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It is a requirement of The Society that all vertebrates (and Octopus vulgaris) used in experiments are humanely treated and, where relevant, humanely killed.

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For experiments involving neuromuscular blockade, the abstract must give the type and dose, plus the methods used to monitor the adequacy of anaesthesia during blockade (or refer to a paper with these details). For the preparation of isolated tissues, including primary cultures and brain slices, the method of killing (e.g. terminal anaesthesia) is required only if scientifically relevant. In experiments where genes are expressed in Xenopus oocytes, full details of the oocyte collection are not necessary. All procedures on human subjects or human tissue must accord with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission; authors must tick the appropriate box to indicate compliance.

SA01

Understanding liver metabolism in NAFLD

Leanne Hodson[1]

[1]University of Oxford, United Kingdom

The liver is a key metabolic organ that undertakes a multitude of physiological processes. It serves as an intermediary organ between exogenous (dietary) and endogenous energy supply to extrahepatic organs, with hepatocytes rapidly transitioning back and forth between the metabolic tasks of energy storage and supply. Given its pivotal role in regulating systemic metabolism, perturbations in hepatic metabolism can impact on metabolic disease risk. For example, the accumulation of intra-hepatocellular triglyceride (IHTG), which likely results from an imbalance between fatty acid delivery to the liver, hepatic fatty acid synthesis and fatty acid removal (via oxidation or export as triglyceride (TG)) from the liver.

Humans spend the majority of the day in a postprandial, rather than postabsorptive state and when dietary fat and carbohydrates are consumed, a series of complex metabolic processes ensures that these nutrients are absorbed, transported around the body and stored appropriately. As the liver plays a major role in regulating fat and carbohydrate metabolism, perturbations in these metabolic processes have the potential to impact on metabolic health. For example, whether fatty acids are partitioned toward oxidation or esterification pathways appears to be dependent on a number of metabolic factors; not least ambient insulin concentrations. Moreover, the nutrient content of the diet appears to play a key role in intrahepatic fatty acid partitioning.

This talk will review insights gained from undertaking studies using in vivo and in vitro models of human liver metabolism and discuss how metabolic and nutritional state may alter hepatic fatty acid partitioning. The usefulness of these models in understanding the aetiology and development of NAFLD will be highlighted

SA02

Glucose-dependent insulinotropic polypeptide regulates food intake and body weight via the area postrema in mice

Jo Lewis[1], Fiona Gribble[2], Frank Reimann[3]

[1]University of Cambridge, UK

The development of dual agonists for the glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide receptor (GLP-1R and GIPR respectively) is a landmark moment in the treatment of type 2 diabetes and obesity. In preclinical and clinical studies, GLP-1R-GIPR co-agonism improves glycemia and reduces body weight superior to GLP-1R agonism alone, however the location and role of GIPR activation remains incompletely understood. Evidence suggests that long acting GIPR agonists act in the CNS to reduce food intake and body weight via an inhibitory GABAergic neuronal population. Here we demonstrate that acyl-GIP decreases food intake in lean mice but not in mice with deletion of GIPR in the area postrema, a component of the hindbrain's dorsal vagal complex (DVC), which lies at the caudal end of the brainstem and integrates sensory information from the gastrointestinal tract, controls food intake and aversive responses. Furthermore, we demonstrate this population is responsible for alleviating the avoidance response, a proxi for an antiemetic action in mice, to PYY by acyl-GIP. Our data demonstrate that long acting GIPR agonists depend on GIPR signalling in the area postrema to decrease food intake and body weight.

SA03

Involvement of astrocytes in the regulation of food intake and glucose homeostasis

Kate Ellacott[1]

[1]University of Exeter Medical School, UK

Precise control of energy homeostasis, the balance between food intake and energy expenditure, is essential for human and animal health. An imbalance between energy intake and expenditure can contribute to the development of obesity. The brain co-ordinates feeding behaviour by integrating information transmitted via hormonal, nutrient, and neural inputs from the periphery. The hypothalamus is the brain's main integratory hub controlling long-term energy homeostasis. Direct neuronal projections connect the hypothalamus to the brainstem and, in turn, the vagus nerve bi-directionally connects the brainstem to the digestive tract. Pharmacological and genetic studies in rodents have begun to unravel the neural circuits regulating energy homeostasis and how these change during obesity. While most of the field has historically focused on neurons, our group has contributed to understanding how non-neuronal cells such as glial cells called astrocytes contribute to these key regulatory circuits. This talk will summarise studies from our group examining a role of astrocytes in the regulation of feeding and glucose homeostasis, and reflect on the mechanisms by which these cells integrate neuroendocrine and nutritional cues to impact physiology and pathophysiology. Data will also be presented on our efforts to refine animal models of metabolic disease to improve animal welfare and translatability of data to human physiology.

SA04

Food-entrainment of the circadian timekeeping in the brainstem satiety centre

Lukasz Chrobok[1], Charlotte Muir[2], Tanya Chonkria Kaur[3], Iliana Veneri[4], Timna Hitrec[5], Michael Ambler[6], Anthony Edward Pickering[7], Hugh David Piggins[8]

[1]University of Bristol, UK, [2]University of Bologna, Italy

Introduction: The timing of food intake is a critical determinant of metabolic health, with misaligned feeding schedules contributing to obesity, cardiovascular disease, and type-2 diabetes. While the suprachiasmatic nucleus (SCN) is well established as the master circadian pacemaker, emerging evidence highlights the importance of extra-SCN clocks in metabolic regulation. In our work, we have identified the dorsal vagal complex (DVC)—a brainstem satiety hub composed of the area postrema (AP), nucleus of the solitary tract (NTS), and dorsal motor nucleus of the vagus (DMV)—as a previously unrecognised site of autonomous circadian timekeeping. The DVC integrates visceral and hormonal signals to control feeding and energy balance, and we hypothesised that it may play a role in encoding daily patterns of food intake. Here, we investigated whether and how feeding schedules entrain circadian gene expression in the DVC.

Methods: Adult C57BL/6J mice (8–12 weeks, both sexes) were housed under a 12:12h light-dark cycle and assigned to one of four time-restricted feeding (TRF) conditions: ad-libitum or 6-hour feeding windows during the early light (ZT0–6), late light (ZT6–12), or early dark phase (ZT12–18) for a week. Animals were then culled at 4 or 6h intervals over a 24h period. The DVC was isolated and analysed using qPCR, NanoString nCounter, and RNAscope in situ hybridisation to assess rhythmic expression of clock genes and neurotransmitter receptor genes. Rhythmicity and phase shifts were analysed using sine wave fitting. All experimental procedures were approved by the University of Bristol Animal Welfare and Ethical Review Body and conducted under the UK Animals (Scientific Procedures) Act 1986, under a valid Home Office project licence.

Results: Under ad-libitum conditions, 23 and 32 transcripts (out of 84 tested) showed significant 24h rhythmicity in the AP and NTS, respectively (n=5/each of 6 timepoints; $p<0.05$). TRF (n=16 per feeding condition) caused robust phase shifts in clock gene expression across DVC subregions, aligning with feeding time rather than the light-dark cycle. Notably, rhythmic expression of key neurotransmitter receptor genes also followed the timing of food availability. The precision of food entrainment was significantly greater in the NTS compared to the AP ($p=0.0223$, paired t-test), suggesting regional differences in metabolic clock plasticity. Altogether, these results indicate that feeding time is a dominant cue for circadian programming in the DVC.

Conclusion: We demonstrate that circadian timekeeping in the brainstem satiety and timekeeping centre is malleable and entrainable by meal timing. This newly discovered circadian node may be an essential component of the broader food-entrainable oscillator network, acting independently of the SCN. Our findings have implications for the design of chrono-nutrition strategies, suggesting that aligning feeding schedules with endogenous brainstem clocks may help optimise satiety signalling and metabolic health. Ongoing behavioural studies are investigating whether this mechanism contributes to food anticipatory activity and exploring neural mechanisms of DVC's food entrainment.

SA05

Circadian clock control of adipocyte function

Louise Hunter[1]

[1]University of Manchester, United Kingdom

The molecular circadian clock is a transcription-translation feedback loop which serves as a timekeeping mechanism in most nucleated mammalian cells. Work from our lab and others has been directed at understanding the contribution of the clock to adipocyte function and response to obesity. In particular, we have focused on the role of REV-ERBalpha (NR1D1), a core clock protein and transcriptional repressor. In a transgenic mouse with adipocyte-targeted Nr1d1 deletion, we see enhanced adiposity with diet-induced obesity, with diminished ectopic lipid deposition, and relative sparing from obesity-related pathology. In my talk, I will discuss our past and present efforts to understand the mechanism underlying this remarkable 'healthy' obesity phenotype.

SA06

Hyperinsulinemia as a primary cause of obesity

James Johnson[1]

[1]University of British Columbia, Canada

The global prevalence of obesity and overweight were 16% and 43% in 2022 according to the World Health Organization. The rise in these numbers increases the risk of many diseases including diabetes, heart disease, dementia, and cancer. The systemic and physiological causes of obesity are multi-level and multi-factorial, and may differ between individuals. In this lecture, evidence will be reviewed from human studies and animal models supporting the concept that hyperinsulinemia is a key causal driver in the development of obesity. Physiological mechanisms will be addressed. Potential interventions will be discussed, as well as the potential requirement for individualized approaches to match the physiology of the patient. The limitations of our current knowledge, as well as a roadmap for future studies will be discussed.

SA07

Neuroimaging of appetite and addiction

Anthony P. Goldstone[1]

[1]Imperial College London, United Kingdom

There is evidence from pre-clinical studies that food intake and appetitive gut hormones, including ghrelin and glucagon-like peptide-1 (GLP-1), not only alter appetite, but also food and non-food reward processing, as well as addictive behaviours influencing eating behaviour. There is more limited evidence from human studies. This talk will review our human studies using multi-modal phenotyping, including functional MRI, to study the food-gut-brain axis in eating and addictive behaviours.

Using a platform of experimental medicine outcome measures including functional MRI paradigms with a food picture evaluation task (to assess cue reactivity or anticipatory reward) [Goldstone AP et al. Eur J Neurosci 2009; Scholtz S et al. Gut 2014; Goldstone AP et al. AJCN 2014; Goldstone AP et al. JCEM 2016], we have studied the effects of food intake, acute administration of orexigenic hormone acyl ghrelin, and comparison of Roux-en-Y gastric bypass (RYGB) with gastric banding surgery for obesity, and acute pharmacological suppression of satiety gut hormones post-bariatric surgery, on eating behaviour and food cue reactivity. Our more recent studies have examined (i) the longitudinal effects of RYGB surgery, endoscopic insertion of the duodenal-jenunal bypass liner (Endobarrier) device for obesity and diabetes (compared with standard medical management), (ii) in our Gut Hormone in Addiction (GHADD) study, the effects of acute infusion of the GLP-1 analogue, Exenatide, in obesity, ex-smokers or abstinent alcohol dependence, and (iii) differences in eating and addictive behaviours in adults with obesity with vs. without binge eating symptoms, while (iv) ongoing studies are examining the role of the recently identified endogenous anorexigenic liver/intestinal hormone LEAP2 that is an inverse agonist at the ghrelin GHSR receptor.

Changes in appetite and food cue reactivity with fasting or after food intake appear contributed to by reciprocal changes in gut hormones such as acyl ghrelin and LEAP2. Reduced food cue reactivity and motivation for food after RYGB surgery appears related to post-surgical exaggerated satiety gut hormones PYY and GLP-1 responses. Our results also indicate: (i) a potential for GLP-1 analogues in preventing smoking cessation weight gain, (ii) enhanced wanting and liking of high fat food, impaired non-food related motor response inhibition (impulsivity) and alterations in negative emotional reactivity in adults with vs. without binge eating symptoms, and (iii) a stimulatory effect of GHSR signalling on striatal high-energy vs. low-energy food cue reactivity, that is attenuated by LEAP2 acting as a satiety hormone.

SA08

Understanding how the MRAP2 accessory protein facilitates metabolic GPCR function

Caroline Gorvin[1]

[1]University of Birmingham, United Kingdom

The melanocortin-2 receptor accessory protein-2 (MRAP2) is a single transmembrane protein that interacts with metabolic G protein-coupled receptors (GPCRs) including the melanocortin receptor 4 (MC4R) and the ghrelin receptor (GHSR) to potentiate their signalling. Human mutations in MRAP2 cause obesity, with hyperglycaemia and hypertension. However, functional studies that have only measured the effect of MRAP2 variants on MC4R-mediated cAMP signaling have produced inconsistent findings and most do not reduce MC4R function. Moreover, there are unanswered questions regarding how MRAP2 forms heterodimers with GPCRs, the structural regions involved in facilitating GPCR signalling and how human mutations in MRAP2 affect receptor signalling and trafficking. Here we used single-molecule microscopy and a range of signalling assays to demonstrate that: i) MRAP2 variants which have been identified in overweight or obese individuals impair MC4R function by multiple signalling pathways; ii) that MRAP2 directly interacts with the melanocortin receptor 3 (MC3R) that regulates timing of sexual maturation, rate of linear growth and lean mass accumulation; and iii) MRAP2 has unique structural regions that help facilitate GPCR signalling. I will discuss how these studies have revealed new insights into the molecular mechanisms by which MRAP2 regulates GPCR function and how these pathways are disrupted by obesity-associated MRAP2 genetic variants.

SA09

Metabolic Small Talk: Metabokine Interorgan Signalling in Metabolic Health and Disease

Lee Roberts[1]

[1]University of Leeds , UK

Metabolites were considered intermediates or end-products of metabolism, passive participants changed by metabolic processes. There is emerging evidence of metabolites, which function to mediate cellular signalling and interorgan crosstalk, regulating local metabolism and systemic physiology. These signals have been termed metabokines. There is impetus to uncover novel metabokine signalling axes to understand how these are perturbed in metabolic diseases and determine their utility as therapeutic targets.

This talk will provide an overview of our research identifying novel metabokine signals:

1) A bioactive non-protein β-aminoacid secreted from skeletal muscle during exercise, which induces adipose tissue thermogenesis and liver β-oxidation with anti-obesity and anti-diabetic effects (1).

2) Monocarboxylic acids secreted from thermogenic adipose tissue, which induce a thermogenic phenotype in white adipose tissue and oxidative energy metabolism in skeletal muscle to reduce adiposity, increase energy expenditure and improve glucose and insulin homeostasis in models of obesity and diabetes (2).

3) A lipid released from skeletal muscle in response to lipotoxcity in obesity, which functions as a cell non-autonomous paracrine signal propagating Endoplasmic Reticulum stress with implications in the development of metabolic disease (3).

Our discoveries further our understanding of obesity, and the potential therapeutic role that metabokines may have to treat obesity and related cardiometabolic diseases.

SA10

Novel adipocyte signature genes

Alexander Bartelt[1]

[1]Technical University of Munich, Germany

Adipose tissue plays a pivotal role in metabolic homeostasis, yet the molecular signatures defining distinct adipocyte populations are only beginning to be unveiled. This lecture presents recent advances in the identification of novel adipocyte signature genes, revealed through integrative epigenetic, transcriptomic and single-cell analyses. We explore how these genes distinguish white, beige, and brown adipocytes, shedding light on their specialized functions in energy storage and thermogenesis. Particular attention is given to regulatory pathways, intercellular communication, and implications for obesity and metabolic diseases. Understanding these unique gene signatures opens new avenues for targeted therapeutic strategies in the treatment of metabolic disorders.

SA11

Precision nutrition in obesity: Implications for the prevention of cardiometabolic risksubmission

Ellen Blaak[1]

[1]Department of Human Biology, Maastricht University Medical Centre+, The Netherlands

Obesity and type 2 diabetes (T2D) are major global health concerns, yet long-term outcomes of dietary prevention strategies remain suboptimal. One limitation is that current nutritional guidelines fail to account for individual metabolic variability. Variations in insulin resistance (IR) across diverse tissues, including adipose tissue, muscle, and liver, exert a pivotal influence on cardiometabolic risk, modulating the body's nutrient processing in response to dietary interventions. Even within cohorts exhibiting overweight and normal glucose tolerance, discernible phenotypes of liver or muscle IR can be delineated, characterized by distinct microbial, metabolomic, lipidomic, and adipose tissue transcriptome profiles.

Specifically, dietary intervention tailored to liver (LIR) or muscle insulin resistance (MIR) phenotypes led to improved insulin sensitivity and cardiometabolic health (PERSON), independent of weight loss, demonstrating the potential of precision nutrition. To further refine this approach, we employed data-driven clustering to stratify individuals based on glucose homeostasis and body composition. Using an iterative Hierarchical Clustering of Principal Components on a large cohort (The Maastricht Study), we identified six metabotypes. These were validated and classified with high accuracy (71–75%) using a Random Forest model and inform a current precision nutrition study.

Obesity and T2D correlate with microbial dysbiosis, with initial microbial composition emerging as a potential determinant in lifestyle-induced cardiometabolic health outcomes. Indeed, a small part of the benefits of metabotype-tailored diets in PERSON may be mediated by gut microbial metabolism. Nevertheless, to induce pronounced effects on host metabolism through microbial modulation, a more robust approach may be required based on the amount and type of dietary fibers. These insights into microbiota and host tissue metabolism will be deliberated within the framework of devising targeted lifestyle strategies to ameliorate cardiometabolic health in individuals with obesity.

Keywords: obesity, precision lifestyle strategies, insulin resistant phenotype, nutrition, microbiota composition, diet, cardiometabolic health

SA12

Technologies for understanding functional relationships between metabolites and proteins

Edward Chouchani[1]

[1]Harvard Medical School, US

Protein regulation of metabolic processes is foundational to biology. However, a comprehensive view of functional relationships between metabolites and proteins is lacking. I will discuss a new mass spectrometry-based approach that leverages genetic diversity to nominate functional relationships between hundreds of metabolites and thousands of proteins in living tissues.

SA13

"Insights into Obesity and Metabolic Disease: Lessons from the Greenlandic Inuit"

Torben Hanson[1]

[1]University of Copenhagen, Denmark

In this talk, I will explore how integrative genomic and proteomic approaches are advancing our understanding of obesity and diabetes susceptibility across diverse populations. Drawing on recent work at the University of Copenhagen, I will first discuss key genetic determinants of cardiometabolic traits in the Greenlandic population, where unique genetic architecture—shaped by Arctic adaptation—has revealed high-impact variants that significantly influence obesity and diabetes risk. Furthermore, I will share emerging data from proteomic studies in children, highlighting early molecular signatures linked to metabolic health trajectories. By integrating genetic and protein-level insights across age groups and ancestries, this work underscores the importance of diversity in precision medicine.

C01

Hydroxysteroid 17β-dehydrogenase 13 (Hsd17b13) knockdown attenuates liver steatosis in high-fat diet obese mice

Nimesh Mody[1], Shehroz Mahmood[2], Dawn Thompson[3], Justin Rochford[4]

[1]University of Aberdeen, United Kingdom

Hydroxysteroid 17β-dehydrogenase 13 (HSD17B13) loss-of-function gene variants are associated with a decreased risk of metabolic dysfunction-associated steatotic liver disease (MASLD). Our RNA-seq analysis of steatotic liver from obese mice ± fenretinide treatment identified major beneficial effects of fenretinide on expression of hepatic genes including Hsd17b13. We sought to determine the relationship between Hsd17b13 expression and MASLD and to validate it as a therapeutic target by liver-specific knockdown. Hsd17b13 expression, which is unique to hepatocytes and associated with the lipid droplet, was elevated in multiple models of MASLD and normalised with the prevention of obesity and steatotic liver. Direct, liver-specific, shRNA-mediated knockdown of Hsd17b13 (shHsd17b13) in high-fat diet (HFD)-obese mice (n = 8–9 male C57BL/6J per group), markedly improved hepatic steatosis with no effect on body weight, adiposity or glycaemia. shHsd17b13 decreased elevated serum alanine aminotransferase (ALT), serum fibroblast growth factor 21 (FGF21) levels, and markers of liver fibrosis, for example, expression of Timp2. shHsd17b13 knockdown in HFD-obese mice and Hsd17b13 overexpression in cells reciprocally regulated expression of lipid metabolism genes, for example, Cd36. Global lipidomic analysis of liver tissue revealed a major decrease in diacylglycerols (e.g. DAG 34:3) with shHsd17b13 expression and an increase in phosphatidylcholines containing polyunsaturated fatty acids (PUFA) for example, phosphatidylcholine (PC) 34:3 and PC 42:10. Expression of key genes involved in phospholipid and PUFA metabolism, for example, Cept1, was also reciprocally regulated suggesting a potential mechanism of Hsd17b13 biological function and role in MASLD. In conclusion, Hsd17b13 knockdown in HFD-obese adult mice was able to alleviate MASLD via regulation of fatty acid and phospholipid metabolism, thereby confirming HSD17B13 as a genuine therapeutic target for MASLD and the development of liver fibrosis.

C02

Palmitate Exposure Impairs Autophagic Flux and Associated Signalling Pathways in Immortalised Human Primary Myotubes

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[1]Department of Sport and Exercise Sciences, Institute of Sport, Manchester Metropolitan University, United Kingdom, [2]Department of Life Sciences, Manchester Metropolitan University, United Kingdom

INTRODUCTION

Lipid infiltration into skeletal muscle in conditions such as obesity and type II diabetes is hypothesized to interfere with proteostatic signalling, leading to increased proteolysis and atrophy(1). Evidence from other cell types/tissues suggests lipid infiltration can inhibit autophagy(2), a cellular recycling process essential for the maintenance of skeletal muscle mass and quality(3), however this has not been investigated in human skeletal muscle. Therefore, we aimed to investigate the effect of high-fat exposure on autophagic flux and associated signalling events in immortalised human primary myotubes.

METHODS

Immortalised human primary myotubes (C25/KM155 line) were exposed to palmitate (PAL, 500uM), or control media (CTRL), for 24h. Myotubes were then either immediately collected (BASAL, n=7 per condition) or media changed to EBSS for 5h to deprive cells of nutrients (STARVE, n=5 per condition), to activate autophagy. A subset of cells were incubated with Bafilomycin A1 (100nM) for 2h prior to collection to assess pre-lysosomal autophagic flux. Immunoblotting was then completed for LC3 (autophagic flux) and a range of associated signalling targets. Two-factor independent measures ANOVAs were used to assess differences between conditions, with significance threshold set at $p<0.05$. Data is presented as Mean ± SD.

RESULTS

Main effects of condition and nutrient deprivation (both $p<0.001$) were observed for pre-lysosomal autophagic flux whereby STARVE elevated flux in both CTRL and PAL (2.2-fold & 3-fold respectively) but overall was lower in PAL. This was not mirrored by more commonly used LC3II/I ratio, where no differences were observed, suggesting this 'static' measure of autophagy does not reflect flux. Further main effects of condition showed AMPK-mediated ULK1^{Ser555} phosphorylation and Beclin1 protein content to be lower in PAL irrespective of nutrient availability ($p\leq0.036$), indicating a potential impairment of autophagy induction in PAL. Markers of mTORC1 activity (p-ULK1^{Ser757}, p-RPS6$^{Ser240/244\ \&\ Ser235/236}$) displayed main effects of nutrient deprivation whereby STARVE was lower than BASAL irrespective of condition ($p\leq0.026$). However, when expressed relative to basal mean values, the reduction in p-RPS6$^{Ser240/244\ \&\ 235/236}$ following STARVE was less pronounced with PAL (72±2% vs. 83±6%, p=0.009 & 66±4% vs. 74±3%, p=0.005 respectively).

CONCLUSION

We show that palmitate exposure impairs absolute pre-lysosomal autophagic flux but not responsiveness to nutrient withdrawal. This impairment may be due to reduced autophagy induction displayed by lower Beclin1 content and AMPK-mediated ULK1^{Ser555} phosphorylation. These data provide initial evidence of a potential detrimental effect of high-fat/lipid exposure on autophagy in human skeletal muscle which warrants further research in vivo.

C03

Acriflavine improved non-alcoholic fatty liver disease by preventing obesity and metabolic syndrome

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[1]University of Ibadan, Nigeria, [2]University of Medical Sciences, Ondo, Nigeria

Introduction: Non-alcoholic fatty liver disease (NAFLD), a spectrum of liver disorders ranging from simple steatosis to steatohepatitis related cirrhosis is emerging as the leading cause of liver morbidity and mortality. While the exact cause of the disorder is presently unknown, obesity and other components of the metabolic syndrome are suggested to play causal role in its incidence and progression. Presently, there is no medication for NAFLD and this has prompted research efforts to understand its pathogenesis and find cure for the disorder. Acriflavine, has been reported to possess activity in the treatment of obesity, right ventriclar hypertrophy and right ventricle systolic pressure. Based on this, we hypothesized that acriflavine would be an effective remedy for NAFLD.

Aim/objectives: This study was designed to explore the pathogenesis of NAFLD, the potentials of acriflavine to act as remedy for the disorder, and to investigate probable mechanisms through which this might occur in wistar rats.

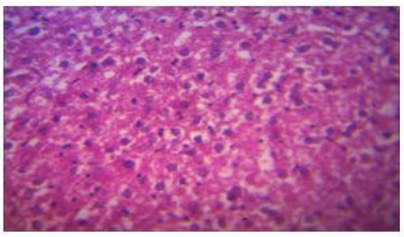
Methods: Twenty male Wistar rats (100-120g) were randomly divided into four groups (n=5/group) namely: Control group given feed and water, Fructose (F) group given high carbohydrate high fat diet (HCHFD) and fructose sweetened drink (FSD) (0.10g/mL), Acriflavine (ACH) group given feed, water and acriflavine (100 mg/kg/day), and F+ACH group given HCHFD, FSD and acriflavine (100mg/kg/day) for 14 weeks. At 14 weeks, blood pressure, heart rate, anthropometric variables and fasting blood glucose were determined. Diazepam (0.4 mg/kg) and ketamine (40 mg/kg) administered intramuscularly into the left hind leg were used as anaesthesia. Serum lipids, proteins, insulin, bilirubin, and liver enzymes, HOMA-IR, liver weight, lipids, inflammation and oxidative stress were determined. Liver histopatholological examination was also done. The procedures of the experiment were endorsed by University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC/19/0140). Data were compared with ANOVA and P0.05 was considered statistically significant. The procedures of the experiment also conform to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

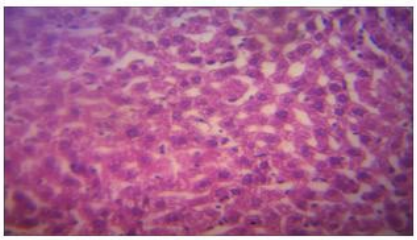
Results: systolic ($p<0.0001$), diastolic ($p=0.0003$), and mean blood pressures ($p<0.0001$), heart rate ($p=0.0004$), body weight gain ($p=0.002$), abdominal circumference ($p=0.0007$), total abdominal fat ($p=0.004$), blood glucose ($p<0.0001$), serum insulin ($p=0.0002$), triglyceride ($p=0.0011$), total cholesterol ($p=0.024$), bilirubin ($p=0.0457$), aspartate aminotransferase ($p=0.0165$), alanine aminotransferase ($p=0.0471$), alkaline phosphatase ($p=0.0063$), total protein ($p=0.0388$), globulin ($p=0.0058$), HOMA-IR ($p<0.0001$), liver weight ($p=0.025$), TNF-α ($p=0.0086$) and IL-6 ($p=0.0074$), hepatic lipids and oxidative stress markers increased significantly in F group when compared with Control while most of them reduced in ACH treated rats when compared with F rats.

Conclusion: The findings of this study showed that a “multiple hit” mechanism underlie NAFLD and also that acriflavine improved NAFLD modestly. This beneficial effect was largely due to the prevention of obesity and components of the metabolic syndrome.

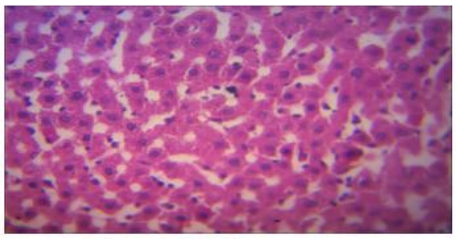
Photomicrographs of the liver

F+ACH group : There is moderate diffuse vacuolar degeneration of hepatocytes

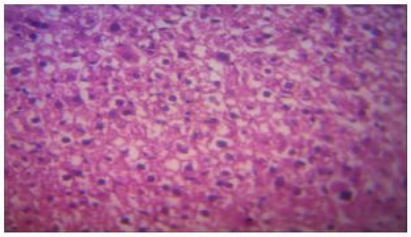




Control group: There is a very mild diffuse hydropic degeneration of hepatocytes



ACH group: No visible lesion seen.



F group: There is severe diffuse vacuolar degeneration of hepatocytes

C04

The Role of Obese Adipose-Derived Extracellular Vesicles in Driving Skeletal Muscle Atrophic Responses

Michael Macleod[1], Joshua Price[2], Caitlin Ditchfield[3], Thomas Nicholson[4], Elpida Tsonou[5], Kostas Tsintzas[6], Simon Jones[7]

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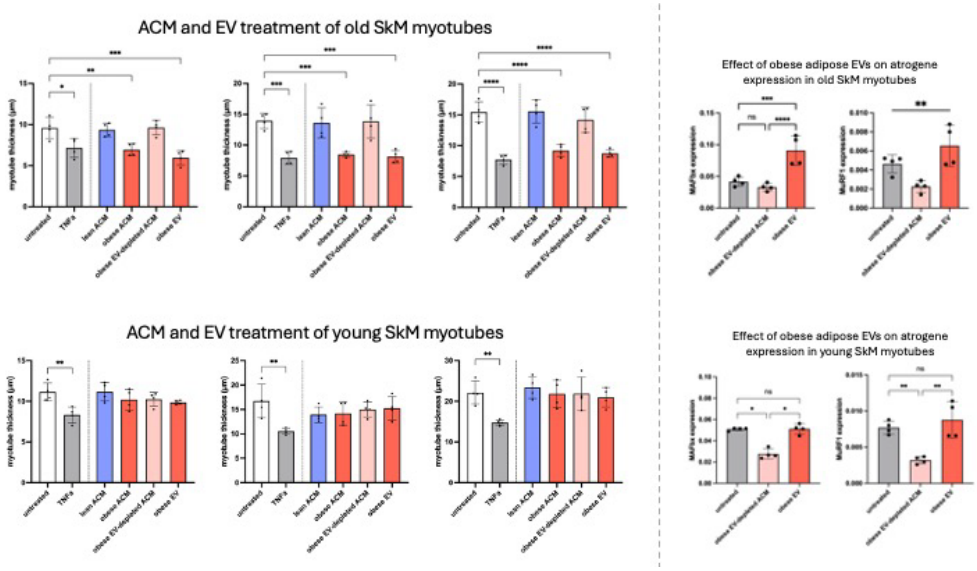
Sarcopenic obesity, characterised by excess adiposity and diminished skeletal muscle mass and function, is associated with frailty and inflammation. Previous work from our group[1] implies a role for dysregulated crosstalk between adipose tissue (AT) and skeletal muscle (SkM) in driving sarcopenic obesity, and increasing evidence demonstrates that extracellular vesicles (EVs) facilitate intercellular communication[2]. We therefore aimed to characterise AT-derived EV profiles across lean and obese subjects, map these to parameters of inflammation and adiposity, explore their effects on SkM, and identify mechanisms of action.

Adipose conditioned media (ACM) was generated from human ex vivo tissue explants collected during joint replacement surgery, and EVs were extracted from conditioned media by overnight ultracentrifugation at 100,000 x g. EV profiles were analysed using ExoView, nanoparticle tracking analysis, nano-flow cytometry, and small RNA sequencing. Human primary SkM myoblasts were isolated from SkM tissue collected peri-operatively during joint replacement surgery. Myoblasts were cultured to confluence before differentiation for 8 days to achieve elongated, multinucleated myotubes. Myotubes (n=3 from donors aged >60, n=3 from donors aged <60) were treated for 24 hr under the following conditions: vehicle PBS control, TNFa positive control, lean ACM, obese ACM, obese EV, and obese EV-depleted ACM. Myotube thickness and nuclear fusion were assessed as functional readouts, and RNA was extracted from treated myotubes to assess modulation of gene expression.

ExoView indicated classical tetraspanin EV marker expression within the population (n=15). Obese AT released fewer EVs than lean AT, with consistently higher EV yield from visceral AT compared to subcutaneous AT (n=5), supported by EV-associated protein concentration trends. Small RNA sequencing of AT-derived EVs reveals 7 differentially expressed microRNAs between lean and obese donors (n=15). In myotubes, TNFa, obese ACM, and obese AT-derived EVs (from a pooled source, n=5) all significantly reduced thickness in cells from old donors, but not young donors, and increased expression of atrophic and inflammatory genes compared to untreated vehicle control, lean ACM, and obese EV-depleted ACM treatments. This suggests that the EV fraction of obese ACM drives modulation of catabolic and anabolic pathways mediating SkM atrophy. Interestingly, concurrent treatment of old myotubes (n=1) with obese AT-derived EVs and an antagomir for key microRNAs upregulated in obese AT-derived EV partially yet significantly ablates the EV-induced elevation of the ubiquitin E3 ligase MAFbx.

The EV fraction of obese ACM appears critical in driving atrophic phenotypes and genotypes in sarcopenic human primary myotubes, with key microRNAs potentially mediating this effect. Interrogating these molecular pathways further in the contexts of adiposity, exercise-like stimulation, and age-related muscle loss will advance understanding of mechanistic drivers of sarcopenic obesity.


ACM and EV treatment of old SkM myotubes
Effect of obese adipose EVs on atrogene expression in old SkM myotubes
ACM and EV treatment of young SkM myotubes
Effect of obese adipose EVs on atrogene expression in young SkM myotubes

C05

Adipose tissue BACE1 regulates thermogenesis and adiposity

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Introduction: Targeting systemic metabolism through the induction of brown adipose tissue (BAT) thermogenesis is an emerging therapeutic strategy for cardiometabolic disease. Activation of BAT thermogenesis reduces key cardiovascular disease risk factors, including weight, ectopic fat accumulation, and dyslipidemia by increasing lipid and glucose oxidation to regulate systemic energy balance. BAT activation occurs through the sympathetic nervous system (SNS), which releases norepinephrine. Norepinephrine binds to beta-adrenergic receptors (beta-ARs) on brown adipocytes, leading to non-shivering thermogenesis in BAT and consequently increased respiration at the whole animal level. The β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) is responsible for the rate-limiting step in the production of β-amyloid (Aβ) peptides and has been implicated in vascular dysfunction in Alzheimer's disease and more recently in obesity and type 2 diabetes.

Aim: This study aims to understand the involvement of BACE1 in the thermogenesis of BAT and to determine if genetic removal of BACE1 could activate BAT, thereby reducing circulating lipid levels and contributing to weight loss.

Method: Adipocyte-specific BACE1 knockout mice (BACE1 AdKO) mice were fed a 60% high-fat diet (HFD) for 12 weeks from 8 weeks of age, compared to control mice (BACE1 flox). After 12 weeks of HFD feeding, mice were weighed then anesthetised with a Ketamine/Xylazine/Acepromazine cocktail and placed into the Comprehensive Lab Animal Monitoring System (CLAMS) to measure oxygen consumption (ml/kg/hr) every 30 seconds. After baseline measurements of oxygen consumption, rectal body temperature was measured before mice were injected with norepinephrine (1mg/kg). Oxygen consumption was measured for a further 60 minutes. At the end of the experiment, body temperature was re-measured and mice were sacrificed. Heart and liver weights were recorded, and tissues were frozen for further analysis.

Results: We identified that specifically knocking out BACE1 in adipose tissue increases oxygen consumption in mice after stimulation with norepinephrine, indicating enhanced BAT thermogenic capacity. The area under the curve (AUC) for oxygen consumption was significantly higher in BACE1 AdKO (n = 4) compared to control mice (BACE1 flox n = 6) (Figure 1A & B). Adipose-specific BACE1 knockout mice maintained body temperature throughout the experiment, with an average increase of 0.2°C, whereas control mice showed an average decrease of 2.1°C (Figure 1C). BACE1 AdKO mice exhibited a 16.8% decrease in body mass compared to control mice (Figure 1D), with significantly reduced liver mass but no significant difference in heart mass between groups (Figure 1E). The change in body mass was not due to differences in mouse size, as tibia length remained unchanged between groups (Figure 1F).

Conclusions: Knocking out BACE1 in adipose tissue results in reduced weight gain and increased norepinephrine-stimulated whole-body respiration, identifying BACE1 as a negative regulator of BAT thermogenesis. Further research will continue to explore BACE1's role within adipose tissue, hypothesising that BACE1 inhibition enhances brown adipose thermogenesis.

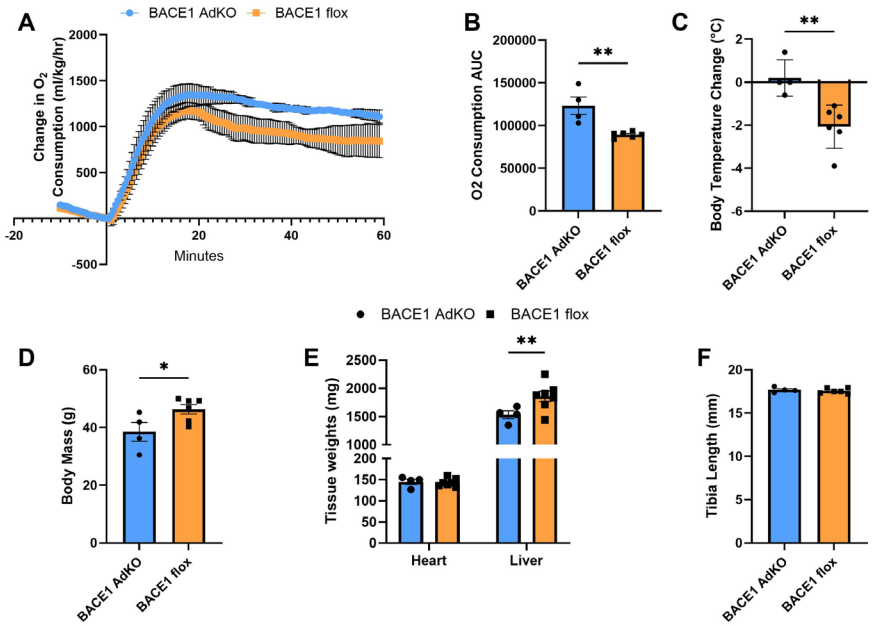

Figure 1. Adipose-specific BACE1 knockout mice exhibit enhanced thermogenesis and decreased body mass. A) Oxygen consumption (mg/kg/hr) of anaesthetized mice after brown adipose tissue (BAT) activation with Norepinephrine S.C. injection (two-way ANOVA with Geisser-Greenhouse correction, $P \leq 0.0001$). B) Area under the curve analysis of change in oxygen consumption (unpaired two-tailed students t-test; ** $P \leq 0.01$). C) Change in boxy temperature before Norepinephrine S.C. injection and 60-min post injection (unpaired two-tailed students t-test; ** $P \leq 0.01$). D) Body maas (g) on the day of experiment after 12 weeks of 60% HFD feeding (unpaired two-tailed students t-test; * $P \leq 0.05$). E) Wet weights of heart and liver (mg) immediately after sacrifice of mice (unpaired two-tailed students t-test; ** $P \leq 0.01$). F) Length of right tibia (mm). BACE1 AdKO = blue, BACE1 flox controls = orange. Data are mean ± SEM with individual data points shown.

C06

Kv1.3 depletion in macrophages improves obesity comorbidities

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Diet-induced obesity comorbidities such as Insulin resistance and hepatic steatosis, have been causally linked to adipose tissue recruited macrophages whose proinflammatory polarization is promoted during adipose tissue mass expansion (1,2). The voltage gated potassium channel Kv1.3 appeared as a putative anti-obesity target back in 2003 when it was shown that the total KO mice are resistant to high-fat diet (HFD) induced obesity (3). Kv1.3 overexpression triggered by proinflammatory stimuli is necessary for macrophages activation (4). Therefore, we aim to better the understanding of the Kv1.3 anti-obesity role by dissecting the specific contribution of macrophages. To do so, we crossed our unique Kv1.3 flox/flox mice with LysM-Cre mice (Jackson Laboratories) obtaining Kv1.3 flox/flox-LysM-Cre mice in which Kv1.3 is specifically knocked out in the myeloid cell lineage including macrophages (named MO-Kv1.3KO). We exposed parental and MO-Kv1.3KO 8-weeks old animals to 45%-high fat diet (HFD) for 12 weeks. At the end of the treatment, we performed glucose tolerance test, insulin tolerance test and sacrificed the animals to collect several fat pads and liver (all procedures were approved by the ethics committee for animal experiments at University of Padova).

After HFD, we could not observe differences in weight (28.22 ± 0.81 vs 27.61 ± 0.84 g; n = 17 vs 8) nor insulin sensitivity between parental and MO-Kv1.3KO female mice. On the contrary, MO-Kv1.3KO males showed a slightly reduced weight (40.32 ± 0.81 vs 37.6 ± 1 g; n = 11 vs 8) which associated to smaller fad pads (interscapular brown, inguinal white subcutaneous and perigonadal white visceral adipose tissues) and improved glucose clearance capacity and insulin sensitivity. When the stromal vascular fraction (SVF) of visceral white adipose tissue was obtained and interrogated using flow cytometry to assess macrophages infiltration, the previously observed sex differences were paralleled. While neither females nor males showed changes in the proportion of F480+ cells (macrophages) in the SVF, in MO-Kv1.3KO males, but not females, macrophages showed a reduced expression of the proinflammatory marker CD11c. This was associated with a lower BodiPY signal showing reduced lipid content only in MO-Kv1.3KO males macrophages. Therefore, affecting the expression of the channel in macrophages have a differential impact on females and males. Hence, our results show that kv1.3 silencing in macrophages does not impair its recruitment into the adipose tissue but disrupts, in a sex dependent way, the vicious cycle that associate fat expansion to insulin resistance.

C07

Loss of CD8+ MAIT cells drives reduced MAIT cytotoxicity in obesity - implications for obesity-associated cancer

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Introduction

Mucosal Associated Invariant T (MAIT) cells are innate, unconventional T cells with specific recognition for antigen presented by MR1 (MHC class-I like molecule). MAIT cells have highly efficient cytotoxic capacity against infected and malignant cells. Previous reports have noted MAIT disruption in the context of obesity and obesity-related morbidities.

Aim

Considering this with the increased risk of cancer in people with obesity we investigated MAIT cytotoxic function in the context of obesity.

Method

MAIT cells were expanded and isolated from the peripheral blood of donors with and without obesity who provided explicit consent in accordance with full ethical approval. MAIT cell characteristics that pertain to cancer risk were assessed in the overall MAIT cell population as well as in previously described phenotypic subsets. Flow cytometry was used to phenotype and measure degranulation (CD107a expression) in MAIT cells. Phenotype was further characterised using RNAseq, while cytotoxicity was confirmed with calcein AM assays. Sufficient power was confirmed with power analyses. All statistical analyses were confirmed appropriate to the nature of the individual dataset (normality, variance, paired etc.) using Graphpad Prism, which was also used to analyse and plot the datasets.

Results

Here we demonstrate a markedly reduced ability for tumour lysis by MAIT cells from people with obesity. We further uncover the importance of MAIT cell subsets in obesity-associated MAIT dysfunction, including cytotoxicity. We show that a MAIT subpopulation ($CD4^+CD8^-$) that is exceedingly rare in lean, metabolically healthy donors makes up a significant proportion of MAIT cells in people with obesity, and that this shift is physiologically relevant. We find that MAIT $CD4^+$ and $CD8^+$ subsets differ in their functionality, with $CD8^+$ MAITs responsible for cytotoxicity. We find the $CD8^+$ compartment of MAITs to be preferentially depleted in people with obesity, and that this change is sufficient to account for previously described obesity-associated MAIT phenotypes, and for the deficiency in MAIT oncolytic function presented here.

Summary

We show for the first time that MAIT cell cytotoxicity is reduced in people with obesity, and that this is driven by a shift in MAIT CD4:CD8.

Conclusion

These findings are of crucial importance to progressing understanding of cancer risk reduction in the context of obesity, and will have critical implications for MAIT cell therapy development.

C08

New Zealand blackcurrant supplementation promotes postprandial insulin secretion without corresponding decreases in plasma glucose in overweight individuals

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Impaired postprandial glucose handling increases the risk of developing insulin resistance in individuals with overweight or obesity. Anthocyanins have been shown to improve postprandial glucose metabolism in both human and in vitro studies; however, mechanistic insights are often derived from models using supraphysiological doses or non-physiological conditions, limiting their relevance to human physiology. Integrating ex-vivo human plasma into cell models offers a more translational approach to understanding anthocyanin bioactivity.

Purpose Explore the effect of short-term supplementation with anthocyanin-rich New Zealand blackcurrant (NZBC) extract on postprandial glucose responses to a high-carbohydrate drink and potential regulatory mechanisms in skeletal muscle.

Methods This study used a combination of in-vivo and in-vitro methods. In study 1, Fifteen overweight (BMI > 25 kg•m^2) sedentary individuals participated in a double-blinded, randomised controlled trial. Participants supplemented with 0 mg, 300 mg, 600 mg, and 900 mg NZBC extract per day (CurraNZ®) for 7-days, and glucose tolerance was assessed on day 7 (n = 15), and again on day 8 (n = 13). In study 2, GLUT4 translocation was determined in L6-GLUT4myc myotubes in vitro treated first with a supraphysiological dose (10 µg•mL^{-1}) of NZBC extract and subsequently with culture medium conditioned with fasting human plasma derived from participants in study 1 after 7 days of 900 mg•d^{-1} NZBC supplementation (n = 4). L6-GLUT4myc myotubes were plasma starved for 3 h before incubation for 40 min in medium containing 10 µg•mL^{-1} NZBC extract, or 2-20% fasting human plasma, after which GLUT4 translocation was determined via colorimetric assay. Results Plasma glucose and insulin concentrations increased following high carbohydrate test drink ingestion (main effect of time, $P < 0.001$). No effect of NZBC dose was observed on 2hr postprandial glucose on day 7 or day 8. However, in response to short-term supplementation with 900 mg•d^{-1} NZBC extract, mean insulin concentrations were significantly greater compared to placebo (NZBC: 40.6 (35.1 – 46.9) µU•mL^{-1}, Placebo: 28.9 (24.6 – 34.3) µU•mL^{-1}, $P = 0.011$). Incubation with a supraphysiological dose of NZBC extract caused a time-dependent increase in cell surface GLUT4myc abundance (1.89-fold increase vs. baseline, $P < 0.001$). Similarly, medium conditioned with fasted human plasma from NZBC-supplemented participants increased GLUT4myc abundance at both 10% (1.88-fold increase, $P < 0.001$) and 20% plasma concentrations (1.63-fold increase, $P = 0.019$).

Conclusion In vivo, the increase in postprandial insulin concentration without a corresponding reduction in plasma glucose may reflect enhanced pancreatic β-cell responsiveness or incretin effects. In-vitro, findings suggest that NZBC extract enhances skeletal muscle GLUT4 translocation under both supraphysiological NZBC extract treatment and conditioned human serum. Although we identify a potential mechanism in vitro, the failure to observe corresponding effects in vivo underscores the complexity of translating cell-based findings to humans and suggests that additional biological factors may limit the bioactivity of NZBC extract in clinical settings.

C09

Canine Genome-Wide Association Study Identifies Novel Candidate Obesity Gene

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Obesity is a highly heritable yet complex condition whose genetic underpinnings remain challenging to fully elucidate. Dogs represent a particularly valuable genetic model for human obesity due to shared environmental influences and breed-specific genetic structures that facilitate robust trait mapping. In this study, we aimed to identify genetic loci associated with obesity using a canine model to provide novel insights into the genetic mechanisms underlying human obesity. Utilising breed-average obesity phenotypes derived from electronic health records of approximately 2.6 million dogs, we performed a multi-breed genome-wide association study (GWAS) encompassing 300 dogs from 36 breeds representing extensive genomic diversity.

Our study identified 19 independent loci significantly associated with obesity (Bonferroni-corrected significance threshold p = 1.28e-05). Fine-mapping using Bayesian Sum of Single Effects (SuSiE) identified high-confidence credible sets (Posterior Inclusion Probability, PIP = 99%). Most lead SNPs were intergenic or intronic, suggesting regulatory functions. Proximal candidate genes included CASP12 (CFA5), LEP (CFA14), GUCY1B1 (CFA15), GPD1L (CFA23), SRXN1 (CFA24), ETV6 (CFA27), COL25A1 (CFA32), EPHB3 (CFA34), and BLOC1S5 (CFA35).

The most significant association mapped to chromosome 13 (lead SNP 13:9231605), a region harbouring R-spondin 2 (RSPO2). RSPO2 is known to cause the “furnishings” coat phenotype in dogs. A complementary GWAS for furnishings corroborated this locus (lead SNP 13:8870149, p=3.45e-60). Bayesian colocalisation analysis using coloc.susie strongly suggested a shared causal variant for both obesity and furnishings at this locus (PP4 = 83.3%), supported by high LD (r2=0.99) between their lead SNPs and substantial overlap in credible sets. RSPO2 is a potent modulator of Wnt signalling, a pathway implicated in adipogenesis and potentially hypothalamic energy balance control. The shared genetics between a morphological trait (furnishings) and a metabolic trait (obesity risk) at RSPO2 highlights how selection for morphological traits may inadvertently influence metabolic processes via pleiotropic gene action.

These findings highlight the translational potential of canine genetic studies in elucidating human obesity biology. The link between RSPO2, Wnt signalling, and adiposity provides novel avenues for exploring metabolic regulation and potential therapeutic targets in both canines and humans.

C10

Is the Exercise-Related Improvement in Glucose Metabolism in Older Adults Dependent on an Increase in Muscle Mass and Reduction in Fat Mass? A Systematic Review and Meta-Analysis

Rocco Sheldon[1], Andrew Zi Xiang Gan[2], Jennifer Tasong[3], Audrey Yap[4], Moiz Ahmad[5], Paulo Roberto Hernandes Júnior[6], Kailash Viswanathan[7], Manas Setti[8], Olivia Jarman[9], Shuayb Hoque-Uddin[10], Tanisha Banerjee[11], Amanda Veiga Sardeli[12]

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Introduction and aims:

Human ageing is associated with increases in fat mass and reductions in muscle mass which impair glucose metabolism, eventually leading to Type 2 Diabetes (T2D).(1) Many studies have hypothesised that body composition may explain exercise-induced improvements in glucose metabolism.(2) We comprehensively integrated the findings of the literature, via subgroup meta-analyses, considering confounding factors such as the presence of T2D and the type of exercise.

Methods:

A search was performed on PubMed, Embase, Scopus, Web of Science, Cochrane Library, and EBSCOhost on 5/8/2024. A systematic review looked for exercise-controlled trials in older adults assessing body composition (fat mass and muscle mass) and glucose metabolism (fasting glucose, fasting insulin, glycated haemoglobin [HbA1c], Homeostatic Model Assessment for Insulin Resistance [HOMA-IR]). Screening, data extraction, and quality assessment were performed by two independent reviewers. We compared the randomized effects of studies that improved and did not improve body composition with exercise training on glucose metabolism, via subgroup meta-analysis (Q-test) on Biostat's Comprehensive Meta-Analysis.

Results:

301 standardised mean differences (SMDs) from included study arms were clustered. Table 1 illustrates that most subgroups (with improvement in body composition or not) showed improvements in markers of glucose metabolism. Subgroups reducing fat mass underwent significantly greater ($p<0.001$) improvements in fasting insulin, HOMA-IR, and HbA1c, but not in fasting glucose ($p=0.14$). On the other hand, the increase in muscle mass was not associated with improvements in any glucose metabolism markers. T2D trials led to higher improvements in fasting glucose, HOMA-IR, and HbA1c, but not fasting insulin, whilst the improvements in glucose metabolism markers were similar between the types of exercise (data not shown).

We tested the influence of body composition within subgroups stratified by diabetes status and training type (Table 1). Within T2D trials, a reduction in fat mass was not necessary to lead to improvements in glucose, HbA1c, and HOMA-IR (insufficient studies testing insulin). Although few T2D trials significantly increased muscle mass, we observed that reductions in HOMA-IR and HbA1c were not dependent on this change.

Within aerobic training (AT), only cohorts reducing fat mass, improved glucose, insulin, and HOMA-IR, but fat mass reduction was not required for reduction in HbA1c. However, improvements in glucose metabolism markers were not dependent on fat mass reduction within resistance training (RT) and

combined training (CT) cohorts. The low number of trials improving muscle mass prevented a conclusive analysis.

Conclusions:

Although improvements in glucose metabolism are not fully dependent on changes in body composition, reductions in fat mass make a significant contribution to exercise-induced improvements in glucose metabolism. Only metabolic improvements generated by AT were fully dependent on fat reduction. A limited number of trials increasing muscle mass (particularly within T2D) with overall improvements in glucose metabolism, suggests the increase in muscle mass was not a necessary adaptation to improve glucose metabolism. Future studies should explore the mechanisms by which RT improves glucose metabolism. A limitation of these cross-sectional associations is that we cannot prove a cause-and-effect relationship, and thus, glucose metabolism adaptations may also be mediating body composition.

Table 1. Subgroup analysis for the effect of reductions in fat mass and increases in muscle mass on markers of glucose metabolism.

			Glucose (SMD)			HbA1c (SMD)			Insulin (SMD)			HOMA-IR (SMD)		
			K	**PE [LL, UL]**	**P diff**	**K**	**PE [LL, UL]**	**P diff**	**K**	**PE [LL, UL]**	**P diff**	**K**	**PE [LL, UL]**	**P diff**
	Overall	Overall	**115**	**-0.49 [-0.62; -0.35]**		**57**	**-0.56 [-0.77; -0.36]**		**65**	**-0.5 [-0.69; -0.31]**		**64**	**-0.61 [-0.8; -0.42]**	
	Fat mass	No change	**61**	**-0.43 [-0.59; -0.27]**	0.14	**29**	**-0.32 [-0.5; -0.14]**	**<0.01**	**32**	**-0.21 [-0.39; -0.03]**	**<0.01**	**34**	**-0.33 [-0.51; -0.15]**	**<0.01**
		Reduction	**18**	**-0.89 [-1.46; -0.31]**		**9**	**-2 [-2.94; -1.07]**		**14**	**-1.43 [-2.01; -0.85]**		**14**	**-1.91 [-2.59; -1.23]**	
	Muscle mass	No change	**44**	**-0.22 [-0.42; -0.02]**	0.13	**21**	**-0.41 [-0.69; -0.12]**	0.62	**32**	**-0.35 [-0.61; -0.09]**	0.67	**29**	**-0.43 [-0.7; -0.16]**	0.39
		Increase	**4**	**-1 [-1.98; -0.02]**		**3**	**-0.55 [-1.02; -0.07]**		3	-0.54 [-1.38; 0.3]		**7**	**-0.63 [-0.98; -0.28]**	
T2D	Fat mass	Not measured	**14**	**-0.65 [-1.03; -0.26]**	0.22	**11**	**-0.48 [-0.7; -0.26]**	**0.03**	**10**	**-1.33 [-2.03; -0.63]**	**<0.01**	5	-0.36 [-0.79; 0.07]	**<0.01**
		No change	**11**	**-0.75 [-0.99; -0.5]**		**12**	**-0.45 [-0.77; -0.13]**		10	-0.04 [-0.32; 0.24]		**9**	**-0.34 [-0.58; -0.1]**	
		Reduction	**7**	**-1.38 [-2.11; -0.64]**		**8**	**-2.03 [-3.14; -0.91]**					**9**	**-2.33 [-3.41; -1.24]**	
	Muscle mass	Not measured	**24**	**-0.9 [-1.23; -0.57]**	0.34	**19**	**-1.07 [-1.53; -0.61]**	0.08	**15**	**-0.87 [-1.43; -0.31]**	**0.03**	**15**	**-1.39 [-2.09; -0.7]**	**<0.01**
		No change	**8**	**-0.69 [-0.97; -0.42]**		**9**	**-0.41 [-0.78; -0.05]**		5	-0.13 [-0.48; 0.22]		**5**	**-0.28 [-0.55; -0.01]**	
		Increase	-	-	-	**3**	**-0.55 [-1.02; -0.07]**		-	-	-	**3**	**-1 [-1.57; -0.42]**	
AT	Fat mass	Not measured	**16**	**-0.46 [-0.76; -0.17]**	0.50	**8**	**-0.51 [-0.91; -0.12]**	**0.04**	**10**	**-0.37 [-0.65; -0.09]**	**<0.01**	**7**	**-0.22 [-0.42; -0.02]**	**<0.01**
		No change	14	-0.33 [-0.67; 0.01]		7	-0.45 [-0.96; 0.06]		7	0.05 [-0.19; 0.3]		10	-0.22 [-0.47; 0.02]	
		Reduction	**14**	**-0.77 [-1.43; -0.1]**		**4**	**-2.57 [-4.15; -1]**		**10**	**-1.18 [-1.81; -0.55]**		**7**	**-1.58 [-2.3; -0.86]**	
	Muscle mass	Not measured	**30**	**-0.58 [-0.84; -0.31]**	**<0.01**	**12**	**-0.92 [-1.47; -0.36]**	0.76	**16**	**-0.58 [-0.9; -0.27]**	**0.02**	**13**	**-0.69 [-1.06; -0.31]**	0.58
		No change	13	-0.24 [-0.75; 0.28]		**7**	**-0.77 [-1.48; -0.07]**		10	-0.44 [-1.06; 0.19]		10	-0.59 [-1.18; 0.01]	
		Increase	**1**	**-1.41 [-1.87; -0.95]**		-	-	-	**1**	**-1.34 [-1.79; -0.88]**		**1**	**-0.93 [-1.37; -0.5]**	
RT	Fat mass	Not measured	**10**	**-0.77 [-1.27; -0.28]**	0.09	5	-0.44 [-0.93; 0.05]	**<0.01**	5	-0.4 [-1.31; 0.51]	**<0.01**	6	-0.2 [-0.49; 0.08]	**<0.01**
		No change	**28**	**-0.53 [-0.81; -0.24]**		13	-0.25 [-0.53; 0.02]		**15**	**-0.34 [-0.65; -0.04]**		**15**	**-0.4 [-0.72; -0.08]**	
		Reduction	**1**	**-1.29 [-1.92; -0.66]**		**1**	**-3.36 [-4.25; -2.47]**		**1**	**-2.72 [-3.51; -1.93]**		**1**	**-4.2 [-5.23; -3.18]**	
	Muscle mass	Not measured	**22**	**-0.91 [-1.16; -0.65]**	**<0.01**	**12**	**-0.61 [-1.11; -0.1]**	0.19	9	-0.66 [-1.4; 0.07]	0.42	**11**	**-0.64 [-1.2; -0.08]**	0.68
		No change	16	-0.06 [-0.33; 0.2]		7	-0.2 [-0.53; 0.13]		12	-0.32 [-0.69; 0.04]		10	-0.35 [-0.78; 0.08]	
		Increase	**1**	**-2.43 [-3.2; -1.66]**		-	-	-	-	-	-	1	-0.58 [-1.18; 0.02]	
CT	Fat mass	Not measured	10	0.02 [-0.19; 0.24]	**0.01**	6	-0.14 [-0.4; 0.13]	0.23	4	-0.21 [-0.5; 0.08]	0.14	3	-0.19 [-0.52; 0.15]	**0.05**
		No change	**19**	**-0.37 [-0.54; -0.2]**		**9**	**-0.33 [-0.57; -0.08]**		10	-0.13 [-0.43; 0.16]		9	-0.27 [-0.57; 0.03]	
		Reduction	3	-1.33 [-2.91; 0.25]		4	-1.14 [-2.42; 0.14]		**3**	**-1.89 [-3.6; -0.18]**		**6**	**-2.03 [-3.46; -0.6]**	
	Muscle mass	Not measured	15	-0.29 [-0.66; 0.07]	0.57	**9**	**-0.52 [-1.02; -0.02]**	0.49	5	-0.93 [-1.99; 0.13]	0.37	**4**	**-2.28 [-4.48; -0.08]**	0.19
		No change	**15**	**-0.36 [-0.55; -0.17]**		7	-0.26 [-0.55; 0.03]		10	-0.26 [-0.65; 0.12]		9	-0.33 [-0.67; 0.02]	
		Increase	2	-0.12 [-0.53; 0.29]		**3**	**-0.55 [-1.02; -0.07]**		2	-0.12 [-0.5; 0.25]		5	-0.56 [-1.06; -0.07]	

Legend: AT, Aerobic training; CT, Combined training; HbA1c, Glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; K, Number of studies; LL, Lower limit; P diff, P value for difference between subgroups; PE, Point estimate; RT, Resistance training; T2D, Type 2 diabetes; UL, Upper limit.

C11

A crossover trial of passive movement training, with and without blood flow restriction, to examine postprandial blood glucose levels

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Introduction

Exercise is a well-documented preventative and therapeutic intervention for both the risk of, and management of type two diabetes (1). Many populations are unable to exercise, which may contribute to a greater risk of type two diabetes. For example, type two diabetes prevalence in the spinal cord injury (SCI) population is 2.52 times greater than the general population (2). Populations which are unable to exercise, such as those with SCI, are unable to access conventional exercise.

Therefore, it is imperative that effective alternatives to conventional exercise are investigated as methods of achieving greater glycaemic control compared to an exclusively sedentary lifestyle. Passive movement training (PMT) may be beneficial in this regard as increased skeletal muscle blood flow has been demonstrated to be associated with greater glucose uptake from the blood to muscle during acute exercise (3). This may also apply to PMT.

This crossover study examines the effectiveness of PMT, with and without blood flow restriction (BFR), at minimising postprandial glucose excursions compared to that at rest.

Methods

Prior to recruitment and data collection, ethical approval was obtained from Lancaster University's Faculty of Health & Medicine Research Ethics Committee (FHM-2024-4434-SA-2). The trial was pre-registered at ClinicalTrials.gov (NCT06704126). The trial has been conducted in accordance with the latest version of the Declaration of Helsinki.

Seven healthy males (mean ± SD) (aged 25.3 ± 6.4 years; height 1.80 ± 0.1 m; weight 82.2 ± 9.9 kg; BMI 25.4 ± 2.3 kg/m^2) completed three 150-minute visits to Lancaster University's Human Performance Laboratory in a fasted state. Fasted blood glucose and insulin measures were obtained immediately prior to a standardised meal being consumed (energy 522 kcal; carbohydrate 112.5g; of which sugars 51.6g; protein 6.9g; fats 4.0g; fibre 3.3g).

Once 30 minutes had elapsed from fasting measurements, either PMT only, PMT + BFR or control (CTRL) protocols commenced for 30 minutes. The PMT elements were conducted using an isokinetic dynamometer (S3, Biodex, New York). These were conducted in a randomised sequence in each participant. A further 90-minute period of glucose and lactate monitoring followed. Throughout the session entirety, venous samples were drawn at five-minute intervals via an antegrade fitted cannula, and glucose and lactate concentrations were analysed.

Preliminary results

Differences in glucose area under the curve (AUC) during the final 120 minutes were analysed by way of a repeated measures ANOVA. No significant differences were observed between CTRL, PMT or PMT+BFR treatments (CTRL 522.8 ± 95.9 mmol min/L, PMT 512.1 ± 96.4 mmol min/L, PMT+BFR 483.9 ± 137.2 mmol min/L ($p = 0.612$).

Conclusion

These preliminary results suggest undertaking PMT or PMT+BFR training have no significant effect on post-prandial blood glucose excursion. This may be due to insufficient blood flow being present to allow an observed effect, a lack of demand for skeletal muscle glucose uptake or insufficient participants to allow a significant effect to be seen at present. Further glucose excursion data and insulin analyses will be presented via conference once data collection is complete.

C12

Palmitic Acid-Induced Insulin Resistance and Inflammatory Responses in SH-SY5Y Cells

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Background

Obesity is a major risk factor for the development of type 2 diabetes mellitus (T2DM), with chronic high-fat intake promoting systemic insulin resistance and inflammation. The brain regulates key metabolic processes, and emerging evidence suggests that brain insulin resistance may influence eating behaviours and energy homeostasis, contributing to the pathophysiology of obesity and T2DM.

Aims

This study aims to explore the impact of palmitic acid exposure on insulin sensitivity and cellular stress responses in SH-SY5Y cells.

Methodology

SH-SY5Y cells were treated with palmitate at physiologically relevant concentrations (100 -– 500 μM) to model high-fat dietary conditions. The impact of palmitate treatment on cell viability and metabolic activity was determined through Trypan Blue and MTT assays. Changes in insulin sensitivity were assessed by quantifying levels of phosphorylated Akt (p-Akt) via Western blot analysis. Markers of inflammation (IL-1α, IL-8), oxidative stress (GPX1, CAT) and palmitoylation enzymes (ZDHHC1 and ZDHHC12) were also evaluated using quantitative PCR.

Results

Cell viability using Trypan Blue assay revealed no increase in cell death following palmitate exposure after 24 and 48 hours (n = 2). Significant reductions in metabolic activity were observed after 24, 48, and 72 hours palmitate treatment via MTT assay. At 24 hours, there was a significant reduction in metabolic activity at 100 μM (48.5% ± 6.4%), 200 μM (49% ± 2.9%), 300 μM (48.5% ± 6.13%), 400 μM (47% ± 9.2%) and 500 μM (45.5% ± 11%) (n = 4, $p < 0.0001$ vs vehicle control for all concentrations). Similar trends were observed at 48- and 72 -hours palmitate treatment. Insulin-stimulated Akt phosphorylation was significantly reduced following palmitate treatment by 43% (±0.05%) at 200 μM and 45% (±0.07%) at 300 μM (n = 3, $p < 0.001$ vs. vehicle control). Additionally, 200 μM palmitic acid upregulated the pro-inflammatory cytokine IL-8 six-fold (645% ± 2.63%, n = 4, $p < 0.01$) and the ER stress marker DDIT3 four-fold (425% ± 2.63%, n = 4, $p < 0.01$), while also increasing the oxidative stress marker SOD1 two-fold (199% ± 0.74%, n = 4, $p < 0.05$). ZDHHC12, a palmitoylation enzyme involved in regulation of NLRP3, was reduced by 41% (± 0.2%, n = 4, $p < 0.05$), potentially leading to the disinhibition of NLRP3 inflammasome.

Conclusions

These findings demonstrate that palmitic acid impairs neuronal insulin signalling, reduces metabolic activity and induces cellular stress in SH-SY5Y cells. Our findings suggest that saturated fatty acids alone may disrupt key neuronal pathways involved in metabolic regulation. Ongoing research is focused on exploring the potential protective effects of unsaturated and polyunsaturated fatty acids, and their ability to counteract the palmitic acid induced impairment of insulin signalling and cellular stress markers in the brain.

C13

Brown Adipose Tissue Gene Expression is Altered in Mice Harbouring a Missense Mutation in Zinc Finger Homeobox-3

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Zinc finger homeobox-3 (ZFHX3) is a transcription factor implicated in multiple functions including circadian regulation in the hypothalamus and regulation of signalling pathways in peripheral tissues. We recently demonstrated a novel role for ZFHX3 in whole animal metabolic phenotype, with a missense mutation mouse model similar to a known human mutation, leading to shorter body length, lower body weight, food intake and circulating metabolic hormones, with altered hypothalamic gene expression (1). While the previous work hypothesised that these changes are in the brain, here we aimed to investigate peripheral changes in adipose tissue that may be driven by the Zfhx3Sci mutation. Objectives: This study investigated differential gene expression of (a) Zfhx3 in adipose tissues, (b) Zfhx3 in a rhythmic and dietary intervention in white adipose tissue (WAT) of wildtype mice, and (c) in adipose tissues of Zfxh3Sci/+ mice vs wildtype littermates. Methods: Animal experiments were conducted at MRC Harwell and University of Bradford according to the Animals (Scientific Procedures) Act, 1986, under UK Home Office Project Licenses 30/3206, P0D6AA50D and P6165EED1, with local ethical approval. Experiment a: 12 week old female C57B6/N mice were humanely killed and multiple adipose tissues were collected (n = 3-5; inguinal, i-; gonadal, g-; perirenal, p-; and mesenteric, m-; WAT; and interscapular brown adipose tissue, iBAT). Experiment b: 8 week old male and female C57B6/J mice were housed in a 12 h:12 h light:dark light cycle with access to high fat diet (HFD) or nutrient matched control diet (NMCD) for 6 weeks and tissue iWAT was collected at 4 h intervals over a 24 h diurnal timecourse (n = 5-6). Experiment c: male and female Zfxh3Sci/+ mice and wildtype littermates (n = 9-12) had ad libitum access to standard rodent chow and water in a 12 h:12 h light:dark light cycle until 12 months old when they were humanely killed (1). Multiple adipose tissues were collected (iWAT, gWAT, pWAT, mWAT and iBAT) and mRNA extracted followed by qPCR analysis for (a,b) Zfhx3, (b) circadian genes (Per2, Dbp) and adipogenic genes (Ppara, Pparg, Srebp1); and (c) genetic markers of adipogenesis (Pparg, Cebpa, Cebpb, Vegfa, Adipoq), glucose transport (Glut1, Glut4), lipid metabolism (Plin1, Fasn, FAbp4) and thermogenesis (Ucp1, Cidea, Dio2). Statistical comparison was by 2-way ANOVA unless indicated. Results: C57B6/N female mice had significantly different expression of Zfhx3 in visceral adipose depots (one way ANOVA) with differences between gWAT and pWAT vs subcutaneous iWAT approaching significance in post hoc tests (Tukey, $p = 0.069$ and $p = 0.084$ respectively). HFD shifted circadian gene expression ($p < 0.0001$), and increased expression of Ppara ($p < 0.05$), but Zfhx3 expression did not differ by timepoint. In Zfxh3Sci/+ mice, very little expression differences were detected in WAT tissues, but in iBAT had significant increased expression of Cebpb, Adipoq, Plin1, Dio2, Ucp1 ($p < 0.05$), Vegfa and Cidea ($p < 0.01$). Conclusion: Zfhx3 was differentially expressed in visceral and subcutaneous adipose tissue and lower with HFD challenge. Zfhx3Sci may be involved in BAT physiology, with increased thermogenic gene expression in these leaner mice.

C14

Molecular effects of physical activity and body composition: a systematic review and meta-analysis

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Physical activity (PA) is an important lifestyle intervention that can influence public health and help tackle the current global obesity challenge. The UK government has issued recommendations on regular PA levels, however individual responses to PA vary, and sedentary lifestyles present a significant risk to public health. Causes of obesity are complex and multi-faceted; genetic variations are a contributing factor, with specific genes such as the fat mass and obesity-associated (FTO) gene known to be strongly involved with obesity and related health conditions. Epigenetic studies examine the effect of PA as an external influence on the control of genes and associated molecular pathways, without affecting the DNA sequence itself. Methylation of DNA sequences in some gene promoter regions (CpG sites) have been found to be affected by PA, affecting gene transcription and expression. An increase in methylation levels (hypermethylation) restricts the action of the gene, whereas a decrease in methylation (hypomethylation) reduces control and regulation of the gene.

This systematic review and meta-analysis aimed to identify specific CpG sites affected by various levels of PA in genes and pathways associated with obesity. The review was registered with PROSPERO and followed PRISMA guidelines. Databases searched included PubMed, SportDISCUS, Embase, Scopus, and Web of Science. Epigenomic DNA methylation analysis studies performed on adult participants with no underlying health conditions were included, using adipose, skeletal muscle or blood sample tissue types. Population studies or PA intervention studies were considered. Articles were screened and selection decisions recorded using Rayyan AI software. Stated CpG sites were extracted from study data and compiled, with gene locations confirmed using the Epigenome-Wide Association Studies (EWAS) catalogue. Six studies comprising of 770 participants were selected for inclusion in this meta-analysis. A total of 257 CpG sites were identified as significantly differentially methylated in physically active participants, and 134 of these CpG sites were located in 92 genes associated with obesity-related pathways. Genes identified as differentially regulated in multiple tissue types and studies are JAZF1 (insulin signalling, and lipid and carbohydrate metabolism pathways) and NAV1 (mTOR signalling pathway). Multiple differentially methylated genes belonged to pathways associated with the lipid metabolism or insulin signalling pathway. To conclude, the current epigenomic meta-analysis showed that PA levels induce differential DNA methylation changes on genes affecting metabolism. To further understand the positive molecular effects of PA, these candidate genes should be investigated further and compared between various levels of a physically active population.

Late Breaking Posters

C15 The influence of sacropenic obesity on osteoarthritis in a young active population
Oliver O'Sullivan, University of Nottingham, UK

C16 The anti-inflammatory effects of three different dietary supplement interventions
Dr Amrita Vijay, University of Nottingham, UK

C17 Brain alterations to sweet taste sensing In obesity and type 2 diabetes
Tobias Long, University of Nottingham, UK

C18 miR-10b-5p: A key regulator of adipogenesis
Dr Nikoletta Kalenderoglou, Nottingham Trent University, UK

C19 Sex-specific metabolic responses to 8-week low energy diet intervention: a secondary analysis of the preview diabetes-risk study Nottingham cohort
Deema Alogaiel, University of Nottingham, UK

C20 Identifying the mechanisms of the gut-brain axis to sweet sensing in diabetes
Dr Colette Milbourn, University of Nottingham, UK

C21 Altered reward-related brain areas in obesity: A systematic neuroimaging review
Amal Ajeebi, University of Nottingham, UK

C22 Inhibition of carbohydrate digestion in the gut, food preference and metabolism
Jack Marples, University of Nottingham, UK

C23 Potential role of carnosine to ameliorate inflammation in metabolic diseases
Joshua Awoke, Nottingham Trent University, UK

C24 Disparities in GLP-1 and GIP responses to small intestinal glucose infusion between people with well- and poorly-controlled type 2 diabetes
Yixuan Sun, University of Nottingham, UK