviour have seen its prescription adjunct to standard treatment options in the management of major depression. However, the mechanism underpinning this effect is poorly understood, a factor limiting widespread uptake of this option.

Disruption of tryptophan metabolism in depressed patients is characterised by increased tryptophan metabolism along the kynurenine pathway. Exercise-interventions may help to control excess kynurenine production improving mood and cognition. It is currently unclear what exercise intensity and duration produce these effects on a long-term basis. The goal of this study was to examine how a 12-week exercise programme can impact on brain function via regulation of tryptophan metabolism along the kynurenine pathway in healthy sedentary adults.

Participants were randomised into groups: sedentary control; high, moderate or low dose exercise (3 sessions/week). Monthly fitness and psychometric assessments were performed throughout to establish a baseline profile and monitor the changes associated with increased exercise.

We present preliminary measures of mood, stress, anxiety and depression specific relative to changes in fitness, stratified by exercise intensity. The optimised exercise protocol will inform a forthcoming study in a depressive cohort to use exercise to reduce the impact of increased kynurenine on mood and cognition.

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PC63

Polyphenol enriched tomatoes protect against atherosclerotic plaque development in ApoE^{-}/$^{-}$ mice by modifying cholesterol efflux and inflammation

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Polyphenols are known to protect against cardiometabolic diseases although very few studies have been carried out in the context of a food matrix. We fed ApoE^{-}/$^{-}$ mice with diets containing standard red tomatoes or tomatoes expressing different polyphenols. Mice were sacrificed by exsanguination under isoflurane and perfused with 0.9% Saline and EDTA via cardiac puncture. Effects of the diets on aortic sinus plaque size and macrophage infiltration were determined using immunohistochemistry, fluorescent microscopy and quantification using ImageJ and Gene expression by qRT-PCR. The effects of anthocyanin metabolites, resveratrol and resveratrol phase II conjugates on the expression of genes involved in inflammation and Reverse Cholesterol Transport (RCT) were determined in PMA differentiated THP1 macrophages. All gene expression experiments were repeated at least three times. Data were not normally distributed, so Mann Whitney test was used with Holm adjustment for multiple comparisons as outlined by Chen *et al* 2017. Data are presented as medians (Mdn). Aortic sinuses of mice fed flavonols + anthocyanins (Mdn = 14.21, n =19) and those expressing resveratrol (Mdn = 15.02, n = 19) had significantly reduced plaque sizes compared to those fed the red tomato control diet (Mdn = 18.73, n = 20), U = 94.50, P = 0.008 and U = 101.0, P = 0.0129 respectively. In comparison to controls (Mdn = 9.276, n =11), mice on the flavonol + anthocyanin diet (Mdn = 4.878, n =11) but not the resveratrol diet (Mdn = 7.804, n =11) had reduced aortic sinus macrophage infiltration U = 30, P = 0.05 and U = 52.00, P = 0.60 respectively. Diets supplemented with tomatoes expressing flavonols or isoflavones alone had no effect on plaque size, nor did they influence macrophage infiltration. In THP1 macrophages, in the absence of TNF-α as an inflammatory stimuli an anthocyanin metabolite phloroglucinaldehyde (PGA) significantly increased *ABCA1* gene expression (Mdn = 2.0 vs 1.0 in controls), U = 1.0, P = 0.001 and decreased ABCG1 (Mdn = 0.66 vs 1.02 in controls), U= 4, P = 0.001, PGA also increased *TNF-α* gene expression

(Mdn = 4.171 vs 0.96 in controls), U = 0.0, P = 0.001. In contrast, resveratrol increased *ABCG1* gene expression both in the absence (Mdn =1.77 vs 1.1 in controls), U = 2, P = 0.001 and in the presence (Mdn =2.2 vs 0.96 in controls), U = 0.0, P = 0.001) of TNF-α. In the presence of TNF-α resveratrol also reduced gene expression of *TNF-*α (Mdn = 0.34 vs 1.3 in the controls), U = 0.0, P = 0.001 and IL-10 gene expression (Mdn = 0.36 vs 1.4 in the controls) U = 0.0, P = 0.001. Thus, in the context of tomato food matrix, anthocyanins and resveratrol may act through differential pathways involving macrophage infiltration and reverse cholesterol transport to reduce atherosclerotic plaque development.
Chen, *et al* 2017

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PC64

The cardiotoxicity of Phenanthrene

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The disease burden from ambient air pollution is becoming increasingly apparent. Despite the lung being the first site of exposure for air pollution, numerous epidemiological studies show strong correlations between air pollution and cardiovascular disease. Inhaled particulate matter (PM) can interact directly with airway epithelia but the surface chemicals on PM, like polyaromatic hydrocarbons (PAH), are also known to exert a cardiovascular response. The focus of our current work is phenanthrene (Phe), a small 3-ringed member of the PAH family which is ubiquitous in air and water fossil fuel-based pollution. We have examined the mechanisms underlying the cardiotoxicity of Phe on the intracellular calcium cycling pathways in zebrafish and sheep ventricular myocytes, in both healthy and diseased animals. Compared to paired controls Phe exposure reduced intracellular calcium transient amplitude in zebrafish ventricular cardiomyocytes field stimulated at room termperature by 7 % (P<0.05; Wilcoxen test) and increased the rate of transient decay by 30% (P<0.05; Wilcoxen test). Interestingly these effects differ in the mammalian system. Ventricular myocytes from wild-type sheep stimulated at 37 °C showed an 12 % reduction in intracellular calcium transient amplitude compared to paired controls (P<0.001; Wilcoxen test), no change in decay rate was observed upon Phe exposure. To try and elucidate whether Phe exposure effects are exacerbated by underlying cardiovascular conditions, ventricular cardiomyocytes isolated from a sheep myocardial infarction model were used in the intracellular calcium cycling experiments. Similarly to the wild-type, no change in decay rate was observed upon Phe exposure, whilst a 10 % reduction in intracellular calcium transient amplitude compared to paired controls (P<0.001; Wilcoxen test). This work indicates the disruptive effect of Phe on the zebrafish and sheep myocardial function,

but suggests that susceptibility may not be increased after periods of impaired cardiovascular function.

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Sheep myocardial infarction model was established and maintained by Charlene Pius and Barbara Niort.

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PC65

The effect of calcium co-ingestion during endurance exercise on exogenous glucose oxidation in healthy men

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The benefits of exogenous carbohydrate availability for optimal endurance exercise performance are well-established. Research has identified maximal exogenous glucose oxidation rates of ~1 g/min[1], which are thought to be limited by intestinal absorption. However exogenous glucose oxidation rates may be increased if the intrinsic activity of the intestinal glucose transport system (SGLT1 and GLUT2) can be upregulated. As SGLT1 is saturated under conditions of high glucose availability[2], GLUT2 translocation is particularly important when large amounts of carbohydrate are consumed. The presence of extracellular calcium in rodent intestine has been found to increase GLUT2 translocation to the apical membrane[3]. Whether this translates to increased intestinal glucose absorption and oxidation during endurance exercise in humans, is unknown. Therefore, the aim of this study was to investigate the effect of calcium co-ingestion during endurance exercise on exogenous glucose oxidation in healthy men. The relative utilisation of exogenous glucose was hypothesised to be higher with calcium-glucose co-ingestion compared to glucose ingestion alone.

Eight healthy male volunteers completed a 120-minute cycling bout (50% peak power output), ingesting either 1.2 g/min dextrose (CON) or an identical solution containing 2000 mg calcium (CAL), distributed across eight 100 ml boluses consumed every 15 minutes. After a minimum 5-day washout period, participants performed the alternate trial in a randomised counterbalanced order. Single-breath expired air samples were collected every 15 minutes and analysed using isotope ratio mass spectrometry to estimate exogenous glucose oxidation (analysis ongoing). Whole-body substrate oxidation was estimated using indirect calorimetry for quantification of total carbohydrate and fat utilisation. Ratings of perceived exertion (RPE) and gut discomfort, blood glucose and lactate concentrations, and heart rate, were also recorded every 15 minutes. Values are means ± S.E.M, compared by paired *t*-test.

No significant differences were observed in total carbohydrate (CON vs. CAL; 265.9 ± 14.1 vs. 274.1 ± 16.1 g) or fat (51.8 ± 6.7 vs. 49.2 ± 5.3 g) oxidation between trials (Figure 1). There were also no significant differences between trials in heart rate, RPE, gut discomfort, blood glucose or blood lactate concentrations (p = .11 – .84). The results thus far suggest no effect of calcium on the characteristics of fuel use during endurance exercise in men. However, mass spectrometry analyses may yet show an increased contribution of exogenous glucose to total carbohydrate oxidation with calcium, suggesting a maintenance of endogenous stores. If demonstrated to have a functional effect, there may be a role for calcium in contemporary nutritional guidelines for endurance exercise performance.

Figure 1: Whole-Body Substrate Oxidation

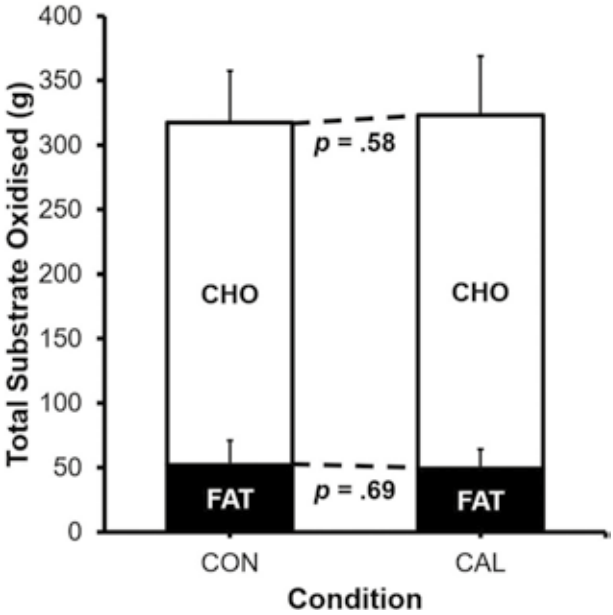

Figure 1: Mean total carbohydrate (CHO) and lipid (FAT) oxidation between the glucose only (CON) and glucose-calcium (CAL) conditions. Error bars represent S.E.M; n = 8.

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Mace OJ *et al*. (2007). *J Physiol* 580, 605-616

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PC66

Contractility of Mural Cells Within Mouse Spinal Cords Induced by Acute Angiotensin II Treatment.

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The microvasculature of the neurovascular network acts as a protective barrier preventing toxic and foreign agents accessing the nervous system and supplying the integral nutrients for the nervous system to function and survive. Diabetic neuropathic pain has previously been associated with reduced perfusion of the spinal cord (1). Reduced microcirculation can be implemented in part by vasoconstrictive processes. Angiotensin II receptor type 1 (AT1) facilitates vasoconstriction and is present on several cell types including pericytes. Pericytes have been identified to cause vessel constriction (2) and highlighted within the vasculature of diabetic patients (3). Angiotensin II (AngII), a vasoactive molecule, acts through AT1 and has been observed to be elevated within neuropathic diabetic patients (4) and associated with development of pain (5). We hypothesis that reduction of microvessel diameter within the spinal cord could be a result of pericyte facilitated constriction, activated by acute effects of AngII on its receptors. The microvessels of spinal cords were investigated within adult male C57BL/6J mice (30g). Anaesthetised (using ~2% isoflurane), animals were administrated with AngII (100nm i.t., N=3) or phosphate saline buffer (age matched control+vehicle, N=3) for 10 minutes. Animals were cardiac perfused with 4% paraformaldehyde. Spinals cords were removed via laminectomy and cryoprotected (30% sucrose) overnight (4ºC). 50μm tissue sections were cut for further analysis. Immunofluorescent confocal imaging of tissue sections were performed after DAPI (nucleus staining), PECAM-1 (endothelial cell) and NG2 (pericyte) labelling (Hulse *et al.*, 2018). Fiji analysis of vessels were performed, followed by Multiple and unpaired t-test statistical analysis via GraphPad Prism with values including mean ± S.E.M. Microvessels identified in spinal cord were overall reduced (NS) in vasculature volume (4.78 ±0.22 vs. 6.83 ±1.03 P<0.1 Unpaired T-test N=3) and number (12.74 ±3.53 vs. 24.64 ±5.41 P<0.2 Unpaired T-test N=3) following AngII treatment compared to vehicle controls. Subsequently, microvessel diameter in relation to pericyte proximity was determined. PECAM-1 positive vessel diameters were recorded in reference to pericyte soma body represented by NG2 and DAPI labelling. Analysis demonstrated a reduction (NS) in mean vessel diameter in AngII vs. vehicle group (soma body 6.76 vs. 7.70 P<0.129 Multiple T-test N=3). Although results demonstrated no statistical significance, there is an overall reduction in diameter of vessels in relation to the localisation of pericyte cell bodies within the dorsal horn of spinal cords within AngII treated group vs. vehicle group. This may be supported by reduced vasculature volume and number resulting from vasoconstriction. Further use of experimental repeats would be used in future to determine significance.

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PC67

Dance training Improves the strength explosive in university students

O. Cárdenas

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The dance must be performed quickly and with specific degree of intensity in accordance with the nature of the rythm, which leed in changes in explosive strength of the shoulders, arms and Lower limbs. (Uzunovic, Kostic, & Zivkovic, 2010). It is are also positive for the individuals because of its favorable effects on diabetes, osteoporosis, neurological conditions and also promote healthy living, funand social life interaction. (Keogh, Kilding, Pidgeon, Ashley, & Gillis, 2009). Objective: To determine the effects of dance in relation to strength explosive of university students. Methods: With institutional ethical approval eleven male university students dance players (mean age: 19, 55 ± 3.21 years, mean height: 165,36 ± 8, 86 cm, body mass: 60,30 ± 12,66 kg.) Participated in this study. Athletes performed three countermovement jump (CMJ) before and after of dance practice (DP). A progressive dance program was carried out over a period of 4 weeks (three per week, with a total of 12 sessions) (15 minutes of warm up and 165 minutes per session) The intensity was measured by means of the effort perception scale. The program was taught by a professional rumba instructor who dictated the basic steps, with forward, backward, transversal and rotational direction along with the simplest movements of different musical genres (salsa, merengue, bachata, joropo, urban dance). A total of 16 variables were analyzed through CMJ testing and simultaneously quantified with BTS 6000 force platform. Results: Significant correlations (r) were found next to the p value of the intervention group, on the countermovement jump variables: flight time: (FT, p=0,0009, r=0,99), Peak Velocity (PV p=0,002, r=0,95), Peak Power

(p=0,006, r=0,94), Power (p=0,001, r=0,98). Conclusions: the musicals rhythm sundry and dance shows an increase in power, velocity and force in lower limbs, because the repetitive neuromuscular stimulation influence over muscle fibers to generate increase nervous impulses like a health benefits in individuals.

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

PC68

Can high intensity interval training reduce fear of hypoglycaemia and improve glycaemic control in people with type 1 diabetes

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Many patients with type 1 diabetes (T1D), avoid exercise due to the potentially large drop in blood glucose that is associated with moderate intensity continuous exercise and the associated risk of hypoglycaemia. Recent work from our laboratory suggests that, unlike moderate intensity exercise, high intensity interval training (HIIT) does not reduce blood glucose during exercise in people with T1D. Although this data provides promising evidence, more information is required on HIIT's effects on daily glycaemic control. Therefore, the aim of this study was to determine the effects of HIIT on inter-day glycaemic control in people with T1D compared to moderate intensity continuous training (MICT) and no exercise at all. This study was provided with NHS research ethics before commencing. Participant's with type 1 diabetes (Mean ± SD, n=2, Age 21.5 ± 0.7 years, Height 1.69 ± 0.01 metres, Weight 83.8 ± 3.6 kg, BMI 29.4 ± 1.6 kg/m^2, T1D duration

12.3 ± 2.5 years) completed a randomised crossover study consisting of three 2-week interventions; 1) HIIT, 2) MICT and 3) a control intervention with no structured exercise training (CON). During the HIIT (6x1min intervals at >80% max heart rate) and MICT (30min at 60-70% max heart rate) interventions, six training sessions were completed. Throughout the 2-week intervention, glycaemic control was measured using an Abbot freestyle flash glucose monitor. Insulin dose, carbohydrate consumption and physical activity were also monitored throughout the intervention. During the 24-hour period after exercise, there were no significant differences ($P > 0.05$) in the mean number of hypoglycaemic episodes (HIIT 1.7, MICT 2.2) and the average time spent in level 1 (L1) and 2 (L2) hypoglycaemia (L1: HIIT 8.9%, MICT 7.2%; L2: HIIT 4.8%, MICT 2.1%) between the exercise interventions. Glycaemic variability, measured as standard deviation (SD) and coefficient of variation (CV) (SD: HIIT 3.7, MICT 3.4; CV: HIIT 42.9, MICT 41.7) was also not significantly different between interventions ($P > 0.05$). This is the first study to use the American Diabetes Association guidelines to assess glycaemic control following exercise in people with T1D. It is also the first study to measure carbohydrate consumption, insulin dose, and physical activity to provide a robust assessment of factors affecting glycaemic control in people with T1D. As such, this study has the potential to inform future guidelines on exercise for people with T1D. Due to the small sample size at this time-point, an overall conclusion cannot be determined for these results, however from the data collected; glycaemic control is similar across all interventions.

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

PC69

Effect of pirimiphos-methyl on hormone concentration during estrous cycle in rats

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The female reproductive function is under the control of the hypothalamus and the pituitary. The synchronous secretion of oestradiol, progesterone, luteinizing and follicle-stimulating hormones are essential for the reproductive functions in female rats. Pesticides among other factors like drugs, environmental factors, lifestyle and diet are capable of having adverse effects on reproductive functions. Pirimiphos-methyl, a broad spectrum, non-cumulative organophosphate insecticide is widely used especially in Africa to protect food against pests. Since the female reproductive function is under the control of the hormones, this present study was designed to evaluate the effect of pirimiphos-methyl on hormone concentration during the estrous cycle in female Sprague-Dawley rats. Rats (150-190g)

were divided into groups A, B, C and D of 24 rats per group and daily administered with pirimiphos-methyl (0, 10, 60 120 mg kg $^{-1}$, orally) respectively for a period of 21days. Daily vaginal smears were performed to determine the pattern of the estrous cycle. After 21 days, rats were sacrificed and blood was collected at the various phases of the estrous cycle for hormonal assay. Values are means ± SEM compared by one-way ANOVA. Results show alteration in the level of hormones during estrous cycle as shown in Table 1. The result suggest that administration of pirimphos-methyl caused an imbalance in the serum hormone levels thus leading to irregularity in ovarian function and alteration in the duration of estrous cycle.

Hormone concentration during estrous cycle in rats administered with Pirimiphos-methyl an organophosphate

Hormone concentration	Groups	Phases of estrous cycle			
		Proestrus	Estrus	Metestrus	Diestrus
Oestradiol (pg/ml)	A	12.7 ± 0.47	10.45 ± 0.19	6.92 ± 0.21	7.88 ± 0.22
	B	10.25 ± 0.34*	10.43 ± 0.15	8.75 ± 0.33*	10.39 ± 0.29*
	C	9.32 ± 0.39*	10.24 ± 0.17	10.42 ± 0.17*	10.97 ± 0.21*
	D	8.50 ± 0.88*	11.73 ± 0.31*	12.65 ± 0.21*	15.13 ± 0.31*
Progesterone (ng/ml)	A	10.27 ± 079	22.44 ± 0.88	35.45 ± 0.72	40.35 ± 0.49
	B	13.12 ± 0.38*	12.66 ± 0.71*	18.92 ± 0.38*	17.21 ± 0.43*
	C	19.76 ± 0.41*	19.38 ± 0.63*	35.37 ± 0.69	26.30 ± 0.75*
	D	18.37 ± 0.54*	33.26 ± 0.31*	33.34 ± 0.74	26.82 ± 0.69*
Leutinizing hormone (IU/L)	A	38.39 ± 0.71	41.91 ± 0.37	35.77 ± 0.75	37.37 ± 0.53
	B	35.05 ± 0.29	31.39 ± 0.82 *	35.80 ± 0.75	34.91 ± 0.47*
	C	37.51 ± 1.21	38.41 ± 0.84 *	39.79 ± 0.94*	33.82 ± 0.73*
	D	44.74 ±1.27*	40.13 ± 1.00*	40.36 ± 0.72*	36.17 ± 1.17
Follicle stimulating hormone (ng/ml)	A	4.88 ± 0.19	3.94 ± 0.19	9.68 ± 0.58	5.81 ± 0.26
	B	9.91 ± 0.64*	8.34 ± 0.58 *	4.68 ± 0.34*	10.08 ± 0.49*
	C	10.31 ± 0.46*	4.93 ± 0.36*	3.29 ± 0.50*	2.60 ± 0.19*
	D	15.74 ± 0.88 *	5.67 ± 0.34*	2.71 ± 0.22*	2.79 ± 0.37*

Group A, B, C and D - administered with 0, 10, 60 and 120 mg kg-1 of pirimiphos-methyl; Values are Mean ± SEM; * $p < 0.05$ compared to group A.

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PC70

Modulatory Roles of Coconut Oil and Lauric Acid on Testicular Parameters in Diabetic Male Wistar Rats: A Comaparative Study

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Diabetes mellitus impairs male reproductive function (1). Coconut oil (CO) is reported to possess anti-diabetic properties (2) and ameliorative effects on impaired testicular parameters (3). Lauric acid (LA) is the most abundant constituent of coconut oil (4). This study thus sought to investigate some testicular parameters in diabetic male wistar rats treated with lauric acid and coconut oil. The animals were divided into 6 groups of five (n=5) as follows: Group I (NC): received distilled water (1ml/Kg b.w), Group II (DUT): Diabetic untreated, Group III (DM + LA 90): Diabetic treated with LA (90 mg/Kg b.w), Group IV (DM + LA 180): Diabetic treated with LA (180 mg/Kg), Group V (DM + LA 360): Diabetic treated with LA (360 mg/Kg) and Group VI (DM + CO): Diabetic treated with CO (1.42 ml/Kg b.w). Diabetes was induced by an intraperitoneal injection of Streptozocin (65 mg/Kg). Treatments were for 4 weeks after which the animals were sacrificed by cervical dislocation. Semen parameters and serum testosterone level were evaluated. Compared to the normal control rats, there was a significant decline ($p < 0.05$) in, serum testosterone, sperm concentration, percentage of motile, normal and viable sperm cells in diabetic untreated rats. Compared to the diabetic untreated rats, sperm concentration, percentage of motile, normal and living sperm cells in diabetic rats treated with coconut oil were significantly higher ($p < 0.05$). Compared to the diabetic untreated rats; the percentage of normal sperm cells in diabetic rats treated with 360 mg/Kg LA, was significantly higher ($p < 0.05$). The impaired testicular parameters were largely ameliorated by coconut oil. Lauric acid however failed to completely mitigate these impairments, thus contradicting the assumption that the improvement of reproductive function by coconut oil in the male diabetic rats is attributable to lauric acid.

Table 1: Semen analysis in diabetic male wistar rats treated with Lauric acid and Coconut oil

GROUPS	Motile sperm cells (%)	Normal sperm cells (%)	Viable sperm cells (%)	Sperm concentration (1 million/ml)
NC	71.33 ± 2.33	79.67 ± 4.84	68.33 ± 4.84	51.93 ± 1.85
DM-UT	25.67 ± 2.40 a	42.67 ± 2.67 a	22.00 ± 4.36 a	6.20 ± 1.15 a
DM + LA 90	25.00 ± 2.00 a	57.50 ± 2.50 a	25.00 ± 5.77 a f	8.88 ± 1.91 a f
DM + LA 180	8.33 ± 1.67 a b c f	31.50 ± 2.50 a f	11.6 ± 4.41 a f	4.30 ± 1.12 a f
DM + LA 360	12.33 ± 1.76 a b c f	61.67 ± 5.46 b d	20.00 ± 5.00 a f	6.80 ± 0.30 a f
DM + CO	79.00 ± 1.53 c	61.33 ± 1.86 a b	58.33 ± 4.41 b	40.17 ± 1.70 a b

a- significant compared to NC ($p < 0.05$); b - significant compared to DUT ($p < 0.05$); c – significant compared to D + LA 90 – significant compared to D + CO ($p < 0.05$); f – significant compared to DM + CO ($p < 0.05$)

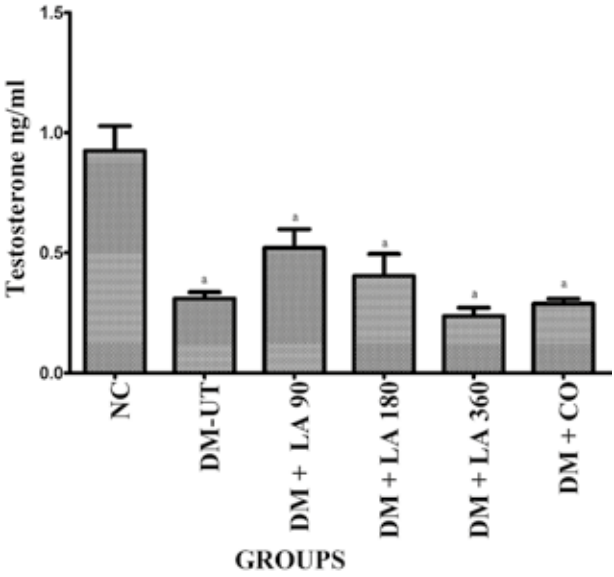

Figure 2: Serum testosterone level in diabetic male wistar rats treated with lauric acid and coconut oil

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PC71

Measuring Muscle Function in Healthy Adults to Interpret Motor Capacity in Children with Cerebral Palsy.

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Cerebral Palsy (CP) affects approximately seventeen million people worldwide (Graham *et al.*, 2016). One of the most consistent abnormalities observed in children with CP is toe walking, characterised by excessive plantar flexion (Wren *et al.*, 2005), which may be due to an increased sarcomere resting length. Evidence has suggested that CP muscle fibres contain elongated sarcomeres (Mathewson *et al.*, 2015; Leonard *et al.*, 2019), which leads to the postulation that the shortened plantar flexor muscles may facilitate optimum ankle torque production (Frisk *et al.*, 2019). If optimal torque production occurred at shorter plantar flexor fascicle lengths in CP patients, this would imply that toe walking serves as a functional benefit by partially maintaining ankle moment during gait, in comparison to typically developed (TD) individuals. To test this hypothesis, we needed to establish if TD subjects, walking in their natural heel-toe pattern, operate with their fascicle lengths at optimal.

Nine TD volunteers (Height 167.03 ± 5.16cm; Mass 75.40 ± 14.88kg; Age 29.00 ± 10.86years) participated in the experiment. Evoked isometric contractions of the plantar flexors at twelve different ankle angles were conducted (foot in relation to the tibia: 100°- 65°), with corresponding fascicle length of the medial gastrocnemius (MG) recorded using B-mode ultrasound. The fascicle length - ankle torque relationship was established for each individual, with the fascicle length producing the highest torque set as the optimal. The zone of optimal functioning was then determined using the fascicle lengths at approximately 80% of peak torque, on the ascending and descending curve. Additionally, participants were asked to walk in the laboratory while 3D motion capture and simultaneous ultrasound imaging of the MG were recorded. Trials were recorded for the subjects' natural heel-toe walking pattern and during voluntary toe walking.

During the stance phase of gait while heel-toe walking, the fascicles appeared to function in an isometric manner, with fascicle lengths residing within, or close to, the zone of optimal functioning. During toe walking, the fascicle lengths of the MG resided below the zone of optimal functioning. Consequently, the fascicles of the MG in TD individuals during normal gait appeared to operate in a mechanically efficient manner, within or close to the zone of optimal functioning. When investigating CP patients, we expect shorter MG fascicles at peak ankle plantar flexor torque in comparison to TD. Further, we expect the shortened fascicles during toe walking to correspond with their zone of optimal functioning. If this hypothesis proves true, effective treatment of toe walking in CP may be achieved by interventions aimed at normalising the resting sarcomere length of their plantar flexors.

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PC72

Adenosine deaminase 2 restores the endothelial glycocalyx of blood outgrowth endothelial cells.

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Background
Glycosaminoglycans (GAGs) are polysaccharide chains that contribute to the formation of a mesh-like layer on the surface of the endothelium: the glycocalyx. The integrity of this layer is vital for endothelial cell homeostasis and its disruption leads to endothelial dysfunction. Endothelial damage associated with certain systematic vasculitides has recently been linked to autosomal recessive loss-of-function mutations in the *ADA2* gene. Direct effects of the ADA2 protein on the endothelium are unclear, although ADA2 is known to have a GAG binding site.

We isolated blood outgrowth endothelial cells (BOECs) in order to study the repair potential of ADA2 on the endothelial glycocalyx, in healthy volunteers.

Methods

BOECs were isolated and cultured from healthy donors. Firstly, peripheral blood mononuclear cells (PBMCs) were extracted from whole blood, cultured in EBM-2 media on collagen-coated plates, for 7-21 days, until colonies formed with characteristic cobbled-shaped morphology. BOECs were confirmed, using flow cytometry, to have classical endothelial cell surface marker expression (CD31, CD144, low CD34). BOECs (n=3) were cultured on chamber slides for 2-3 days until confluent and then incubated with GAG degradative enzymes for 2 hours; confirmed by staining. Cells were then treated with either ADA2 (10 U/L) or untreated and allowed to recover for a further 18 hours. The samples were then fixed, blocked and stained with primary antibodies specific for the GAGs; Heparan sulfate (HS) and Chrondroitin sulfate (CS). The slides were stained with FITC secondary antibodies and Hoechst nuclear stain. Samples were analysed by confocal microscopy, and data acquired over 3 random fields was evaluated using Image J software.

Results

100% of BOECs expressed both HS and CS, with GAG coverage over the entire cell. Following enzymatic treatment, HS and CS expression was removed. Incubation with ADA2 for 18 hours restored the original GAG coverage. Over this time, untreated BOECs (media alone), expressed significantly less GAGs than with ADA2 ($p<0.001$) and were restored to only 52% (+/- 3.6% SEM) of their initial coverage. It took a further 18 hours before untreated BOECs recovered their full glycocalyx expression.

Conclusions

Glycocalyx removal precedes endothelial damage. This is the first time that the promotion of glycocalyx and GAG repair by an exogenous enzyme, ADA2, has been reported. ADA2 accelerated BOEC glycocalyx recovery following GAG degradation and BOECs widely expressed the two most commonly expressed GAGs. This data provides a suggested mechanism by which ADA2 protects against endothelial damage. Therefore, in ADA2-deficient patients, this may contribute to the endothelial damage and ensuing inflammation and vasculitis that is observed.

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

PC73

The effects of sprint interval training in the fasted and carbohydrate-fed states on regulators of the intracellular nicotinamide adenine dinucleotide pool in human skeletal muscle

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Nicotinamide adenine dinucleotide (NAD+) is a cofactor with roles in many biochemical processes. NAD+ can be synthesised from exogenous dietary sources and via endogenous salvage pathways. Regulators of this system include nicotinamide phosphoribosyltransferase (NAMPT), nicotinamide riboside kinase 2 (NMRK2), nicotinamide n-methyltransferase (NNMT), and nicotinamide mononucleotide adenylyltransferase 1/3 (NMNAT1/3). Ageing is marked by a decline in NAD+ biosynthesis, contributing to metabolic dysfunction. Thus, developing strategies to regulate NAD+ biosynthesis across the lifespan has garnered interest. Sprint interval training (SIT) has emerged as a potent metabolic stimulus, inducing beneficial musculoskeletal adaptations. Low energy conditions, such as the fasted state, have been investigated for their potential to upregulate NAD+ biosynthetic pathway activity. This study compared the effects of fasted (FAST) and carbohydrate-fed (FED) SIT on NAD+ biosynthetic pathway gene expression in skeletal muscle of recreationally active males. This study received local ethical approval and was conducted in line with the Declaration of Helsinki. Healthy, recreationally active (VO_2 max < 50 $ml.kg^{-1}.min^{-1}$) males (n=18) were randomised to complete SIT under FAST or FED conditions. Participants attended the lab after an overnight fast (≥10 h) and ingested 0.33 $g.kg^{-1}$ body mass (BM) of non-caloric placebo (FAST), or 0.91 $g.kg^{-1}$ BM maltodextrin (FED). Forty-five minutes post feeding, participants underwent SIT consisting of 4 X 30 s "all out" cycle sprints at a resistance of 7.5 % BM, interspersed with 4 min recovery. Muscle biopsies were obtained under local anaesthetic from *m. vastus lateralis* at rest (pre-feeding) and 3 h post-exercise. RNA extracted from skeletal muscle underwent multiplex PCR analysis to determine *mRNA* expression of *NAMPT*, *NMRK2*, *NNMT*, *NMNAT1*, *NMNAT3,* and the reference gene *UBE2D2*. Paired and independent t-tests determined within and between-group differences in gene expression fold changes from baseline. *NMRK2*, *NAMPT*, and *NNMT* expression increased in both groups following SIT ($p < 0.05$). *NMNAT1* was unchanged and *NMNAT3* was decreased from baseline in FAST only ($p < 0.05$). No between-groups differences were observed for Δ*NMRK2*, Δ*NMNAT1*, or Δ*NMNAT3*. Both Δ*NAMPT* and Δ*NNMT* were upregulated to a greater extent in FAST compared with FED ($p < 0.05$). In summary, these findings indicate that SIT is a potent regulator of a network of genes modulating NAD+ biosynthesis and that there is divergent regulation of some, but not all these genes in response to FAST compared with FED SIT. This may have implications for health across the lifespan.

Future research should investigate the efficacy of FAST compared with FED SIT on the regulation of this system in ageing populations.

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PC74

Effects of hypoxia on Notch1 and Nrf2 signalling in human neuroblastoma SH-SY5Y cells: consequences for hypoxia-induced neurogenesis

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Inherent to the pathophysiology of both ischemic and haemorrhagic stroke is the depletion of tissue oxygen levels. While brain ischaemia can elicit irreparable brain injury, it primes a defence mechanism to boost the formation of new neurons from known neural precursor cell niches in the adult brain, contributing to repair and partial recovery of brain function. Fuller understanding of this mechanism could inform therapeutic strategies to enhance this endogenous repair process. Hypoxic microenvironments stabilise hypoxia-inducible factor 1-alpha (HIF1-α), reported to independently promote key stem/precursor-cell protection and regulatory pathways involving Nrf2 and Notch1 signalling. In the present study, these signalling events have been investigated as a possible mechanism underlying hypoxia-induced neurogenesis. Human neuroblastoma cells (SH-SY5Y), possessing neural precursor properties, were exposed to room air (18kPa O_2) or hypoxia (1kPa O_2) for 2 - 72h. Whole-cell lysates were immunoblotted for HIF-1α, Notch1 full-length and intracellular domain as well as Nrf2 and its downstream targets heme oxygenase-1 (HO-1) and NAD(P)H dehydrogenase quinone 1 (NQO1). We also assessed cell viability, proliferation and redox signalling in cells loaded with L-012 followed by exposure to hypoxia (1h) and reoxygenation (1h). Cell viability was unaffected by hypoxia but proliferation decreased after 72h hypoxia (18 kPa $37.97\pm3.43x10^6$ cells/ml vs 1 kPa $1.13\pm3.23x10^6$ cells/ml, n= 4, $P<0.05$). After 2h, HIF1-α protein levels were increased significantly and remained elevated until 16h. Full-length Notch1 protein

expression was also elevated by hypoxia, while Nrf2 and downstream targets HO1 and NQO1 were unaffected. Acute hypoxia alone did not stimulate L-O12 luminescence but subsequent reoxygenation increased L-012 luminescence, which was abrogated by PEG-SOD and unaffected by PEG-CAT, both reactive oxygen species (ROS) scavengers. Our findings confirm HIF1-α stabilisation following hypoxia and changes to Notch1 expression in human neuroblast-like cells. Conversely, neither hypoxia-induced neuroblast proliferation nor Nrf2-signalling changes were found, contrary to previous reports. Ongoing experiments are investigating changes in neurogenic-markers and notch1-downstream signalling.
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PC75

Gender differences in the effect of ghrelin on pancreatic islet survival

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Background and aims: Ghrelin is expressed in a number of tissues including stomach, hypothalamus and islets of Langerhans. It inhibits insulin secretion and promotes islet survival. In addition, ghrelin displays sexual dimorphism with regards to plasma levels in humans and rodents, and orexigenic responses in rodents. However, most studies relating ghrelin to islet function have investigated islets from male rodents and sex unspecified human islets. The aim of this study was to explore whether there are any sex-specific differences in mRNA expression of *Ghrelin*, ghrelin receptor (*Ghsr*) and oestrogen receptor 1 (*Esr1*) in murine islets, and whether treatment with acyl-ghrelin (AG) affects their expression levels as well as apoptosis in islets from both groups. It was also tested whether any effect observed is conveyed via the Growth Hormone Secretagougue Receptor type 1a (GHSR1a).
Materials and methods: Oestrus cycle stage was determined by haematoxylin and eosin (H&E) staining of vaginal smears. Islets from male and female CD-1 mice were incubated with 10 or 100 nmol/l AG +/- 5 μmol/l GHSR1a antagonists YIL781 or +/-100 nM Liver expressed antimicrobial peptide-2 for 48h and exposed to TNF-α (1000 U/ml) and IL-1β (50 U/ml) for 20h. Apoptosis was determined by measurement of caspase 3/7 activity, gene expression by quantitative PCR and protein expression by Western blot.
Results: H&E staining showed that the majority (60%) of female mice used were synchronised at metestrus stage with the remaining at oestrus stage (31%), (n=48). Treatment with 10 and 100 nmol/l AG reduced cytokine-induced apoptosis

compared to control in islets from female (45±4% (10 nmol/l), p<0.0001 and 35±4% (100 nmol/l) p<0.001, n=6, One-Way ANOVA), but not male mice (113±8% (10 nmol/l) p>0.6 and 113±10% (100 nmol/l), p>0.6, n=6). Expression of *Ghrelin* was not significantly different in stomach and hypothalamus of male and female mice (both p>0.05, n=5, Student's T-test). *Ghsr* mRNA expression was 2-fold greater in islets from female mice (p<0.05, n=4, T-test), however *Ghrelin* and *Esr1* (p>0.5,n=3-4) mRNA expression levels were not significantly different between the two groups. Following incubation with 100 nmol/l AG, mRNA expression of *Ghrelin*, *Ghsr* and *Esr1* (p>0.1, n=3-4, T-test) remained unchanged in both groups. GHSR expression was not significantly different in the islets from male and female mice at protein level (p=0.09, n=3, T-test). Treatment with either GHSR antagonists did not reverse the observed protective effect of ghrelin in islets from female mice. However treatment with YIL781 resulted in significant reduction in apoptosis (47±6% (5 μmol/l YIL781), p<0.0001 and 35±4% (5 μmol/l YIL781+100 nmol/l AG), p<0.001, n=6, One-Way ANOVA).
Conclusion: Our results suggest a differential effect of ghrelin on islet survival in male and female mice which may be independent of endogenous islet ghrelin receptor.

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

PC76

Correlation between systolic blood pressure responses to maximal exercise test and dynamic resistance exercise in hypertensive men

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Introduction: Maximal exercise test (MET) is recommended for hypertensives before engagement in exercise training[1,2]. Blood pressure (BP) responses during MET allows to identify subjects with exaggerated BP responses during aerobic exercise[1]. Nowadays, dynamic resistance exercise (DRE) has also been shown to reduce BP in hypertensives[2,3]; however, this kind of exercise produces a huge increase in BP during its execution, being important to verify whether BP measured during MET can also identify subjects with exaggerated BP responses during DRE. For that, the first step is to observe if systolic BP (SBP) responses during MET and DRE are correlated. Thus, this was the objective of the present study.

Methods: Twenty-eight medicated hypertensive men (52 ± 9 years) underwent a cycle ergometer MET (3-min of 30w warm-up followed by increments of 30W/min to exhaustion). Auscultatory SBP was measured pre-MET and at 30W, 60W, 90W, 120W and peak effort. After at least 48h, the subjects executed a DRE (one leg extension, 3 sets of maximal repetitions at 50% of 1RM and 90s of interval between the sets). Photoplethysmographic SBP was continuously measured and averaged for 3 min pre-DRE. In addition, the peak values of each set (S1, S2 and S3) were also obtained. Pre-exercise values were compared by paired t-test. In addition, Pearson's correlations and linear regressions were calculated between the absolute values of SBP during MET and the increase in SBP (DeltaSBP = peak – pre-SBP) observed in each set of the DRE. P<0.05 was considered significant.
Results: Pre-MET and pre-DRE values were similar (124±14 vs. 126±16, P=0.382). There were no significant correlations between SBP measured at 30W MET and the DeltaSBP obtained for all the sets of DRE (p>0,05). However, as Figure 1 shows, SBP measured at 60W, 90W and 120W presented significant correlations with DeltaSBP of S2 (60WxS2 - r=0.41, p=0.028; 90WxS2 - r=0.47. p=0.011; and 120WxS2 - r=0.54, p=0.002). In addition, peak SBP at MET also correlated significantly with DeltaSBP of S2 and S3 (S2- r=0.69, p<0.000; and S2 - r=0.49, p=0.008).
Conclusions and Implications: In treated hypertensive men, SBP measured at submaximal and peak workloads of a MET positively correlate with SBP increase obtained in the second set of a DRE, while peak SBP at MET also correlates with SBP increase obtained at the third set. These results suggest that MET can be used to identify hypertensive men that may present exaggerated SBP responses during DRE.

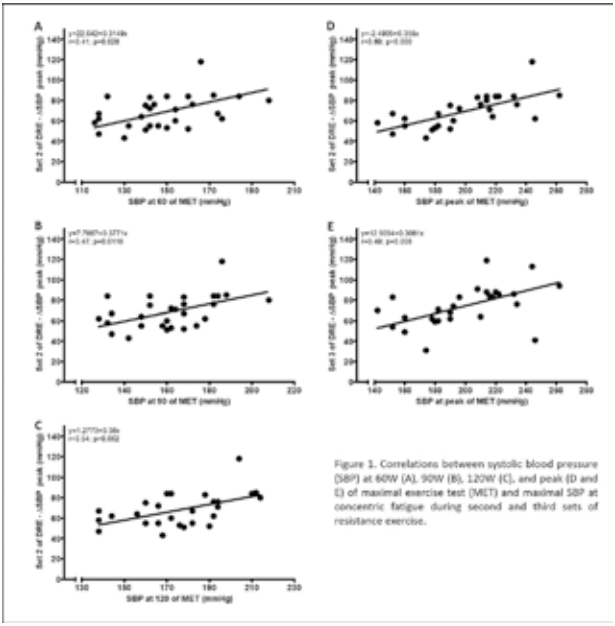

Figure 1. Correlations between systolic blood pressure (SBP) at 60W (A), 90W (B), 120W (C), and peak (D and E) of maximal exercise test (MET) and maximal SBP at concentric fatigue during second and third sets of resistance exercise.

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PC77

Influence of Replicatively Ageing and Nutrition on C_2C_{12} Murine Myoblast Fusion

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Introduction:With ageing the ability to retain skeletal muscle mass and function declines (sarcopenia), with associated "anabolic resistance" being reported. However, the impact of anabolic agents on ageing muscle cell adaptation has not been studied. The aim was therefore to utilise models of cellular ageing in the absence or presence of leucine or HMB to improve myoblast fusion. The hypothesis to be challenged was that replicatively aged cells show compromised fusion vs. control but that this would be rescued by amino acid supplementation.
Methods:Control and replicatively aged C_2C_{12} murine myoblasts were supplemented with 10 mM leucine or HMB. Myotube formation was assessed morphologically, biochemically (CK, LDH, Akt, mTOR, ERK, p38 signalling) and at the gene expression level at 0 h, 24 h and 96 h. All experiments were repeated 3 times in duplicate and analyses completed using one and two-way ANOVA.
Results: Although control cells showed significant fusion ($P < 0.05$) with time, replicatively aged myoblasts did not fuse and displayed significant reductions in CK (6-fold; $P < 0.05$) and significant increases in LDH (3.5-fold: $P < 0.05$) at 96 h vs. control. Akt, mTOR and ERK signalling over 24 h were all significantly decreased (8-fold, 3-fold and 2-fold: all $P < 0.05$) vs. control. Significant suppression of myogenin, IGF-I, IGF-II (200-fold, 19-fold, 52-fold: all $P < 0.05$) and relative increases in myostatin (12-fold; $P < 0.05$), with no differences in amino acid transporters were observed in replicatively aged vs. control at 96 h. However, replicatively aged cells treated with leucine significantly (2-fold: $P < 0.05$) increased Akt signalling at 120 minutes compared to untreated controls. HMB significantly increased Akt and mTOR at 120 minutes (both 3-fold and $P < 0.05$) compared to untreated control. Despite these positive signalling responses to leucine and HMB supplementation, the aged cells were still incapable of fusion by all parameters assessed.
Conclusion:Replicatively aged myoblasts failed to fuse basally and with supplementation. They were responsive to leucine and HMB treatment at a signalling level,

however, this was without impact on fusion. Therefore, while potentially displaying "anabolic resistance" at the level of fusion, the aged cells are not resistant to protein supplementation per se, warranting further investigation.

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PC78

Comparisons between young South Asians and White Europeans in the effects of acute mental stress and slow breathing on baroreflex sensitivity and heart rate variability

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South Asians (SAs) have a greater risk of cardiovascular disease including hypertension, (CVD) than White Europeans (WEs). Whether this reflects disturbances in autonomic control of the cardiovascular system and whether this might be present an early adulthood has not been tested. Thus, we performed studies on young WEs and SAs (10, 8 respectively, aged 19-24; equal numbers of men and women in each group) in which arterial blood pressure (ABP), heart rate (HR), ECG and respiration were recorded at rest, during mental stress (3 min Colour Stroop test) and during 5 min slow breathing (6 breaths/min). Baroreflex sensitivity (BRS) was calculated by the sequence method as change in R-R interval evoked by spontaneous up and down sequences in systolic pressure (SP) at rest and during mental stress and as the relationship between R-R interval and the fall in SP evoked by standing from a squat position before and following mental stress. Heart rate variability (HRV) was computed in the time- and frequency-domains during mental stress and slow breathing. Comparisons within and between WEs and SAs were done by paired and un-paired t-tests respectively.

At rest, WEs and SAs had similar mean ABP (mABP, 82.2±2.1 vs 82.0±2.8 mmHg), HR (70.1±3.4 vs 64.5±4.5beats/min) and BRS (up-sequence: 1.1±0.09, down-sequence: 1.2±0.05 vs 1.2±0.04 and 1.3±0.06ms/mmHg). Mental stress evoked similar increases in mABP and HR in WEs and SAs, but was accompanied by less depression of BRS in SAs than WE especially during down-sequences (to 0.69±0.11 vs 1.1±0.07*ms/mmHg, *: $P<0.05$, WE vs SA). Further, in the time domain, RMSSD and pRR50, indices of vagal activity, were depressed during mental stress in SAs only, whereas following mental stress BRS during squat to stand, an index of cardiac sympathetic activity was depressed in WEs only (0.6±0.04 to 0.3±0.09* vs 0.6±0.09 to 0.5±0.11ms/mmHg). During slow breathing, ABP tended to decrease in both WEs and SAs (P,0.05; P=0.09 respectively), but SDRR increased in SAs only.

These results suggest that baroreflex regulation of ABP is disturbed in young adult SAs relative to WEs, with SAs showing more persistent vagally-mediated

tachycardia in response to falls in ABP during mental stress than WEs. Moreover, whereas, WEs showed depressed baroreflex sympathetically-mediated tachycardia for several minutes following mental stress, this was not the case in SAs. Such disturbances in SAs may be early markers of future CVD and hypertension in SAs. However, the finding that increased vagal activity in young SAs but not WEs raises the possibility that practice of regular slow breathing may help restore respiratory-cardiovascular interactions in SAs and so help limit CVD.

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PC79

Proton-mediated activation of epithelial sodium channels is affected by the presence of a positively charged amino acid residue in the palm-domain of the δ-subunit

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Epithelial sodium channels (ENaCs) are sodium-selective ion channels which regulate salt and water balance in vertebrates. There are four different ENaC subunits (α, β, γ and δ) which can assemble in either the αβγ- or δβγ-combination. The physiological function of δβγ-ENaCs is poorly understood. Using *Xenopus laevis* as a model, we have recently shown that δβγ-ENaC is profoundly activated by protons, whereas αβγ-ENaC is not (Wichmann *et al.* 2019). The pH sensitivity of the closely related proton-sensitive ion channel ASIC1a (acid-sensing ion channel 1a) is linked to the amino acid residue K211 which is located in the palm domain between neighbouring channel subunits. We therefore hypothesise that conservation of the positive charge at the corresponding position in *Xenopus* δ-ENaC (δR279) could account for proton-sensitivity of this ENaC isoform. The absence of the positive charge in the α-ENaC subunit (αM282) may prevent proton-induced channel activation.
Site-directed mutagenesis was used to replace the δR279-residue with the corresponding methionine (M) present in the α-ENaC subunit. Both wild-type (WT δβγ-ENaC) and mutant (δR279Mβγ-ENaC) ion channels were expressed in *Xenopus laevis* oocytes. All procedures involving *Xenopus* oocytes were approved by the Animal Welfare Ethical Review Board of Newcastle University (ID 630). The activity of the expressed ion channels was measured using the two-electrode voltage-clamp technique at a holding potential of -60 mV. Oocytes were perfused in a Na^+ containing solution (Wichmann *et al.* 2019) at pH 7.4 or pH 6.0. The ENaC inhibitor amiloride (100 μM) was used in order to determine ENaC-mediated current fractions (ΔI_{ami}). All data are presented as means ± standard error of the mean.

At pH 7.4 (Figure 1), ΔI_{ami} in oocytes expressing WT δβγ-ENaC were 5.35 ± 1.36 μA (n = 8) and were significantly larger than in those expressing δR279Mβγ-ENaC (0.48 ± 0.19 μA, n = 8, $p < 0.05$, one way ANOVA followed by Dunn's multiple comparison test). The ΔI_{ami} of both channels were increased under pH 6.0 (Figure 2). Compared to pH 7.4, pH 6.0 activated δR279Mβγ-ENaC by 13.42 ± 4.42 fold (n = 8), whereas WT δβγ-ENaC was only activated by 2.01 ± 0.17 fold (n = 8, $p < 0.001$, Mann-Whitney U-test).
In conclusion, δR279Mβγ-ENaC showed a reduced activity at neutral pH but was significantly stronger activated by protons compared to wild type δβγ-ENaC. This suggests that the δR279-residue is important for proton-sensitivity of δβγ-ENaC.

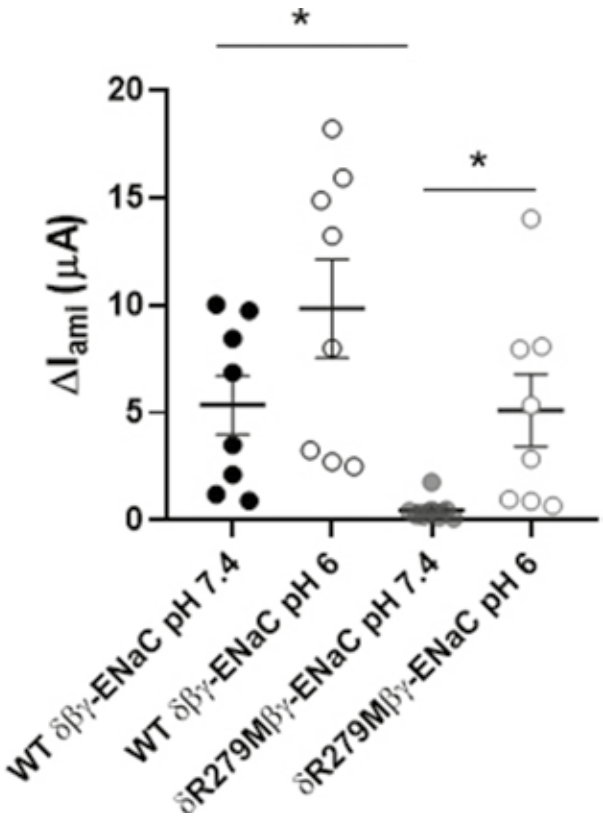

Figure 1: Amiloride-sensitive currents (ΔI_{ami}) of oocytes expressing wild-type (WT) δβγ-ENaC and δR279Mβγ-ENaC mutant at pH 6.0 and 7.4. * $p \leq 0.05$.

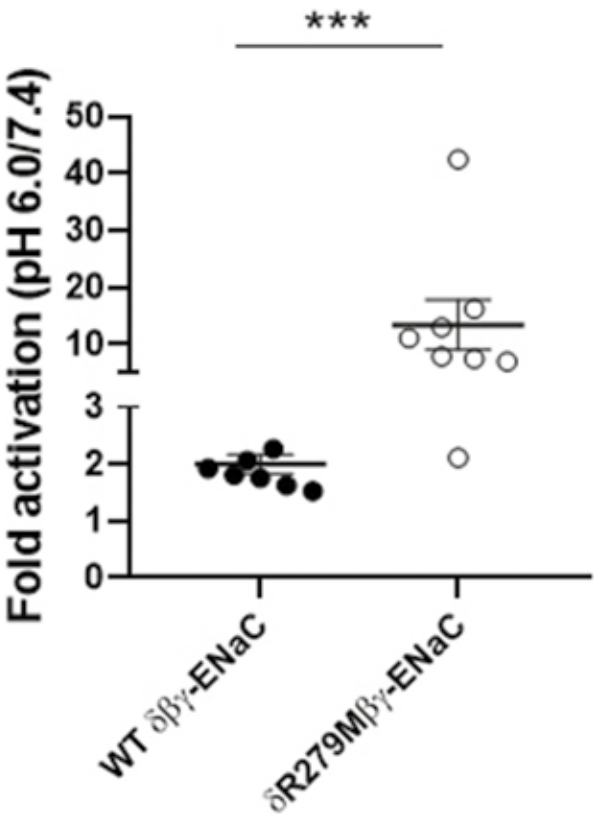

Figure 2: Fold-activation of WT δβγ-ENaC and δR279Mβγ-ENaC as calculated from ΔI_{ami} at pH 6.0 and pH 7.4.

Wichmann L *et al.* (2019). *J Biol Chem* 294, 12507-12520

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PC80

Dynamic Proteome Profiling of C2C12 Differentiation.

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Adult muscle consists of multinucleated myofibers that originate from myogenic precursor cells during embryonic development. The gene programme of muscle differentiation is well described, but currently there is paucity of data regarding protein synthetic responses during muscle cell fusion. We used stable isotope (deuterium oxide, D_2O) labelling of murine C2C12 muscle cells *in vitro* to investigate the synthesis of individual proteins during early and latter stages of differentiation.

Upon reaching 80 % confluency, myoblasts were transferred to differentiation medium supplemented with D_2O to label newly synthesised proteins during early (0-24 h) or later (72-96 h) periods of differentiation. Samples were analysed by liquid chromatography-mass spectrometry and data from control and D_2O-labelled cells were used to calculate protein fractional synthesis rates (FSR). Samples were also spiked with a yeast protein (alcohol dehydrogenase) to facilitate reporting of synthesis and abundance data in absolute terms.

Proteomic analysis of 152 proteins and one-way ANOVA (n=3, per group) detected 55 proteins that exhibited significant ($P<0.05$, false discovery rate of <1 %) differences between early and late differentiation. Ribosomal proteins were enriched during early differentiation, whereas myofibrillar and metabolic enzymes became more abundant during later differentiation. The median (first – third quartile) FSR (%/h) during early differentiation 4.1 (2.7-5.3) was ~2-fold greater than during later differentiation 1.7 (1.0-2.2), which equates to absolute (fmol/h/ug total protein) synthesis rates of 0.64, (0.38-1.2) and 0.28, (0.1-0.5), respectively.

When expressed in relative (%/h) terms the top 5 synthesised proteins during early differentiation were cytosolic actin (ACTC), myc box-dependent-interacting protein 1 (BIN1), myosin light chain 6 (MYL6), protein phosphatase 1-alpha (PP1A) and peroxiredoxin-5 (PRDX5), whereas ubiquitin-40S ribosomal protein S27a (RS27A), stress-induced-phosphoprotein 1 (STIP 1), PP1A, troponin C, slow (TNNC1) and ADP-ribosylation factor 1 (ARF1) were ranked the highest in later differentiation. The interpretation of data was different when expressed in molar (fmol/h/ug total protein) terms. ACTC, alpha enolase (ENOA) and RS27A, were all top-ranked proteins in both early and later differentiation. Additionally, glyceraldehyde 3-phosphate dehydrogenase (G3P; 4.8 fmol/h/ug protein) and Peptidyl-prolyl cis-trans isomerase A (PPIA; 4.0 fmol/h/ug protein) were also high in early, while Desmin (DESM; 1.8 fmol/h/ug protein), and vimentin (VIME; 1.6 fmol/h/ug protein) were high later during differentiation.

In conclusion, the biological interpretation of protein synthesis data can differ when results are reported in absolute or relative terms. This has consequences when studying cellular adaptation or the stoichiometry of multi-protein complexes.

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PC81

Baker's Yeast Prevents Carbon Tetrachloride-Induced Lipid Peroxidation in Rats' Model

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Our previous study showed that baker's yeast produced a protective effect against CCl_4 induced liver pathological change. We, therefore, examined the effects of baker's yeast against CCl_4 induced lipid peroxidation as determined by the formation of thiobarbituric acid reactive substances in the liver of Sprague Dawley rats (180-270g).(1) In-vivo: Rats were divided into 3 groups [n=5]. Group1 were injected with olive oil 1ml/kg intraperitoneally. Group2 were injected with 1ml/kg CCl_4 intraperitoneally dissolved in equal volume of olive oil. Group3 after receiving 200mg/rat oral yeast dissolved in distal water for 5 days, rats were injected with 1ml /kg CCl_4 dissolved in equal volume of olive oil intraperitoneally. The rats were humanely killed using co2 anesthesia followed by Cervical dislocation in accordance with national guidelines. Livers were taken and homogenated and the assay for lipid peroxides were performed using thiobarbituric acid reaction method as described by Ohkawa et al. (1979). (2) Ex-vivo: homogenates from the control group were divided into 3 groups (n=5). Group1 contains only liver homogenate (5ml), group2 and 3 contain 5 ml of liver homogenates plus 50 μm CCl_4, as well, 200mg yeast was added only to group3. The malondialdehyde [MDA] was determined in the supernants as thiobarbituric acid reacting material as described by Albro et al. (1986). The results were expressed as nmol of MDA formed per minute per milligram protein.

It was demonstrated that the exposure to CCl_4 significantly increases ($p < 0.001$) lipid peroxides levels in both in-vivo and ex-vivo methods in the CCl_4 untreated rats to 18.5±1.4 nmol of MDA for in-vivo and 2.05±0.01 nmol of MDA for ex-vivo as compared to normal group 6.38±0.56 nmol of MDA for in-vivo and 0.15±0.1 nmol of MDA for ex-vivo. It also noted that the administration of the yeast significantly reduced lipid peroxidation both in the in-vivo and ex-vivo test ($p <0.001$) to 6.12±0.6 nmol of MDA for in-vivo and 0.14±0.01 nmol of MDA for ex-vivo as compared to CCl_4 untreated group. These results suggest that the protective effects of baker's against CCl_4 induced lipid peroxidation in the liver is due at least partly to its glutathione antioxidant properties (Wiedeman et al., 2018), the two glutaredoxin genes

required for Protection against Reactive Oxygen Species (Luikenhuis et al.,1998) and high choline content which promotes phosphatidylcholine Synthesis; a vital for the integrity of the cell membranes (Varela-Moreiras et al.,1995).

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PC82

Objectively-measured physical activity associates with cardiometabolic risk factors and carotid intima-media thickness in rheumatoid arthritis

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Rheumatoid arthritis (RA) is an inflammatory disease characterized by accelerated atherosclerosis and increased cardiovascular risk (1). Recent data indicate that physical activity (PA) is associated with reduced cardiovascular risk in RA (2); however, it is still unclear which factors underlie the cardioprotective role of PA in this population. The aim of this study was to investigate the associations between objectively-measured PA and cardiovascular risk factors (i.e. blood pressure [BP], fasting glucose and lipids and C-reactive protein) and vascular remodeling (i.e., carotid intima-media thickness [cIMT]) in RA. Thirty six post-menopausal women with RA (64±8 years, 28.6±7.7 $kg.m^{-2}$) took part in this cross-sectional study. This study followed the principles of the Declaration of Helsinki and was approved by the local Institutional Ethics Committee. PA levels were assessed by means of a triaxial accelerometer. Time spent in sedentary, light-intensity and moderate-to-vigorous (MVPA) activities was determined using Freedson cutpoints (3). Blood samples following a 12-h fasting were taken to determine plasma glucose,

lipid profile, and C-reactive protein. BP was assessed for 24 h using an ambulatory blood pressure monitor. Systolic and diastolic BP (SBP/DBP) mean levels and SBP/DBP load (% of values above normative values) were determined for 24-h, awake and asleep periods (4). cIMT was assessed using a high-resolution ultrasound (Logiq E, General Electric, USA). Patients remained in the supine position with their head slightly extended, while the transducer (3.0-10.0 MHz) was positioned perpendicularly to the orientation of the common carotid artery (CCA), 1-2 cm below the bifurcation, in the longitudinal plane (5). Measurements were performed in the far wall of the right CCA and were analyzed by the Cardiovascular Suite software (QUIPU, Italy). Pearson's correlation coefficients were calculated to test the association between PA levels and BP, traditional cardiovascular risk factors and cIMT. Significance level was set at $p \leq 0.05$. Weekly MVPA (min/week) was negatively associated with mean cIMT ($r=-0.49$, $p=0.02$) and SBP load during sleep ($r=-0.43$, $p=0.04$). Daily steps were negatively associated with 24h-SBP ($r=-0.43$, $p=0.04$), asleep-SBP ($r=-0.47$, $p=0.02$), and SBP load during 24-h ($r=-0.41$, $p=0.05$), awake ($r=-0.41$, $p=0.05$) and asleep ($r=-0.52$, $p=0.01$) periods. Sedentary time and light-intensity PA were not associated with any cardiovascular parameter. In conclusion, higher MVPA levels associate with reduced ambulatory SBP and with improved vascular health in post-menopausal women with RA. This latter emerges a potential new mechanism by which PA may play a cardioprotective role in RA, a hypothesis that requires further validation in a prospective fashion.

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PC83

The Transient Receptor Potential Vanilloid 4 Channel Contributes To Pregnant Human Myometrial Contractility

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Introduction
L-Type voltage-operated Ca^{2+} channels provide the route for bulk Ca^{2+} influx to promote rhythmic uterine contractions during labour. However, they are not the only mediator of Ca^{2+} entry into myometrial cells. TRPV4, a non-selective cation channel, is hypothesized to be capable of activating spontaneously in myometrial cells in the absence of agonist stimulation. This study investigated the contribution of TRPV4 channels to spontaneous and oxytocin stimulated contractions in human pregnant myometrium in vitro.
Methods
Twenty-two biopsies of human myometrium were obtained, with written informed consent, from term (38.6 ±1.6 weeks) non-labouring pregnant women during elective caesarean section (Ethics No. EC00/137). Tissues were dissected and mounted in an organ bath, immersed in physiological salt solution and placed under 29.34mN of tension to establish spontaneous contractions. Subsequently, myometrial strips were exposed to cumulative concentrations (1nM-10μM) of TRPV4 agonist (GSK101 - GSK1016790A) or antagonist (RN17 - RN1734), at 60 min intervals. Myometrial tissues were also pre-treated (60 min) with RN17 (200nM or 1μM) and subsequently treated with GSK101 or oxytocin (1nM-10μM). Data are given as mean ± S.E.M, a p value ≤ 0.05 was deemed statistically significant. Data were analysed using two-way ANOVA with Bonferroni correction to determine differences between treatment and DMSO control groups (% response MIT - mean integral tension or amplitude).
Results
GSK101 produced a dose-dependent inhibitory effect on amplitude (2.30% ±0.45, n=7) and MIT (8.01% ±2.55, n=7) of spontaneous contraction of human myometrium vs control group (n=7). GSK101 (TRPV4 agonist) had the opposite effect to that expected; it decreased contraction amplitude by 33.61% ±7.35 (n=7, p=0.0006) and MIT by 27.53% ±10.17 (n=7, p=0.0191) vs control group (n=7). Pre-treatment of myometrial tissues with RN17 reduced subsequent oxytocin stimulated contractions [% MIT by 47.16 ±27.10, (200nM RN17 +oxytocin, n=4) and 74.32 ±22.14 (1μM, n=4) vs control group (n=3)]. The % change of the amplitude of tissues pre-treated with 200nM and 1μM of RN17 was 19.60 ±9.77 (n=4) and 6.18 ± 20.46 (n=4), respectively. Pre-incubation of myometrial strips with 1μM RN17 reduced the inhibitory effect of GSK101 on the % MIT by 42.02 ±15.68 (n=4),

reaching statistical significance at 10μM of GSK101 (p=0.0071). Pre-incubation with 200nM RN17 also reduced the inhibitory effect of GSK101 by 71.25% ±11.70, reaching statistical significance at 1μM (p= 0.0010) and 10μM (p= 0,0084).
Conclusions
Using the antagonist RN17, these results demonstrate TRPV4 modulates spontaneous and oxytocin-induced contractility in human myometrium. The TRPV4 agonist GSK101 supressed myometrium contractions; potentially through activation of BK channels or off-target effects.
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PC84

Circulating biomarkers of antioxidant status and oxidative stress in people with cystic fibrosis: a systematic review and meta-analysis

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Problem statement: Cystic fibrosis (CF) is a multi-system, life-shortening autosomal recessive disease that affects more than 70,000 people worldwide. Common co-morbidities of CF include obstructive lung disease, pancreatic insufficiency and diabetes mellitus, which contribute to exacerbated free radical production. Additionally, antioxidant status in CF may be compromised due to CF transmembrane conductance regulator deficiency and malabsorption of fat-soluble micronutrients. Consequently, oxidative stress may play an important role in the pathophysiology of CF. This review, therefore, aimed to quantify CF-related circulatory redox imbalances and summarise their relationships with clinical outcomes. Methods: A protocol was registered on PROSPERO (Reference: CRD42018094241). To quantify the extent of CF-redox abnormalities, systematic searches of the Medline, CINAHL, CENTRAL and PsycINFO databases were conducted. Mean biomarker content in whole blood, plasma, serum or erythrocytes from people with clinically-stable CF and non-CF controls within the included studies were used to calculate the standardized mean difference (SMD) and 95% confidence intervals (95% CI) for

meta-analysis. Results: Forty-nine eligible studies were identified, including a total of 1,702 people with CF and 1,583 controls, in which 25 biomarkers were eligible for meta-analysis. Notably, meta-analyses revealed that protein carbonyls (SMD: 1.13, 95% CI: 0.48-1.77), total F_2-isoprostane 8-iso-prostaglandin $F_{2\alpha}$ (SMD: 0.64, 95% CI: 0.23-1.05) and malondialdehyde (SMD: 1.34, 95% CI: 0.30-2.39) were significantly higher, and vitamins A (SMD: -0.66, 95% CI -1.14–0.17) and E (SMD: -0.77, 95% CI: -1.31–0.23), β-carotene (SMD: -1.80, 95% CI: -2.92–0.67), lutein (SMD: -1.52, 95% CI: -1.83–1.20) and albumin (SMD: -0.98, 95% CI: -1.68–0.27) were significantly lowered in the plasma or serum of people with clinically-stable CF versus controls. Fat-soluble vitamin concentrations were positively correlated with indices of lung function and nutritional status, and negatively correlated with age and leukocyte counts. Additionally, markers of lipid peroxidation were positively correlated with age and leukocyte count, and negatively correlated with nutritional status. Conclusions: This systematic review and meta-analysis supports the concept that some circulating biomarkers of oxidative damage and antioxidant capacity are abnormal in people with clinically stable-CF versus controls. These observations implicate redox imbalances in the pathophysiology of CF-related lung disease; however, further research is required to fully understand the implications of oxidative stress on clinical practise, and on common co-morbidities such as lung disease, pancreatic insufficiency and diabetes mellitus.

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PC85

Neuropathological effects of hippocampal amyloidosis induced in a mouse model by Amyloid-β_{25-35} peptide

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Successful memory encoding and information storage require specific patterns of synchronous activity in neural circuits and networks. These processes rely on the accurate equilibrium between excitatory and inhibitory neurotransmission systems driven by different neuronal types. During preclinical stages of Alzheimer's disease (AD), there is an imbalance between both systems mainly due to synaptic dysfunction induced by amyloid-β (Aβ) peptide. Prior to accumulation of the histopathological hallmarks of the disease, this scenario leads to hippocampal hyperexcitability, disrupted oscillatory activity patterns and cognitive deficits. The molecular mechanisms underlying these alterations remain unclear but functional evidence point

to alteration of neuronal excitability playing a pivotal role in early Aβ-induced AD pathogenesis. Previously, $A\beta_{25-35}$ has been proposed as the biologically active fragment of Aβ, and has been shown to induce major neuropathological signs related to early AD stages. Unlike other Aβ isoforms with high clinical relevance such as $A\beta_{1-42}$, $A\beta_{25-35}$ does not form ion-permeable pores in neuronal membrane. Although it has been extensively used to explore acutely the pathophysiological events related with neuronal dysfunction induced by soluble Aβ forms, it is still unknown whether its toxic effects on hippocampal performance mimic the actions of other clinically relevant species.

Here, we have systematically examined the effects of $A\beta_{25-35}$ on the physiological role of the hippocampus at different complexity levels (from synaptic to behavioral levels), with special emphasis on synaptic plasticity processes. Mice were prepared for chronic intracerebroventricular injections, hippocampal electrical stimulation and electrophysiological recordings *in vivo* to correlate neural activity to hippocampal-dependent learning and memory tasks such as novel object recognition and open field habituation tests. Our data reveals that $A\beta_{25-35}$ alters hippocampal synaptic properties (excitability and synaptic plasticity) and triggers an imbalance in the excitatory/inhibitory neurotransmission that causes aberrant network activity and early cognitive impairments with high similarities to $A\beta_{1-42}$ effects. Thus, our results confirm the potentiality of the model to study preclinical stages of hippocampal amyloidopathy at the molecular, synaptic, circuit, and behavioral levels.

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PC86

Modulatory role of Zingiber officials supplements on liver enzymes in type 2 diabetes in rabbits

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Diabetes mellitus, a chronic metabolic disease associated with insidious tissue damage and dysfunctions. Monitoring vital organs functioning status such as the liver is given less attention in many studies. Liver enzymes (alanine aminotransferase (ALT), aspartame aminotransferase (AST) and alkaline phosphatase (ALP)) are clinical indicators that give clue on early detection of disease complications and or adverse effect of therapeutical agents in use.
The aim of the present study is to assess the modulatory role of Zingiber officinale supplements on liver enzymes levels in type 2 diabetes in rabbits. And ethical approval was gotten from Ahmadu Bello University ZARIA Kaduna-Nigeria (ABUCAUC/2017/048).
Twenty male rabbits were grouped into four groups (n=5).
Group 1 were treated with standard animal feeds (SAF) throughout the experiment. Groups 2, 3 and 4 were treated with high fat diet for eleven weeks to induced type 2 diabetes.
Thereafter, group 2 were treated with SAF only, group 3 were treated with standard anticholesterol drugs (cholestran 0.26 mg/kg) and group 4 was treated with the study supplement (12.5 %) for six weeks.
At the end of the treatment blood samples were collected via cardiac puncture and were assay using standard laboratory procedures. Data generated were analyses using SPSS version 20.1.
The results revealed a significant increase in aspartame aminotransferase (AST) and alkaline phosphatase (ALP) activities in group 2 compared to that in group 1. While in group 4 all liver enzymes (ALT, AST and ALP) activity shows a significant decrease when compared to their levels to that of group 2.
In conclusion, Zingiber officials supplementation down-regulate elevated liver enzymes associated with high fat diet induced type 2 diabetes in rabbits.

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PC87

Types of circadian profile of peripheral augmentation index in healthy adolescents

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Arterial stiffness (AS) assessment is a relevant approach in diagnostic of arterial hypertension. AS consists of two components: structural and functional and the last one is most important factor in regulation of blood pressure (BP) during a day in adolescents. Nowadays there is a lack of data about parameters of AS in children and adolescents, especially about their circadian variation. Augmentation index is the parameter of arterial stiffness that characterise the reflected pulse wave. When the augmentation index increses it means that arterial stiffness increses. The aim was to find out typological features of circadian profile of augmentation index in healthy adolescents. We recruited 354 healthy adolescents (170 boys, 184 girls) from 12 to 17 years old to participate in the study. We performed ambulatory blood pressure monitoring by portable monitor, that allows also to analyze pulse waves and arterial stiffness parameters. We analyzed peripheral augmentation index and its average daytime and nighttime levels. Augmentation index showed skewed distribution, so for the descriptive statistic we used median and 25^{th} and 75^{th} percentile, Mann-Whitney test for comparison of 2 groups and Kruskal — Wallis test for multiple comparison. The median (25^{th}; 75^{th} percentile) of augmentation index was -60% (-65; -54) in girls and -64% (-70; -58) in boys at daytime, -61% (-66; -52) in girls and -62% (-70; -55) in boys at nighttime. We performed cluster analysis by k-means to distinguish types of circadian rhythm of peripheral augmentation index. As the result we have found three clusters. The first cluster consists of 53% (n=77) girls and 47% (n=69) boys. The second one consists of 59% (n=55) of girls and 41% (n=37) of boys. In the third cluster there were 43% (n=52) of girls and 57% (n=64) of boys. The first type (cluster) of circadian rhythm of augmentation index was relative constant during 24-hours (median -63% during daytime and -64%, during nighttime ($p>0,05$). The second type (cluster) showed a significant 8% increase in the amplitude of the reflected wave at night. The third profile (cluster) showed a significant 12% decrease in the average level of augmentation index at night. The adolescents with the second type of circadian rhythm had significant higher levels of systolic, diastolic and pulse pressure at night in comparison with others ($p<0,05$). Moreover, in the second cluster the percentage of occurrence of insufficient decrease of blood pressure at night (non-dipping blood pressure

profile) was 32% and it was significantly higher than in other groups (p<0,05). Conclusion: there are three types of circadian profile of augmentation index in adolescents. The second type is adverse and should be considered as a new risk factor of arterial hypertension development.

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PC88

Iron deficiency impacts duodenal paracellular calcium absorption via a mechanism involving claudin 2

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Iron deficiency is the most common nutritional deficiency worldwide and has been linked to bone disease and impaired calcium metabolism (1,2). However, how this specifically affects the pathways of intestinal calcium absorption is unknown. Based on previous findings that paracellular calcium absorption is dominant under normal dietary calcium levels (3), we investigated the effect of diet-induced iron deficiency on paracellular calcium absorption. We focused our studies on the duodenum as this is the major segment responsible for iron absorption.

Six-week-old male Sprague-Dawley rats were fed an iron deficient (ID) diet containing 2-6 ppm of iron, or a matched control (C) diet, containing 48 ppm of iron for 2-weeks. On the day of experimentation, the animals were anaesthetised with an intraperitoneal injection of pentobarbitone sodium (45mg/kg) and the effect of iron deficiency on duodenal calcium absorption was tested using the *in vivo* ligated loop technique. A calcium concentration of 100mM was used to favour the paracellular route. At the end of the experiment, serum and plasma samples were collected via cardiac puncture for iron, ferritin and calcium assays. Additionally, duodenal mucosa was collected for RT-qPCR and Western blotting to examine the potential proteins involved in changes in calcium absorption. Following tissue collection, the animals were killed by excising the heart. Values are presented as means ± S.E.M and analysed using an unpaired student's t-test.

A significant decrease in serum iron (C: 19.6 ± 2.3 vs ID: 8.6 ± 1.3 μmol/L p<0.01, n=4-7) and plasma ferritin (C: 172.7 ± 37.9 vs ID: 41.5 ± 10.8 ng/ml p<0.05, n=4) levels confirmed that the rats were iron deficient. Although serum calcium levels were unaffected (C: 2.03 ± 0.02 vs ID: 2.09 ± 0.04 mM, n=11-12), diet-induced iron deficiency significantly increased duodenal calcium absorption (C: 197.7 ± 14.0 vs ID: 265.3 ± 3.2 nmoles of calcium transferred in to 1ml of plasma per 5cm, p<0.05, n=5) after 30 minutes. Iron deficiency upregulated vitamin D receptor (VDR) mRNA (C: 0.0153 ± 0.0008 vs ID: 0.0229 ± 0.0016 a.u. p<0.01, n=5-6) and protein

(C: 0.07 ± 0.02 vs ID: 0.7 ± 0.2 a.u. $p<0.05$, n=5-6) expression, but did not affect circulating vitamin D levels (C: 150.7 ± 7.8 vs ID: 144.0 ± 7.2 fmol/L n=6). Interestingly, mRNA (C: 0.0004 ± 0.0001 vs ID: 0.0035 ± 0.0007 a.u. $p<0.001$, n=5-6) and protein (C: 0.20 ± 0.06 vs ID: 0.67 ± 0.10 a.u. $p<0.05$, n=3) expression of the vitamin D-sensitive claudin 2 also increased.
This is the first study demonstrating that diet-induced iron deficiency increases duodenal paracellular calcium absorption via a mechanism that may involve VDR and claudin 2. Further studies are required to elucidate the cellular mechanisms involved and how these changes relate to the altered calcium metabolism and bone disease seen in individuals with iron deficiency.

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PC89

Comparison between young adults with hypertensive parents and those with normotensive parents on the effects of slow breathing and mental stress on the cardiac component of the baroreflex and heart rate variability (HRV)

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Individuals with hypertensive parents (FH+) have greater risk of hypertension than those with normotensive parents (FH-). Whether autonomic control of arterial blood pressure (ABP) is impaired in young normotensive FH+ is unclear. Thus, in 9 FH- and 11 FH+ (18-25 years), we recorded ABP, ECG, respiration, baroreflex sensitivity (BRS) by the sequence method (changes in R-R interval evoked by spontaneous increases and decreases in systolic pressure (SP)), and as change in R-R interval evoked by the fall in SP that occurs on standing from a squat position, at rest, during slow breathing for 5 min at 6 breaths/min, and during mental stress for 3 min (Colour Stroop test). HRV was computed by time- and frequency domain analyses under each condition. Comparisons with and between FH+ and FH- were done by paired and unpaired t-tests respectively.
FH- and FH+ had similar mean ABP (mABP: 85±0.8 vs 90±2.7 mmHg) and respiration (16.1±1.2 vs 15.9±0.6 breaths/min) at rest. Mental stress increased ABP, HR, and respiratory frequency in both FH+ and FH-, but the increase in ABP tended to be greater in FH+ ($P=0.07$). Vagal indices of HRV (RMSSD and pRR50) decreased during mental stress in FH+, but not FH- (RMSSD: 70.3±14.3 to 45.9±8.8*; 67.2±14.8 to 62.2±23.3 respectively, *: $P<0.05$ within group). Further, Further, BRS was

decreased in response to up- and down-sequences in SP during mental stress in FH+, but during down sequences only in FH- (1.2±0.05 to 1.0±0.05* and 1.2±0.05 to 0.9±0.05*; 1.2±0.08 to 1.0±0.06 and 1.2±0.08 to 0.9±0.08* respectively). Moreover, BRS during squat to stand, was lower under resting conditions in FH+ than FH- (0.48 vs 0.69†; †: P<0.05 FH+ vs FH-), but BRS during squat to stand following mental stress decreased in FH- only (to 0.37*). On the other hand, slow breathing increased BRS during up-sequences in FH+ (1.2±0.05 to 1.3±0.05*), but not FH- (1.18±0.08 to 1.34±0.08ms/mmHg), and increased RMSSD, in FH+ only (70.3±14.3 to 78.9±13.5*).

These results suggest that young, normotensive FH+ tend to show a greater pressor response to mental stress than FH-, which is accompanied by greater depression of BRS and a substantial decrease in the vagal influence over HR. Thus, it seems BRS regulation of ABP via the vagus is disturbed more during mental stress in FH+ than FH-. On the other hand, as BRS during squat to stand is well-maintained following mental stress in FH+ but not FH-, it seems that young FH+ lack central neural depression of the sympathetic component of BRS that occurs during mental stress in FH-. Since slow breathing enhanced the BRS and increased vagal control over HR in FH+, it may be that regular slow breathing may restore vagal-sympathetic regulation of HR and BRS in FH+ and reduce their future risk of hypertension.

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PC90

Acute effects of resistance exercise on appetite and energy intake in older adults.

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Ageing is associated with reductions in appetite and food intake (1) resulting in unintentional weight loss (2). Such weight loss, particularly through muscle mass reduction is associated with muscle weakness and functional decline, which represent significant predictors of poor health outcomes (3) and are contributing factors to frailty in older people. Resistance exercise is crucial in attenuating age-induced muscle loss, whilst muscle mass gains have been proposed as a means of increasing appetite and energy intake (EI) through changes in metabolic demand (4). Exercise intensities > 60% of maximal oxygen uptake have consistently been shown to acutely supress appetite in younger adults in a variety of exercise modes (5), yet little is known about the acute effects of resistance exercise on appetite and energy intake particularly in older adults. Understanding these effects may aid exercise prescription and provide post-exercise feeding strategies to reduce age-related anorexia and ultimately attenuate age-associated reductions in muscle

mass. Therefore, we investigated the effect of an acute bout of resistance exercise on appetite and EI. Twenty healthy, older adults (13 females and 7 males; 68 ± 5 years old, body mass index of 26.2 ± 4.5 kg. m^{-2}) undertook two 5-h experimental trials. On arrival at the laboratory, participants rested for a 0.5-h period before they were given a standardised breakfast. They then rested for a 1-h period before they completed in a randomised crossover design: 1) a 1-h resistance exercise workout followed by 2-h of rest and 2) a control condition where participants rested for a subsequent 3-h period. On cessation of the trials, participants were administered an *ad libitum* pasta meal for the assessment of EI. Composite appetite scores (CAS) were measured throughout using visual analogue scales. A paired samples t-test revealed no difference in EI between conditions. Two-way ANOVA revealed a significant effect of condition (p = 0.007) and time (p <0.001) but no significant interaction effect (p = 0.153) for CAS, which were lower in the resistance exercise condition. Similarly, area under the curve for the entire trial was significantly different (p = 0.007) between conditions and was associated with a small effect size (Cohen's *d = 0.27).* Our findings suggest that an acute resistance exercise bout does not result in EI reductions during an *ad libitum* meal given 2 h post-exercise, in spite of significant main condition effects in CAS. This significance can be attributed to the temporary suppression of appetite during the resistance exercise bout. Given that appetite profiles remained similar between conditions for the rest of the trial, and that feeding is likely to take place post-exercise, this mode of exercise is an appropriate means for optimising muscle mass adaptations by maintaining subsequent EI of older adults.

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PC91

Vitamin C enriched collagen supplementation before resistance exercise does not affect a biomarker of collagen synthesis in healthy young trained men

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Previous research has demonstrated a dose-response of collagen supplementation on a blood-borne biomarker of collagen synthesis (procollagen type I N-terminal propeptide (PINP)) following jump rope (skipping) exercise (Shaw et al., 2017). However, skipping does not overload the muscle-tendon unit (MTU) to the same degree as resistance exercise (RE), therefore these data may linked to bone rather than MTU collagen turnover. We therefore investigated the impact of hydrolysed collagen (HC; 0 g, 15 g, or 30 g) supplementation on serum PINP following high-intensity RE. Ten resistance-trained young men (mean ± SD; age: 26.03 ± 3.25 years; height: 177.9 ± 3.63 cm; mass: 79.7 ± 7.03 kg) participated in three trials (separated by a 7-day washout period) in a double-blind, randomised cross-over design study. In each trial, participants consumed a beverage comprising HC plus 50 mg vitamin C prior to performing 4 sets of 10-RM back-squats. Venous blood samples were collected at REST prior to HC ingestion, 2 h-POST RE, and 6 h-POST RE and the sera were analysed for PINP via ELISA. Serum PINP concentrations for 0 g, 15 g and 30 g HC, respectively, were 20.27 ± 4.64, 19.97 ± 3.53, and 20.56 ± 3.99 ng/mL (REST); 20.52 ± 3.92, 20.12 ± 3.42, and 19.65 ± 4.17 ng/mL (2 h-POST); 21.38 ± 4.42, 19.20 ± 3.71, and 19.77 ± 5.05 ng/mL (6 h-POST). Serum PINP concentration did not change as a consequence of HC dose, time or RE. Our results suggest that there is either no HC dose-response following high-intensity RE on lower-limb MTU collagen synthesis, that serum PINP is not a sensitive biomarker of MTU collagen synthesis, or that serum PINP increased during RE but had returned to resting concentration by 2 h post RE. Skipping is likely a more potent stimulator of bone collagen turnover, for which impact force is more important than high muscle forces, thus potentially explaining the discrepancy between our findings and those of Shaw *et al*. (2017). Previous studies have shown that serum PINP increases during high-intensity exercises (running and drop jumps) but returns to resting levels immediately after finishing exercises (Scott *et al*., 2011; Clifford *et al*., 2019). Future studies investigating the dose-response of collagen supplementation before RE on MTU collagen synthesis should measure serum PINP immediately after RE and ideally include direct measurements of MTU collagen turnover.

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