Experiments on animals and animal tissues

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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Please note, to constitute an acceptable abstract, the Society requires the following ethical criteria to be met. To be acceptable for publication, experiments on living vertebrates and Octopus vulgaris must conform with the ethical requirements of The Society regarding relevant authorisation, as indicated in Step 2 of submission.

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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.

PL05

Breaking barriers, from the mile to the marathon: physiological determinants of sporting success

Andrew Jones[1]

[1]University of Exeter, United Kingdom

In this lecture, Andy Jones will discuss the scientific background to, and legacy of, his involvement in Nike's 'Breaking 2' (men's marathon) and 'Breaking 4' (women's mile) projects. Specifically, he will break down the physiological requirements of these immense challenges, the physical characteristics of the athletes capable of tackling them, and the other factors that need to be taken into account to enable these athletes to fully express their physiological potential. These factors include the environment, footwear innovation, nutrition, pacing and drafting. Lessons learnt from participation in these challenges will be considered with a view to predicting what the ultimate limits to human athletic performance might be.

SA01

Role of ketone bodies in the regulation of skeletal muscle protein metabolism

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Ketone bodies (i.e., acetoacetate (AcAc), beta-hydroxybutyrate (BHB), and acetone) are lipid-derived metabolites produced primarily in hepatic mitochondria. Endogenous ketone body production is markedly amplified during periods of low carbohydrate availability, such as prolonged fasting, starvation, or adherence to a ketogenic diet, where they serve as an alternative oxidative fuel for extrahepatic tissues, including the brain, heart, and skeletal muscle. Beyond their role in energy metabolism, ketone bodies are now increasingly recognized as signaling molecules capable of regulating diverse cellular processes, prompting growing interest in their therapeutic potential across a range of physiological and clinical contexts.

Recently, orally ingested exogenous' ketone supplements have become available that can rapidly induce a transient increase in blood ketone body concentration, referred to as 'acute nutritional ketosis'. Exogenous ketone supplements permit direct testing of the metabolic effects of elevated blood ketone body concentration without the confounding influence of widespread physiological changes associated with fasting, starvation, or ketogenic diets. The availability of exogenous ketone supplements has contributed to increased interest in the prospective therapeutic applications of ketone bodies. An emerging area of interest is the role of ketone bodies in the regulation of skeletal muscle protein metabolism. Historically, ketone bodies were proposed to spare body protein during prolonged fasting by reducing the reliance on amino acids for gluconeogenesis as the brain transitions to ketone bodies as a primary fuel source. This longstanding hypothesis has stimulated renewed investigation into the role of ketone bodies in the regulation of skeletal muscle protein turnover.

This presentation will examine current evidence demonstrating that ketone bodies influence both muscle protein synthesis and muscle protein breakdown. Acute studies in healthy adults indicate that exogenous ketone supplementation can augment postprandial muscle protein synthesis, while experimental models of inflammatory stress suggest ketone bodies may suppress muscle protein breakdown, resulting in a more favorable net protein balance. The mechanisms underlying these effects remain incompletely understood but are thought to involve actions beyond their role as a metabolic fuel, including modulation of intracellular signaling pathways regulating protein turnover, attenuation of inflammatory signaling, and changes in cellular energetics.

This presentation will critically evaluate the mechanistic and translational evidence supporting ketone bodies as regulators of skeletal muscle protein metabolism. Although the current literature suggests ketone bodies may promote an anabolic environment and contribute to the preservation of skeletal muscle under certain catabolic conditions, many questions remain regarding the underlying mechanisms, the influence of age and disease, and the long-term effects of exogenous ketone supplementation on muscle mass and function. By integrating recent advances in ketone body biology

with emerging evidence on skeletal muscle protein metabolism, this presentation will examine the role of ketone bodies in the regulation of muscle protein turnover and discuss their potential as a nutritional strategy to support skeletal muscle health in aging and disease.

SA02

Effects of dietary and supplemental ketosis on carbohydrate and lipid metabolism

Javier Gonzalez[1]

[1]University of Bath, United Kingdom

Ketone bodies are endogenously produced by the liver under conditions of low carbohydrate and/or high fatty acid availability, and can affect metabolism via signalling or substrate effects. Ketone esters provide the possibility to increase circulating ketone body concentrations without the need for low carbohydrate or high fatty acid availability. This may circumvent some of the challenges and risks of ketogenic diets, facilitating more practical approaches to achieving ketosis. In this presentation evidence of the effects of ketogenic diets and ketone esters on carbohydrate and lipid metabolism will be presented. This will include effects of ketogenic diets and ketone esters on energy metabolism and body composition. Effects on glycaemia under diurnal and fasting conditions will be presented, in addition to the response to a meal challenge. Furthermore, potential mechanisms by which glycaemia is affected will be covered, in addition to trade-offs with lipoprotein metabolism.

SA03

Plant protein nutritional quality: What can in vitro models tell us about digestibility and amino acid Bioaccessibility?

Patricia Duque-Estrada[1]

[1]University of Copenhagen, Denmark

Plant proteins are gaining prominence in the transition toward more sustainable, plant-based diets due to their environmental and health advantages compared to animal-derived proteins. However, their nutritional quality is often constrained by suboptimal essential amino acid profiles and limited protein digestibility, influenced by the complexity of the food matrix (including the presence of antinutrients) and the effects of food processing. Processing plays a central role in modulating the nutritional quality of plant proteins, as certain methods, such as soaking, germination, cooking, and extrusion, can reduce antinutrients and improve protein digestibility. However, these processes may also induce undesirable changes, including protein denaturation, chemical modifications, and losses of essential amino acids. This highlights a key paradox: while processing can enhance functionality and sustainability, it may simultaneously compromise aspects of nutritional quality. This presentation will examine how in vitro digestion models can be used to investigate plant protein digestibility and amino acid availability, with a focus on their ability to capture the effects of processing and food matrix interactions. Emphasis will be placed on how these models contribute to mechanistic understanding and how well they reflect in vivo outcomes.

SA04

The brain as a regulator of organismal amino acid homeostasis

Clemence Blouet[1]

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Circulating amino acid concentrations are defended within narrow limits, yet how the organism maintains amino acid homeostasis in the face of variable dietary protein intake remains poorly understood. We propose that, much as it does for glucose, the brain acts as a central regulator of organismal amino acid balance—continuously monitoring protein and amino acid availability and orchestrating the behavioural and peripheral metabolic responses needed to preserve it.

As a molecular entry point into this framework, we identify the low-voltage-activated calcium channel Cav3.1 in hypothalamic POMC neurons as a sensor of the essential amino acid leucine. Postprandial leucine activates mediobasal hypothalamic Cav3.1 to suppress appetite and drive weight loss in response to dietary protein, and its pharmacological activation with SAK3 promotes weight loss and potentiates GLP-1 receptor agonism in diet-induced obesity. Beyond feeding, hypothalamic Cav3.1 signalling coordinates hepatic amino acid metabolism through parasympathetic innervation: loss of this signal—by MBH Cav3.1 deletion or hepatic cholinergic denervation—locks the liver in a fasting-like program and induces FGF21 independently of the hepatocyte integrated stress response, reproducing the full behavioural and metabolic signature of protein restriction even on a protein-adequate diet.

Together, these findings define a brain-to-liver axis in which hypothalamic leucine sensing gates whole-body amino acid handling, positioning central amino acid sensing as a tractable target for obesity and metabolic disease.

SA05

Protein and muscle conditioning: What's new?

Luc van Loon[1]

[1]Maastricht University, The Netherlands

Skeletal muscle protein is constantly being synthesized and broken down, with a turnover rate of about 1-2% per day. This muscle plasticity allows the muscle to remodel to support our exercise goals. The rate of skeletal muscle protein synthesis is regulated by two main metabolic stimuli, protein intake and exercise. Protein ingestion directly elevates muscle protein synthesis rates. The dietary protein derived essential amino acids act as signaling molecules activating anabolic pathways and function as precursors for muscle protein synthesis. When protein is ingested during recovery from exercise, muscle protein synthesis rates are further elevated. This improves the skeletal muscle adaptive response to exercise and, as such, allows more efficient muscle conditioning. New insights with regards to the impact of the quality, amount, and timing of protein ingestion during recovery from exercise will be discussed. Furthermore, the anabolic properties of more sustainable protein sources and the impact of protein processing will be touched upon.

SA06

The role of n-3 fatty acids in muscle health during ageing

Stuart Gray[1]

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Skeletal muscle mass and function decline progressively from the fourth decade of life onwards, often culminating in sarcopenia and a concomitant loss of mobility and independence in older age. Recent estimates indicate that the excess health and social care costs associated with muscle weakness in the UK are approximately £2.5 billion per year. With the proportion of older adults (>65 years) predicted to increase substantially over the coming decades, there is an urgent need to develop effective interventions to preserve or enhance muscle mass and function.

Resistance exercise is the most efficacious strategy to counteract age-related declines in skeletal muscle mass and function. However, adherence to resistance exercise recommendations remains low, particularly among older adults, limiting its effectiveness at a population level. Consequently, nutritional interventions have attracted considerable interest as alternative or adjunctive strategies. Emerging evidence suggests that the long-chain n-3 polyunsaturated fatty acids (LCn-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may represent a promising intervention for promoting healthy muscle ageing. EPA and DHA are obtained primarily from oily fish, yet population intakes are generally low, with a large proportion of individuals consuming insufficient amounts to achieve recommended levels.

Early studies in cell culture and animal models demonstrated that LCn-3 PUFA can stimulate muscle protein synthesis (MPS) and potentially increase muscle mass. Consistent with these findings, epidemiological studies in humans have reported positive associations between oily fish consumption and muscle strength, while acute physiological studies have shown that LCn-3 PUFA supplementation augments the MPS response under hyperinsulinaemic–hyperaminoacidaemic clamp conditions. More recently, chronic supplementation studies have demonstrated improvements in muscle mass and function in older adults following fish oil supplementation. Supporting these observations, our recent work in a larger cohort of older adults (n = 94) demonstrated that 6 months of krill oil supplementation increased grip strength, knee extensor maximal torque, muscle thickness and muscle quality. These findings also suggested that some of the functional benefits occurred independently of changes in muscle mass, alongside increases in the M-wave, raising the possibility that neural adaptations contribute to the observed improvements. Further work is required to determine the mechanisms underpinning these effects. There is also evidence that LCn-3 PUFA supplementation may enhance adaptations to resistance exercise training.

This presentation will review the mechanistic and clinical evidence supporting a role for LCn-3 PUFA in healthy muscle ageing and present our latest findings examining the effects of LCn-3 PUFA supplementation on MPS responses to protein ingestion and resistance exercise in older adults.

SA07

Exercise and Nutrition as Co-Regulators of Skeletal Muscle Adaptation

Brian P. Carson[1]

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The adaptive capacity of skeletal muscle underpins athletic performance, healthy ageing and metabolic health. Skeletal muscle exhibits remarkable plasticity, adapting to exercise through coordinated changes in gene expression, protein turnover, mitochondrial function, extracellular matrix remodelling, and metabolic regulation. While exercise is widely recognised as the primary stimulus initiating these adaptations, nutrition is increasingly understood more than just a source of metabolic substrate, but as an active regulator of the molecular processes that underpin adaptation. Rather than acting independently, exercise and nutrition converge on shared signalling pathways and related metabolic networks to influence the magnitude, specificity and temporal progression of skeletal muscle remodelling.

This presentation will examine the emerging concept of exercise and nutrition as complementary co-regulators of skeletal muscle adaptation. Drawing upon evidence from molecular physiology, human intervention studies and translational research, it will explore how exercise variables, nutrient availability, protein quality, energy status and nutritional timing interact to influence anabolic signalling, mitochondrial biogenesis and metabolic adaptation. The presentation will also highlight recent work investigating the role of dietary proteins and bioactive peptides in modulating post-exercise skeletal muscle responses, alongside emerging opportunities arising from multi-omics approaches that are transforming our understanding of the molecular mechanisms linking nutrition and exercise.

By reframing exercise and nutrition as integrated co-regulators rather than independent determinants of adaptation, this presentation will propose a framework in which optimal skeletal muscle adaptation is viewed as the product of coordinated mechanical and nutritional signalling, with new opportunities to optimise human performance, enhance healthy ageing and develop more precise, mechanism-based nutritional strategies to maximise the benefits of exercise across health and disease.

SA08

Molecular and Mitochondrial Responses to Exercise in Human Skeletal Muscle Myotubes

John Noone[1], Reichelle Yeo[2], Maria Pino[2], Yifei Sun[3], Giovanna Distefano[2], Mark Campbell[4], Katie Whytock[2], Cheehoon Ahn[2], Polina Krassovskaia[5], Donghai Zheng[5], Filip Jevtovic[5], Cynthia Stowe[6], Shannon Emilson[6], Eric Ravussin[7], Nicolas Musi[8], Kim Huffman[9], Nicolas Broskey[5], Bryan Bergman[10], Martin Walsh[3], Bret Goodpaster[2], Joseph Houmard[5], Lauren Sparks[2]

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Introduction: Exercise training improves skeletal muscle mitochondrial energetics and insulin sensitivity, contributing to enhanced metabolic health and reduced all-cause mortality. Skeletal muscle stem cells may retain exercise-induced adaptations through persistent epigenetic, transcriptional and metabolic programming. Whether habitual physical activity and exercise training induce long-term cell-autonomous adaptations in skeletal muscle energetics and molecular regulation remains unclear.

Aim: To determine whether habitual physical activity and exercise training imprint persistent cell-intrinsic adaptations in human skeletal muscle stem cell-derived myotubes and skeletal muscle fibres.

Methods: Human skeletal muscle cells (HSkMCs) were isolated from *vastus lateralis* biopsies collected as part of the MoTrMyo ancillary study within the Molecular Transducers of Physical Activity Consortium (MoTrPAC). MoTrMyo recruited 601 extensively phenotyped participants (habitually active (HA), n=101; sedentary (SED), n=500). Of these subjects, baseline analyses were performed in a selection of habitually active (HA) endurance exercisers (HA-EE), habitually active (HA) resistance exercisers (HA-RE), sedentary individuals (SED) and sedentary individuals with obesity (OS-SED). $CD56^+$ myoblasts were immunopurified, differentiated into myotubes and assessed using high-resolution respirometry under carbohydrate (CHO)- and fatty acid-supported (FAO) conditions, with and without a 24-hour free fatty acid (FFA) challenge. RNA sequencing and DNA methylation analyses were performed in untreated myotubes. Complementary intervention studies assessed the effects of 12 weeks of endurance or resistance exercise training on mitochondrial energetics in HSkMCs (MoTrMyo) and permeabilized skeletal muscle fibre bundles (MoTrEnergetics) from previously sedentary participants. Statistical analyses used linear models adjusting for known covariates, with significance accepted at p<0.05.

Results: HA-EE HSkMCs demonstrated enhanced carbohydrate- and fatty-acid-supported mitochondrial respiratory capacity compared to HA-RE and sedentary groups (SED and OS-SED), alongside greater metabolic flexibility following FFA challenge. Differential gene expression between individual groups was modest; however, comparison of habitually active (HA; HA-EE + HA-RE) and sedentary (SED + OS-SED) participants identified 208 differentially expressed genes. Pathway analyses demonstrated enrichment of RHO GTPase cycling, RAC signaling, myogenesis, muscle contraction, vascular development and cellular differentiation. Integrative RNA sequencing and DNA methylation analyses identified coordinated transcriptomic-epigenetic relationships involving genes and promoter CpG methylation associated with RHO GTPase signaling (DEF6 promoter CpG1389), cell adhesion (F11R promoter CpG1434), protein

tyrosine phosphatase signaling (PTPN20 promoter CpG16413), extracellular matrix organization (AEBP1 promoter CpG14036), peptide processing (LVRN promoter CpG14684) and Ras signaling (TBC1D10C promoter CpG19048). WGCNA identified positive associations between mitochondrial respiration and gene co-expression modules. Complementary intervention studies demonstrated that 12 weeks of endurance or resistance training increased mitochondrial respiratory capacity in both primary HSkMCs (MoTrMyo) and permeabilized skeletal muscle fibres (MoTrEnergetics), with increased Complex II-supported respiration following both interventions.

Discussion: These findings demonstrate that habitual physical activity and exercise training induce persistent adaptations in skeletal muscle energetics evident in isolated HSkMCs and intact skeletal muscle fibres. Habitual exercise was associated with coordinated transcriptomic and epigenetic signatures linked to mitochondrial energetics, cytoskeletal remodeling and cellular differentiation, while intervention studies confirm that exercise training enhances mitochondrial respiratory capacity across multiple experimental models. Collectively, these findings support the concept that habitual physical activity imprints a persistent, cell-autonomous skeletal muscle phenotype and provide mechanistic insight into how regular exercise promotes long-term metabolic health.

SA09

The effect of menstrual cycle phase on muscle protein turnover

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While dietary amino acids and resistance exercise are recognised regulators of muscle protein turnover, skeletal muscle is also exposed to cyclical fluctuations in ovarian hormones across the menstrual cycle. Whether these hormonal fluctuations meaningfully influence skeletal muscle anabolic regulation remains unclear, yet menstrual cycle phase-based exercise recommendations have emerged.

This presentation will synthesise current evidence alongside findings from two complementary stable isotope tracer studies conducted in healthy, eumenorrheic females using gold-standard cycle phase verification under tightly controlled experimental conditions. The implications of these findings for menstrual cycle phase-based training recommendations, inclusion of females, and study design considerations in skeletal muscle physiology research will be discussed.

SA10

Aging muscle and anabolic resistance: from whole muscle to the single-fiber level

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Introduction

Around age 40, muscle mass begins to decline, potentially leading to sarcopenia, a condition associated with frailty and increased fall risk (1). This muscle loss has been attributed to “anabolic resistance”, defined as a reduced capacity to stimulate muscle protein synthesis (MPS) in response to essential amino acids (EAAs) or resistance exercise (REx), potentially due to impaired mTORC1 signalling (2-3). However, it remains unclear whether anabolic resistance and age-related myocellular changes, such as denervation and satellite cell loss, reflect intrinsic aging or are confounded by lifestyle factors, such as inactivity, adiposity, or medications.

Methods and materials
We addressed these questions by recruiting healthy, non-smoking young (n=10, 18-35 years) and older (n=11, 65-75 years) men who were lean, physically active, and free of medications. Participants underwent an acute trial consisting of high-volume unilateral REx (10 sets of 10 reps) followed by ingestion of EAAs (240 mg per kg bw). To investigate age-related muscle decline, we applied several complementary approaches, including stable isotope tracer infusions, immunoblotting, immunofluorescence, single-fiber experiments, and phosphoproteomics.

Results

Rates of MPS were comparable across age groups in response to EAA intake, both alone and after exercise. Similarly, mTORC1 signalling was maintained or more pronounced in older compared with younger men. Notably, older men displayed higher levels of amino acid transporters, nutrient sensors, and mTORC1 pathway activators ($p<0.05$). From the same cohorts, analyses of cross-sections revealed that older men had a lower proportion of type II fibers, with smaller and misshaped type II fibers, as well as fewer satellite cells and capillaries surrounding these fibers ($p<0.05$). In addition, older men showed more denervated and “grouped” muscle fibers compared with young men ($p<0.05$). These findings prompted further investigation of anabolic responses in different fiber types. To enable such analyses, we first developed a new method (THRIFTY) for fiber typing individual fibers, which was valid and shown to be more time-efficient than reference methods. We then applied THRIFTY and examined cell signalling responses to EAA intake alone and in combination with REx in pooled type I and type II fibers. The anabolic signalling response was similar or even more pronounced in older compared to younger individuals, with a more robust response observed in type I than in type II fibers ($p<0.05$). Finally, phosphoproteomic analysis of muscle biopsies from the acute intervention revealed that aging was associated with global suppression of the growth-induced phosphoproteome, despite intact mTORC1 activation in older muscle.

Conclusion

Healthy, lean, physically active older men did not display deficits in MPS or mTORC1 signalling in response to anabolic stimuli, indicating that anabolic resistance is not an inevitable consequence of

healthy aging. Reduced activation of the mTORC1 pathway or an impaired capacity to stimulate MPS is therefore unlikely to be driving muscle loss in this population. Instead, our findings suggest that other mechanisms, including denervation, satellite cell loss, and altered activation of signalling pathways beyond mTORC1, may contribute to age-related muscle decline.

SA11

Sex differences in skeletal muscle response to endurance exercise

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Endurance exercise reduces the risk of metabolic diseases by improving skeletal muscle metabolism, particularly in individuals with overweight and obesity. As biological sex impacts glucose and fatty acid handling in skeletal muscle, we hypothesized sex differences at the transcriptomic and proteomic level in the acute response to exercise and after an 8-week exercise intervention.

We analyzed skeletal muscle biopsies from 25 sedentary subjects (16f/9m) with overweight and obesity using transcriptomics and proteomics at baseline, after acute exercise, and following an 8-week endurance training program. Regulation of sex-specific differences was studied in primary myotubes from the donors.

At baseline, differences were dominated by higher abundance of transcripts and proteins regulating glycogen degradation, glycolysis and other fast-twitch fiber type proteins in males. Females showed higher abundance of proteins regulating fatty acid uptake and storage. Differentially methylated CpG-sites potentially explained up to 60% of transcriptomic and 80% of proteomic sex differences. Acute exercise induced stress-responsive transcripts and plasma myoglobin predominantly in males. Both sexes adapted to 8-week endurance training by upregulating mitochondrial proteins involved in TCA cycle, oxidative phosphorylation, and β-oxidation. Training equalized fast-twitch fiber type protein levels, mainly by reducing them in males. In vivo sex differences in autosomal genes were poorly retained in myotubes but partially restored by sex hormone treatment.

In conclusion, the sex-specific molecular profiles underpin differences in glucose and fatty acid metabolism in female and male skeletal muscle. After just 8 weeks, training attenuates skeletal muscle sexual dimorphism towards a common metabolically beneficial response.

C01

Effects of Anti-Inflammatory and Antioxidant Supplementation on Exercise-Induced Muscle Damage and the Repeated Bout Effect: an interim analysis.

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Introduction: Exercise-induced muscle damage (EIMD), caused by unaccustomed eccentric muscle contractions and subsequent mechanical damage, inflammation, and oxidative responses, is associated with soreness and impaired muscle function. Understandably, athletes often seek to mitigate these consequences by consuming anti-inflammatory (e.g. ibuprofen) and antioxidative (e.g. vitamin C) supplements so as not to interrupt training. However, it remains unclear whether such interventions impair adaptive responses, including the repeated bout effect (RBE), which attenuates subsequent muscle damage. Previous investigations have employed inadequate protocols to assess this.

Objectives: Assess whether anti-inflammatory (e.g. ibuprofen) and antioxidative (e.g. vitamin C) supplements attenuate the development of the RBE.

Methods: 23 adults (mean(SD) age: 22(1), BMI: 23.3(2.3) $kg \cdot m^{-2}$, ♀:♂: 12:11) participated in a randomised, double blind, placebo controlled parallel group study. Participants completed two 20-minute bouts of stepping exercise to a bench 110% of tibia length with the dominant leg always acting eccentrically, separated by 3-weeks. In visit 1, one group consumed vitamin C (1000 mg) 2-h pre-exercise and ibuprofen (400 mg every 8-h) for 48-h post-exercise (SUPP), while the other consumed matched placebos (PLA). In visit 2, the acute effects of supplementation were removed, with all participants receiving placebo; the only difference between groups were the residual effects of treatment from visit 1. To ensure internal validity, the study was described such that it could be interpreted as being a randomised crossover design. This concealed its true objectives in researching the RBE, meaning volitional measures were not influence by the expectation of reduced damage. EIMD was quantified through jump height and force-velocity profiling using a leg press dynamometer at baseline and immediately-, 2-h-, 24-h- and 7-d-post-exercise. Membrane permeability (creatine kinase (CK)) was assessed at baseline and immediately, 1-, 2-, 24-, and 48-h-post-exercise. All research was approved by a University of Bath Research Ethics Committee.

Results: Following the bout of eccentric exercise in visit 1, indices of EIMD were evident in both groups, with reductions in jump height and interpolated maximum power (Pmax) at 2- and 24-h ($p<0.05$), but this did not differ between groups ($p>0.59$). Peak post-exercise CK was increased for both groups ($p<0.05$), with this increase being numerically greater in PLA (SUPP: 32.8(56.2) *vs* PLA: 185.0(286.7) $U \cdot L^{-1}$; $p=0.08$; $d=0.74$). After exercise in visit 2, no between group differences in functional outcomes were observed; however, CK responses suggested an altered repeated bout effect. Compared to visit 1, CK responses in visit 2 were numerically, but not statically, attenuated in PLA (185.0(286.7) vs 121.5(134.0) $U \cdot L^{-1}$; $p=0.21$; $d=0.28$) whilst accentuated in SUPP (32.8(56.2) *vs* 130.1(214.2) $U \cdot L^{-1}$; $p=0.27$; $d=0.62$), and while time*group interaction was not statistically significant, the effect size indicated a potentially meaningful between-group difference throughout visit 2 ($p=0.23$; partial $\eta^2=0.14$; $d=0.78$).

Conclusion: This interim analysis suggests mitigating EIMD indices through supplementation may potentially alter the RBE response of muscle membrane proteins to subsequent eccentric exercise without influencing functional markers. We intend to fulfil the target sample size of 52, and broaden the

analysis of physiological markers to include more comprehensive assessment of muscle damage, oxidative stress, and inflammation.

C02

Semaglutide effects on muscle function and morphology in ageing: implications for nutritional physiology and sarcopenia mitigation.

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Aging causes a progressive decline in skeletal muscle mass and function, alongside shifts in fibre composition that reduce physical capacity. Glucagon-like peptide-1 (GLP-1) receptor agonists, like semaglutide, are widely used to reduce adiposity; however, their effects on aging skeletal muscle remain poorly defined. This study investigated low-dose semaglutide's effects on contractile function and micro-architecture in locomotor (soleus) and respiratory (diaphragm) muscles of young and aged mice.

Young (3-month) and aged (24-month) C57BL/6J mice were assigned to placebo or semaglutide groups (young placebo (YP, n=6), young semaglutide (YS, n=6), old placebo (OP, n=6), old semaglutide (OS, n=7)). Semaglutide was administered for two weeks (60 µg/kg/day, 3 days, week 1; 30 µg/kg/day, 3 days, week 2). In vitro contractile function of the soleus and diaphragm was assessed to determine force–frequency relationships and fatigue resistance (40 Hz stimulation every 2 seconds for 5 mins). A subset of samples underwent immunofluorescent staining for fibre type composition (type I, IIa, IIx/b), cross-sectional area (FCSA), capillarisation indices, and roundness using semi-automatic image analysis.

Body weight remained stable throughout the intervention due to the low doses administered (p=0.3846). Absolute muscle wet mass did not differ between groups; however, hindlimb muscle mass normalised to body weight was reduced in older mice (p=0.0118). Semaglutide reduced subcutaneous (p=0.0010) and visceral (p=0.0071) fat and liver mass (p=0.0021).

Semaglutide exerted age-dependent effects on function. In the soleus, twitch force was reduced in semaglutide-treated aged mice only (p=0.0116). A trend occurred for maximal specific force (N/cm^2), which was lower in older mice receiving semaglutide (p=0.0888). In the diaphragm, semaglutide showed a trend toward reduced twitch force in both age groups (p=0.0696) alongside an age × treatment interaction for maximal force (p=0.0286), indicating impaired force production in aged semaglutide-treated mice. Conversely, fatigue resistance improved following semaglutide treatment in soleus muscle, showing enhanced force maintenance during repeated stimulation (40 Hz every 2 s for 5 min) in both young and aged mice (p=0.0461). Diaphragm fatigue properties were unaffected by treatment or age.

Histological analysis revealed prominent structural remodelling underlying these functional shifts. The treatment and age interaction caused a reduction in type 1 FCSA (p=0.0018), with its SD altered by age (p=0.0073). Type 2a FCSA demonstrated a reduction when considering the interaction of treatment and age (p=0.0019) and age effect (p=0.0405), with its SD showing interaction (p=0.0483), treatment (p=0.0337), and age (p=0.0220) effects. Fibre proportions differed according to age (Type 1 increase: p<0.0001; Type 2a decrease: p=0.0024; Type 2b/x decrease: p=0.0005). Capillary-to-fibre ratio increased with age (p=0.0194) and age × treatment interaction (p=0.0108), while a trend toward reduction occurred with treatment (p=0.0795).

While low-dose semaglutide benefits mouse body composition without weight loss, this is accompanied by unfavourable changes in the older muscle. We observed a reduction in FCSA, while muscle mass

remained unchanged, suggesting an altered muscle composition. These structural changes may contribute to the impaired force-generating capacity observed in aged skeletal muscle. These data highlight the need to consider muscle-specific and age-dependent responses when evaluating the physiological impacts of GLP-1 receptor agonist therapies.

C03

A self-selected vegan diet reduces skeletal muscle mass in healthy older adults: a randomized controlled trial

Jacintha Domic[1], Pol Grootswagers[1], Philippe Pinckaers[2], Max Melchers[3], Luc van Loon[2], Lisette de Groot[1]

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Background: Adopting a plant-based diet may compromise protein intake and impair skeletal muscle mass in older adults.

Objectives: To compare the impact of a 12-week self-selected vegan diet to an omnivorous diet, and a self-selected vegan diet with resistance exercise (RE) on muscle mass, strength and muscle protein synthesis (MPS) rates in older adults.

Methods: This randomized controlled trial included 3 parallel study arms in which 72 healthy older adults aged ≥65y were randomly assigned to follow 1) a self-selected vegan diet (VEG), 2) their habitual omnivorous diet (OMNI), or 3) a self-selected vegan diet with RE (VEG-RE). At baseline and after 12 weeks, thigh muscle volume, thigh muscle fat infiltration, and body fat distribution were assessed using magnetic resonance imaging, appendicular lean mass with dual X-ray absorptiometry, and muscle strength via Biodex. MPS rates were assessed during the first 10 days using a deuterium oxide protocol. Other outcome measures were body mass, dietary intake, and physical activity. Linear mixed models were applied to test differences between VEG and the other groups. Results are presented as estimated marginal mean or change in estimated marginal mean (95%CI).

Results: VEG reduced protein intake with -27.7(-37.3,-18.2), -22.0(-31.7, -12.4), and -19.3(-29.2,-9.37) g/day in week 1, 6 and 12 (all P<0.0001 compared with baseline), which was similar to the decrease in VEG-RE (P>0.05), but different from OMNI (P<0.0001), in which protein intake did not change. Thigh muscle volume and appendicular lean mass significantly reduced in VEG (-0.61(-0.72,-0.50)L; -1.15(-1.46,-0.85)kg) compared to OMNI (-0.01(-0.11,0.09)L; +0.05(-0.23,0.32)kg; both P<0.0001) and VEG-RE (-0.35(-0.47,-0.24)L, P=0.0038; -0.62(-0.93,-0.32)kg, P=0.0033). MPS was lower in VEG (1.09(1.01,1.17)%/day) compared with OMNI (1.27(1.18,1.35)%/day); P=0.0073) and VEG-RE (1.24(1.16,1.33)%/day; P=0.0246).

Conclusions: A self-selected vegan diet reduces protein intake, skeletal muscle mass and MPS in older adults. The negative impact of a vegan diet on skeletal muscle mass can be mitigated by RE.

C04

Protein requirements measured by the Indicator Amino Acid Oxidation method are elevated long-term after Roux-en-Y gastric bypass surgery in adult men

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Rationale: Protein intake recommendations after metabolic and bariatric surgery range from 60 g/day to 2.1 g/kg ideal body weight but is based on weak evidence and does not consider time since surgery[1].

Aim: This study determined long-term protein requirements in adult men after Roux-en-Y gastric bypass (RYGB) using the Indicator Amino Acid Oxidation (IAAO) method.

Methodology: In a randomized, within-subject feeding study, 7 protein intake levels (0.2–2.0 g/kg body weight) were compared in adult men (18–65 years), 1-10 years after RYGB surgery and without diabetes. Each study day followed a 2-day standardized run-in diet, whereafter participants received eight hourly isocaloric meals, with different test protein intakes as free amino acids based on egg protein composition with excess phenylalanine (Phe) and tyrosine. Energy intake was individualized based on measured energy expenditure. From the fifth meal, 1-^{13}C-phe was added, and breath $^{13}CO_2$ measured during isotopic steady state to assess oxidation. Protein requirements were estimated using mixed effects changepoint regression on expired $^{13}CO_2$. Sample size was estimated based on the expected difference in amino acid oxidation between protein intakes below and above the predicted breakpoint, using an effect size derived from prior IAAO data; power analysis (80% power, $\alpha = 0.05$) indicated that 7 participants at each intake level would be sufficient. The study protocol was approved by the Research Ethics Committee of UZ Leuven Hospital and KU Leuven University, registered at ClinicalTrials.gov (NCT06853652), and conducted in accordance with the Declaration of Helsinki, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

Results: Eight participants completed 49 study days [median age 57 years (IQR 38–63)], median 34 months (IQR 18–39) post-surgery, achieving 76% (IQR 66–80) excess weight loss. Estimated average protein requirements were 1.42 g/kg/day (95% Confidence Interval [CI] 1.31–1.53; R^2 0.85).

Conclusion: This study provides the first direct estimate of protein requirements long-term after RYGB. Requirements appear substantially elevated relative to IAAO derived requirements for healthy younger or older men (average 0.93 g/kg, upper 95% CI 1.24 g/kg), potentially reflecting reduced postprandial efficiency in protein utilization[2,3]. Translating these findings into recommendations will need careful consideration of aspects such as protein type, meal timing, sex, obesity and diabetes.

C05

Recycle, Repair, Recover: Investigating the Human Skeletal Muscle Autophagic Response to Muscle Damaging Resistance Exercise and Nutraceutical Supplementation

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Background:

Performing unaccustomed high-intensity resistance exercise (REx) causes acute skeletal muscle (SkM) damage, which must be repaired to enable further training and muscle adaptation. Autophagy, a vital cellular quality-control process, facilitates rodent SkM repair by recycling damaged proteins and organelles via autophagolysosomal vesicles (Call and Nichenko, 2020). Whether this occurs in human SkM remains unclear as previous studies rely on static markers of autophagosomes (i.e. LC3-II/I ratio), which do not reflect autophagic flux (Acheson et al., 2025). Intriguingly, some nutraceutical compounds observed to accelerate human SkM recovery (Tanabe et al., 2015) also exert pro-autophagic effects *in vitro* (Zhang et al., 2016); however, it is unknown if this is the underpinning mechanism *in vivo*.

Aims:

We utilised a novel *ex vivo* autophagic flux assay to investigate whether damaging REx alters human SkM autophagic flux and if nutraceutical supplementation augments autophagy to aid recovery.

Methods:

Twenty young healthy untrained adults were randomised to consume a daily nutraceutical supplement containing curcumin, quercetin, rosehip, piperine, and spermidine (n=10), or a placebo (n=10) for 3-weeks before and 1-week after performing 5 sets of leg extensions and leg presses to failure (80%1RM). Markers of SkM damage, including lower body strength and creatine kinase (CK) in venous blood samples, were measured before, immediately (0h), 24h, 72h, and 1-week post-REx. Vastus Lateralis biopsies were donated before, 24h, and 72h. Tissue was immediately snap frozen or incubated in control or lysosomal inhibition (NH4Cl, leupeptin) medium for 1h to assess autophagic flux *ex vivo*. Autophagy-related proteins and myosin-heavy chain (MyHC) fragments were assessed in sarcoplasmic and myofibrillar SkM fractions via western blots, respectively. LC3-II autophagic flux was calculated as inhibited minus control conditions. Serum CK activity was analysed using an N-acetylcysteine-activated photometric assay. Data were analysed using a mixed-design ANOVA with Holm-Bonferroni corrected post-hoc tests if main or interaction effects were observed ($p<0.05$).

Results:

At 0h, 24h, and 72h post-REx, quadriceps pain increased whilst leg extensor peak isometric torque and jump height were attenuated (all $p<0.05$). Serum CK was elevated at all post-REx timepoints (all $p<0.01$). LC3-II flux was elevated at 72h ($p=0.018$) whilst traditional 'static' markers of autophagic flux (LC3-II/I ratio) remained unchanged. Cytosolic p62 content was increased at 24h and 72h (both $p<0.01$), suggesting increased ubiquitinated autophagic cargo alongside increased autophagic flux. Furthermore, peak MyHC fragmentation, a novel marker of contractile protein damage, was elevated 76% from baseline ($p=0.009$). Autophagy signalling proteins, including phosphorylated/total ULK1, Beclin-1, and ATG4 b, remained unchanged throughout, whereas the autophagy repressor BCL-2 was reduced at 72h vs 24h ($p=0.012$). Intriguingly, lysosomal markers LAMP-2 and v-ATPase B1/2 were reduced at 72h vs 24h ($p<0.05$), indicating that lysosome content was attenuated during elevated flux. Nutraceutical supplementation did not affect recovery or autophagy outcomes.

Conclusion:

Here, we show that unaccustomed REx increases human SkM autophagic flux, an observation not reflected in commonly utilised 'static' measures of autophagy. Furthermore, we posit that lysosome abundance could form an autophagy 'bottleneck' previously observed in mice (Call and Nichenko, 2020). Future studies should utilise *ex vivo* flux assays to understand how exercise, health, and disease modulate human SkM autophagy.

C06

Comparative Effects of Butter, Coconut Oil, and Olive Oil-Based Ketogenic Diets on Liver and Kidney Function in Wistar Rats

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Ketogenic diet (KD) is characterized by high fat, very low carbohydrate, and moderate protein intake, leading to a state of ketosis. Although commonly employed for weight management and therapeutic purposes, its potential impacts on hepatic and renal function remain a subject of debate. This study evaluated the effects of different dietary fat sources in a high fat KD coconut oil, olive oil, and butter on liver enzymes, renal markers, and body weight in albino Wistar rats.

Forty rats were acclimatized for two weeks under standard laboratory conditions with appropriate ventilation, a 12-hour light dark cycle, and constant temperature, following the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 2011). The animals were then randomly assigned to four groups (n = 10). Group A (control) received standard rat chow, while Groups B, C, and D were fed a 65% high fat KD formulated with butter, coconut oil, or olive oil, respectively, for eight weeks. Body weights were recorded weekly. At the end of the experiment, 24-hour urine samples were collected in metabolic cages. Blood was obtained via cardiac puncture under chloroform anesthesia, and serum was separated by centrifugation. Liver function was assessed by determining aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total protein, albumin, and globulin levels. Renal function was evaluated through serum and urinary urea, creatinine, and urinary albumin. Data were analyzed by ANOVA, with significance accepted at $P < 0.05$.

KD feeding significantly reduced body weight compared with control ($P \leq 0.05$). Hepatic enzyme activities increased markedly, particularly in coconut oil fed rats. AST levels were 160.1 ± 9.5 U/L (control), 143.4 ± 8.2 U/L (butter), 196.1 ± 3.9 U/L (olive oil), and 260.1 ± 17.8 U/L (coconut oil). ALT increased from 36.0 ± 3.9 U/L (control) to 91.2 ± 18.7 U/L (coconut oil). ALP increased from 9.90 ± 0.74 U/L (control) to 24.9 ± 2.5 U/L (butter). Total protein and albumin levels decreased significantly ($P = 0.0032$), while globulin remained unchanged.

Renal markers showed no deterioration. Serum urea decreased from 64.20 ± 3.41 g/dl (control) to 39.40 ± 4.70 g/dl (butter), 29.90 ± 1.46 g/dl (coconut oil), and 40.20 ± 2.62 g/dl (olive oil). Serum creatinine fell from 1.42 ± 0.04 mg/dl to 1.05 ± 0.09 mg/dl (butter), 0.85 ± 0.07 mg/dl (coconut oil), and 1.03 ± 0.07 mg/dl (olive oil). Urinary albumin declined from 0.22 ± 0.03 g/dl to 0.19 ± 0.02 g/dl (butter), 0.15 ± 0.02 g/dl (coconut oil), and 0.13 ± 0.01 g/dl (olive oil). Urinary creatinine was 3.84 ± 1.02 mg/dl (control), 3.84 ± 0.90 mg/dl (butter), 1.75 ± 0.46 mg/dl (coconut oil), and 4.31 ± 0.70 mg/dl (olive oil). Albumin:creatinine ratio decreased from 120.6 ± 32.0 to 94.6 ± 23.0 (butter), 174.7 ± 61.8 (coconut oil), and 41.3 ± 8.3 (olive oil).

In conclusion, high fat ketogenic diets induced hepatocellular stress, particularly with coconut oil, but did not compromise renal function. Olive oil based KD exhibited the most favorable hepatic and renal profiles.

C07

Intergenerational and sex-specific effects of gestational exposure to low-level environmental chemical mixtures on skeletal muscle structure

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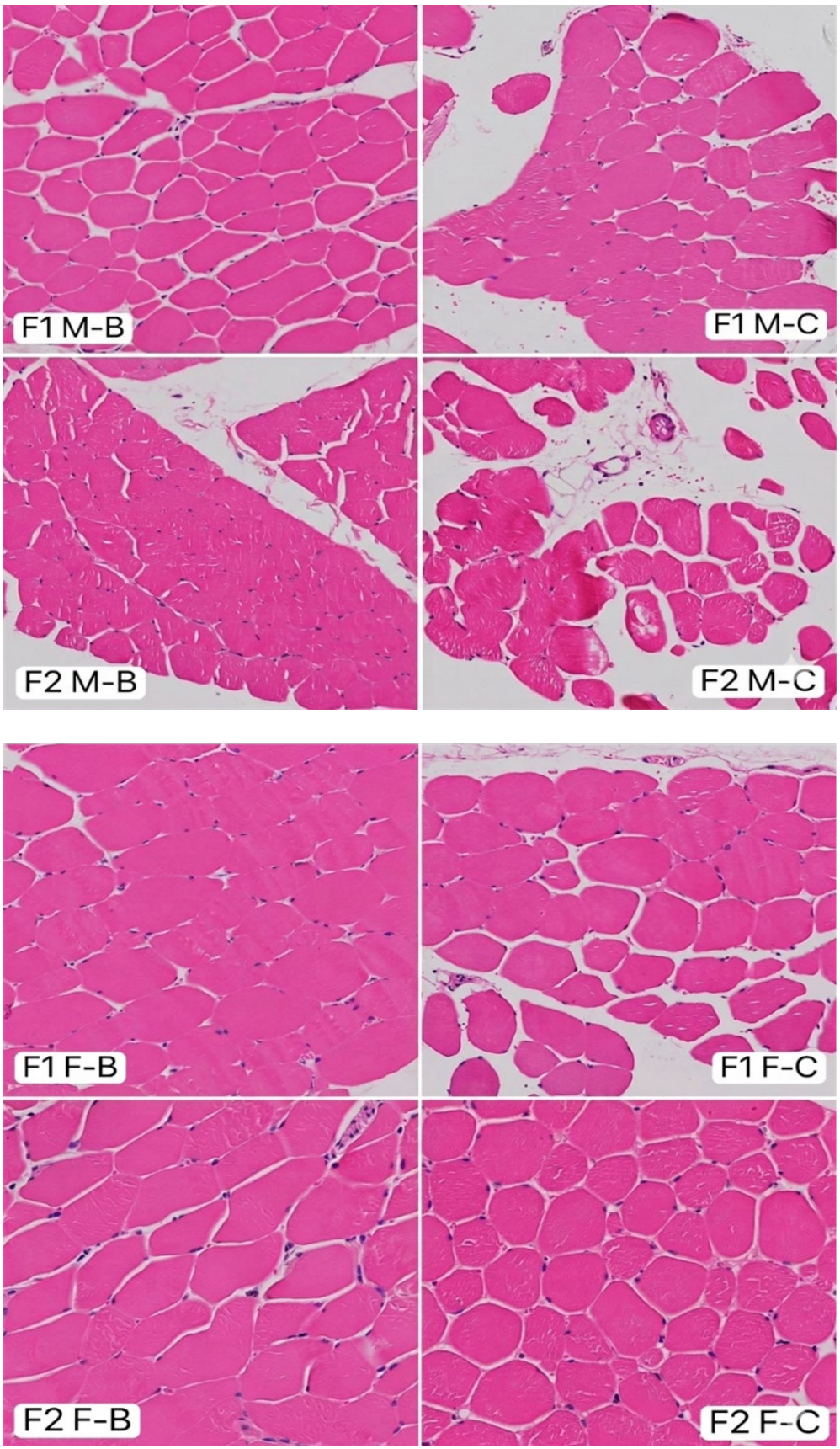
Humans are continuously exposed to complex mixtures of environmental chemicals (ECs), many of which are implicated as risk factors for metabolic disease. Biosolids-treated pastures (BTP) contain environmentally relevant mixtures of anthropogenic chemicals and provide a powerful “real-world” exposure model to understand EC mixture effects on metabolic health across generations. This study investigated sex-specific and multigenerational effects of maternal real-world EC mixture exposure on skeletal muscle, a key regulator of whole-body metabolic health, in adult F1 offspring, and their F2 descendants. Ewes (F0) were grazed on either control (conventionally fertilised) or BTP from one month prior to mating until parturition. All F1 and F2 offspring were maintained on control pastures throughout life. The Longissimus dorsi muscle was analyzed for myofibre cross-sectional area (CSA), fibre size distribution, and muscle regeneration to investigate whether gestational biosolids-treated pasture (BTP) exposure altered skeletal muscle morphology and regenerative characteristics across generations and between sexes. It was hypothesised that biosolids exposure during the sheep gestational stage would affect skeletal muscle metabolic health in offspring, resulting in increased central nuclei percentage and reduced muscle fibre size distribution and cross-sectional area, with effects differing between sexes and persisting across generations. Sample sizes were: F1 females (Control n = 10, BTP n = 10), F1 males (Control n = 9, BTP n = 12), F2 females (Control n = 12, BTP n = 12), and F2 males (Control n = 10, BTP n = 9). Mean myofibre CSA was significantly ($p = 0.015$) lower in F2 compared with F1 animals, with a significant ($p = 0.012$) sex-by-generation interaction. However, there was no effect of real-world EC mixture exposure on mean CSA ($p = 0.83$). Fibre size distribution differed significantly between control and BTP groups, characterised by a shift toward smaller fibres in F1 females ($p = 0.0047$) and in both sexes in the F2 generation, with the strongest effect ($p < 0.001$) observed in F2 males. These findings suggest that gestational BTP exposure altered skeletal muscle architecture and fibre remodelling across generations. In addition, BTP-exposed animals showed a trend ($p = 0.071$) toward increased centrally nucleated fibres, suggestive of muscle regeneration and/or tissue remodelling (independent of sex or generation). These

findings demonstrate that developmental exposure to real-world EC mixtures can reprogram skeletal muscle organisation across sexes and generations, supportive of a heritable biologic memory on muscle structure and metabolic health. All animal procedures were conducted under the UK Animals (Scientific Procedures) Act 1986 and approved by Cochno Farm and Research Centre, University of Glasgow. This work was supported by the National Institutes of Health R01 ES030374.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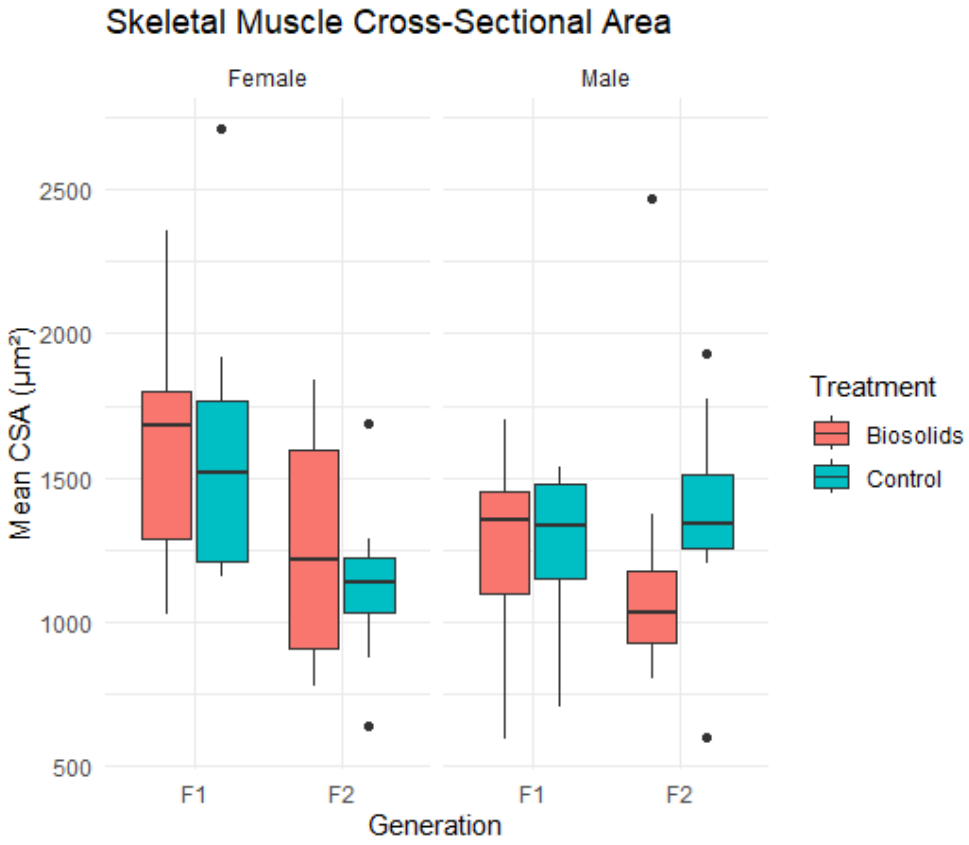
Skeletal Muscle Cross-Sectional Area
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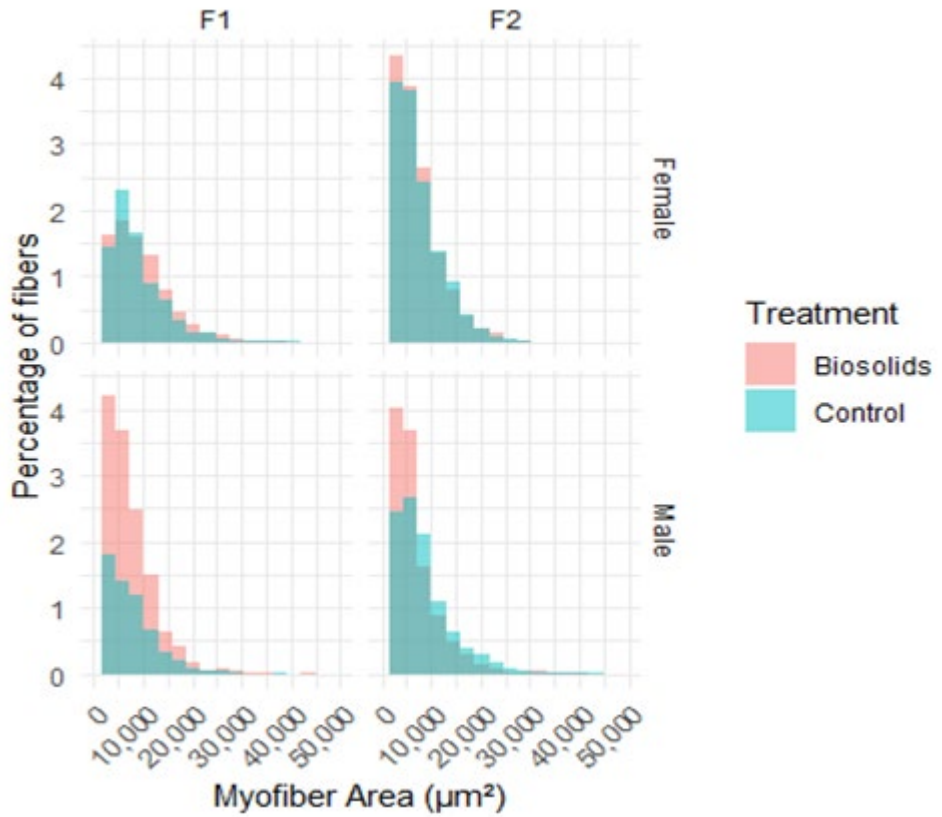
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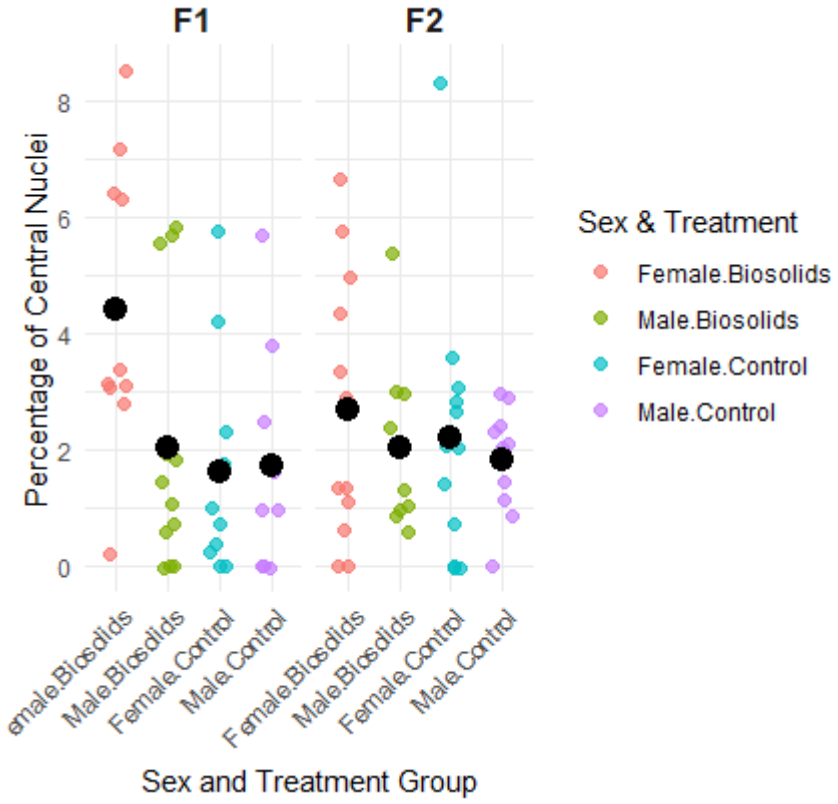
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Female.Biosolids
Male.Biosolids
Female.Control
Male.Control

C08

Diabetes-induced muscular atrophy was reversed by Ocimum gratissimum leaf extract treatment in male Wistar rat

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Introduction: Diabetic muscular atrophy (DMA) is a complication of diabetes mellitus characterized by proximal lower extremity muscle weakness, atrophy, pain, sensory disturbances, and, in severe cases, quadriplegia. Inflammation and oxidative stress are implicated in DMA; hence, therapeutic agents targeting either pathway, in addition to glycemic control, have been found beneficial for ameliorating DMA. *Ocimum gratissimum* (OG), scent leaf, cultivated for its culinary and medicinal values, is documented to possess antidiabetic, anti-inflammatory, and antioxidant properties. However, there is a dearth of information on the effect of OG on DMA.

Aims: This study investigated the effects of the aqueous leaf extract of OG (ALOG) on diabetes-induced muscular atrophy in male Wistar rats.

Method: Twenty male Wistar rats were assigned to four groups: Control, ALOG, Untreated-Diab, and ALOG-Diab (5 rats each). Diabetes was induced with streptozotocin in the Untreated-Diab and ALOG-Diab groups. Control and Untreated-Diab received distilled water, while ALOG and ALOG-Diab received 400 mg/kg ALOG orally for 4 weeks. Body weight and Fasting Blood Glucose (FBG) were monitored weekly. Thereafter, the rats were anesthetized with ketamine and xylazine, and the gastrocnemius muscle was obtained for glycogen determination; the right rectus femoris and extensor digitorum longus muscles were obtained to assess oxidative stress biomarkers and to extract mitochondria for mitochondrial permeability transition (mPT) assays. The left counterpart muscles were fixed for histology and morphometric analysis. Data were expressed as mean ± SEM and analyzed using one-way ANOVA.

Results: Untreated diabetic rats had higher FBG levels and significant weight loss compared with controls. The ALOG-Diab rats showed reduced FBG and body weight loss compared with the Untreated-Diab. Untreated Diab rats had lower gastrocnemius glycogen, increased malondialdehyde, increased mPT pore opening, and decreased ATPase activity; these changes were mitigated in ALOG-Diab rats. Histology confirmed muscular atrophy in Untreated-Diab, which was reversed in ALOG-Diab.

Conclusion: Muscular atrophy was ameliorated by *Ocimum gratissimum* leaf extract, potentially through mechanisms that include enhanced glycogenesis, reduced oxidative stress, and stabilization of mitochondrial permeability transition in the skeletal muscles of diabetic male Wistar rats.

C09

Nicotinamide nucleotide transhydrogenase (NNT) deficiency impairs glucose tolerance and muscle endurance regardless of sex and age in B6JRccHsd mice

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Introduction:

Deficiency of nicotinamide nucleotide transhydrogenase (NNT), a mitochondrial enzyme involved in antioxidant defense, impairs glucose tolerance in male mice [1]. However, females generally show lower levels of oxidative stress than males [2], whereas aging is associated with progressively increased oxidative stress [3], suggesting possible sex- and age-specific effects of NNT deletion. In addition, while skeletal muscle is the primary site for glucose disposal, it remains unknown if NNT deficiency directly impairs muscle function.

Aims:

This study aimed to investigate whether the effects of NNT deficiency on glucose metabolism and muscle function are sex-dependent across the lifespan.

Methods:

All animal handling procedures were conducted in accordance with the EU Directive 2010/63/EU and were approved by the Animal Welfare Committee of Wageningen University (2020.W-0019.004). Female and male congenic BL6JRcc.BL6J-$Nnt^{C57BL/6J}$/Wuhap (Nnt^{Δ}) mice and B6JRcc(B6J)-Nnt^{+}/Wuhap (Nnt^{WT}) mice were utilized for experiments at 3, 6, 12, 18, and 24 months of age (n=13-15 per experimental group). Body weight, body composition, and food intake were measured. Oral glucose tolerance test (OGTT) were performed to assess glucose handling, and glucose and insulin levels were measured via a glucose meter and insulin ELISA kits, respectively. The Rotarod and inverted mesh hanging test (IMHT) was performed to assess motor coordination/flexibility and muscle strength/endurance, respectively. All data are presented as means ± SD. Data at each age point were analyzed using two-way ANOVA followed by Tukey's post hoc test, with genotype and sex as fixed factors. Statistical significance was set at $P<0.05$.

Results:

Female mice exhibited significantly lower body weight and lean mass compared to male mice at different ages ($P<0.0007$). Glucose area under the curve (AUC) during the OGTT was lower in females compared to males at 3 months (1365.2±178.0 female vs. 1605.3±77.0 mmol/L·min male, $P=0.039$) and 12 months (1534.8±236.2 female vs. 1796.3±176.5 mmol/L·min male, $P=0.013$), indicating greater glucose

tolerance. However, this protective effect in females disappeared at older ages (18, 24 months, P>0.224). Serum insulin AUC and basal insulin resistance (HOMA-IR) were lower in females compared to males at both younger (3 months, 54.4-61.2% lower, P<0.0001) and older ages (12, 18, 24 months, 35.3-73.6% lower in females vs. males, P<0.026). Nevertheless, NNT deficiency impaired glucose tolerance, an effect that occurred in both males and females at all ages tested (P<0.039). With respect to muscle function, females outperformed males in motor coordination on the Rotarod (P<0.001 to P=0.066) and muscle endurance during the IMHT (P<0.001 to P=0.063). NNT deficiency did not affect motor coordination (P>0.069 to P=0.745). However, muscle strength and endurance were lower in mice lacking NNT at 3 months (17370±2820 Nnt^{WT} vs. 12394±3398 sec·g Nnt^{Δ}, P=0.009), 18 months (1322±324 Nnt^{WT} vs. 865±11 sec·g Nnt^{Δ}, P=0.02), and 24 months (1341±405 Nnt^{WT} vs. 867±98 sec·g Nnt^{Δ}, P=0.015), independently of sex.

Conclusions:

Females display better glucose tolerance, insulin sensitivity, and muscle endurance compared to males. Despite these sex-driven differences, NNT deficiency drives glucose intolerance in both males and females across all ages, and impairs muscle function during early adulthood and old age.

C10

Chronic Spinal Cord Injury Is Associated with Lower Fat-Free Mass Across All Body Compartments Compared to Non-Injured Controls

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Background:

Spinal cord injury (SCI) results in profound changes in body composition characterised by muscular atrophy below the level of injury (LoI) and increased adiposity. Paralysis of the lower limbs increases reliance on upper limbs for daily mobility and physical activity. Whether this compensatory increase in upper limb activity affects upper limb and total fat-free mass is currently unclear.

Objectives:

To compare whole body and regional (upper limbs, lower limbs and trunk) FFM between individuals with chronic SCI (>1 year) and non-injured controls adjusted for age, sex and height.

Methods:

A retrospective cross-sectional study included individuals with chronic SCI (*n*=54;M=32,F=22; time since injury [TSI] 15±10 years; lesion level C5–L3; tetraplegia, *n*=2, high paraplegia *n*=22, low paraplegia *n*=30; American Spinal Injury Association Impairment Scale [AIS] A–C) and non-injured controls (*n*=37; M=19, F=18). Whole-body dual-energy X-ray absorptiometry (DEXA) quantified total and regional (upper-limb, lower-limb and trunk) FFM. Between-group differences in FFM were assessed using general linear models, with group (SCI vs CON) as a fixed factor and age, sex, and height as covariates. Independent-samples t-tests compared age, body mass index (BMI), and body fat percentage between groups. Associations between LoI, TSI, and FFM were examined using Spearman's rank correlation coefficients (r_s). Data are presented as mean±standard deviation and 95% confidence intervals (CI).

Results:

Age did not significantly differ between SCI and controls (SCI: 47±8 years vs CON: 47±10 years; *p*=0.746). BMI was lower in SCI than controls (25.7±4.4 kg/m^2 vs 28.4±3.1 kg/m^2, respectively; *p*=0.001). Body fat percentage did not significantly differ between groups (SCI: 33±10% vs CON: 33±9%; *p*=0.904). After adjusting for age, sex, and height, individuals with SCI demonstrated significantly reduced lower-limb FFM compared with controls (-9.6 kg, -10.9 to -8.3 kg; *p*<0.001). Adjusted trunk FFM was also significantly lower in SCI (-2.9 kg, -4.1kg to -1.7 kg; *p*<0.001), as was upper-limb FFM (-1.2 kg, -1.9 to -0.6 kg; *p*<0.001). Adjusted total FFM was 10.5 kg lower in SCI compared with controls (8.3 to 12.7 kg; *p*<0.001). No associations were observed between TSI and upper-limb (r_s=0.069, *p*=0.620) or lower-limb FFM (r_s=-

0.166, p=0.232), nor between LoI and lower-limb (r_s=-0.113, p=0.417), upper-limb (r_s=-0.131, p=0.347), or total FFM (r_s=-0.124, p=0.372).

Conclusion:

These data suggest that SCI is associated with lower FFM across all body compartments; however, the reduction is disproportionately greater in the lower-limbs than the upper-limbs. These findings highlight reduced metabolically active FFM and limited compensatory adaptation, with important implications for health and exercise prescription in SCI. For example, such findings may support targeted interventions including neuromuscular electrical stimulation, resistance training (NMES) and/or hybrid exercise modalities such as NMES-RT and functional electrical stimulation aimed at eliciting lower-limb muscular contractions to preserve lean mass and potentially improve metabolic health.

C11

The effect of sleep restriction on physical and subjective markers of recovery after a late-evening simulated soccer match in male team sport players

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Introduction Although adequate sleep is widely recognised as essential for maintaining both physical and psychological health, its specific role in post-exercise recovery, particularly during the days following a training session or competitive event, remains insufficiently understood.

Objectives The aim of the current study is to investigate the effects of a single night of sleep restriction, following a late-evening simulated match, on physical and subjective markers of recovery in team sport players.

Methods Ethical approval was obtained from the Dublin City University Research Ethics Committee (DCUREC/2025/120). In a randomized, crossover design, eleven male team sport players (Tier 3; age: 29.3±5.7 years; body mass: 75.8±7.8kg; height: 1.75±0.04m; %fat: 16.7±4.7) completed two experimental trials consisting of an evening simulated soccer match (Loughborough Intermittent Shuttle Test; 90 min) followed by either a night of regular sleep (RS) or a sleep restriction condition (SR; 4-h sleep opportunity). Total sleep time (h) and sleep quality (10-point Likert scale) was monitored via sleep diaries. Neuromuscular function was quantified using a countermovement jump test, and maximal voluntary isometric and isokinetic contractions of the knee extensors and flexors before, and at 36 and 60-h post-match. Subjective recovery was assessed using 7-point Likert scales and 100-point visual analog scales (VAS) before, and at 12, 36, and 60-h post-match. Intermittent endurance capacity was evaluated via the Yo-Yo Intermittent Shuttle Run Test at 60-h post-match. Data were analysed using a linear mixed model with condition and time as fixed effects, participant ID as a random effect, and baseline data as a covariate. A paired-samples Student's t-test was utilized to evaluate differences in endurance capacity between conditions.

Results Significant reductions in total sleep time (4.0±0.6 vs. 7.4±0.6 h, p=0.003) and sleep quality (5.0±0.5 vs. 7.6±0.5, p=0.001) were observed in the SR condition compared to RS. Following the match, no differences were observed for jump height (F=0.09, p=0.76), isometric and isokinetic strength of knee extensors (F=1.12, p=0.72 and F=0.06, p=0.80, respectively) and flexors (F=0.42, p=0.52 and F=0.66, p=0.42, respectively) between conditions. However, a significant reduction [mean (95%CI):185 (30, 340) m] in endurance capacity was observed in SR (mean±SD:756±322 m) compared to RS (mean±SD:941±492 m) at 60-h post-match (p=0.02, ES=0.80), despite no concurrent differences in lactate (F=0.30, p=0.58) and RPE (p=0.27, ES=0.35). No differences were observed for subjective ratings pertaining to muscle soreness (F=1.04, p=0.31), fatigue (F=0.45, p=0.50), and readiness to train (F=2.85, p=0.09) (all VAS), nor muscular stress (F=1.77, p=0.19), lack of activation (F=1.39, p=0.24), negative emotional state (F=0.57, p=0.45), overall stress (F=2.65, p=0.11), physical performance (F=3.16, p=0.08),

or overall recovery (F=0.25, p=0.61) (all Likert scales). However, mental performance (F=10.3, p=0.002) and emotional balance (F=6.32, p=0.01) were negatively affected in SR compared RS condition.

Conclusion These findings suggest that a single night of sleep restriction may partially impair post-exercise recovery and recovery of function up to 60-h after a simulated soccer match, with endurance capacity being more susceptible than neuromuscular measures such as strength and power. Similarly, mental performance and emotional balance were negatively affected by sleep restriction, while other subjective markers of recovery were unaffected.

C12

Possible sex-specific effects of omega-3 polyunsaturated fatty acids on muscle recovery following acute exercise in older adults

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Introduction: Muscle mass and strength are crucial for healthy aging, but their maintenance depends on muscle recovery capacity that often declines with age. Omega-3 polyunsaturated fatty acids (n-3 PUFA) possibly improve muscle recovery due to their anti-inflammatory effects.

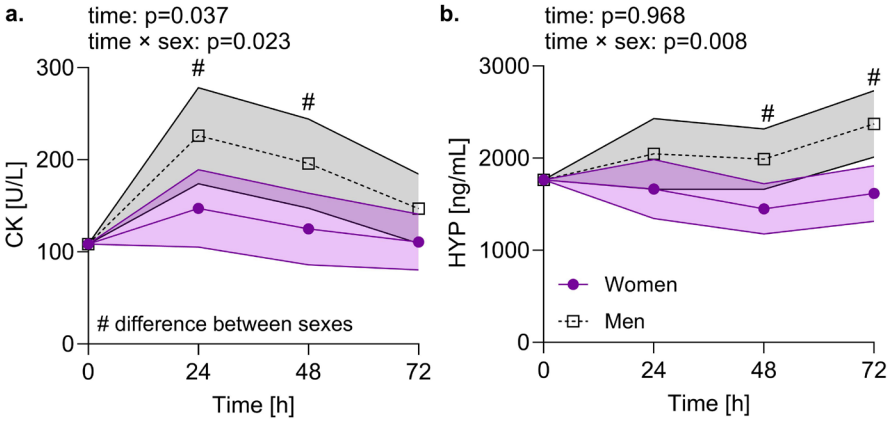
Aims and objectives: Recognizing potential sex-specific differences in inflammation and muscle physiology, we examined whether n-3 PUFA supplementation affects muscle recovery following exercise-induced muscle damage (EIMD) and collagen turnover markers differently in older women and men.

Methods: Forty-three healthy older adults (65-85 years) participated in an eight-week, double-blind, randomized, placebo-controlled trial (German Study Registry number DRKS00032985). Participants ingested either 5 mL n-3 PUFA algae oil (609 mg eicosapentaenoic acid, 1158 mg docosahexaenoic acid; n=21, women: n=13) daily or a placebo oil (sunflower oil; n=22, women: n=13). Plasma n-3 PUFA% index was measured. Following the intervention, participants performed a bout of resistance exercise consisting of 10x10 eccentric-emphasized leg extensions. Maximal voluntary contraction of the quadriceps, countermovement jump height, and perceived muscle soreness were assessed as functional indicators of EIMD. Creatine kinase (CK) and hydroxyproline (HYP) were measured as markers of muscle damage and collagen turnover; high-sensitivity C-reactive protein and interleukin-6 as inflammatory markers using ELISAs over 72-hours post-exercise. Trajectories were analyzed using repeated-measures ANOVA, adjusted for sex, pre-exercise concentrations and plasma n-3 PUFA% index. Data are presented as mean and 95%CI.

Results: Overall, functional recovery and post-exercise molecular markers did not differ between n-3 PUFA supplementation and placebo. Regarding sex differences, functional and inflammatory parameters were similar post-exercise (all $p>0.05$). To maintain a larger group size, we continued with sex-specific analyses adjusted for n-3 PUFA% index instead of group assignment. Men exhibited higher post-exercise CK concentrations than women at 24-hours (+78.9 U/L, 95%CI: 9.61;148, $p=0.027$) and 48-hours post-exercise (+71.0 U/L, 95%CI: 6.82;135, $p=0.031$). This results from women exhibiting only a small CK increase post-exercise (+38.7 U/L, 95%CI: -3.30;80.7, $p=0.070$), which returned to baseline by 48-hours. In men, CK peaked at 24-hours post-exercise (+118U/L, 95%CI: 65.4;170, $p<0.001$) and remained elevated above baseline at 72-hours (**Figure 1a**). HYP concentrations also differed significantly between the sexes (**Figure 1b**), with men having higher post-exercise HYP concentrations. In women, HYP declined from baseline to 48-hours post-exercise (-315 ng/mL, 95%CI: 42.9;588, $p = 0.025$), returning to baseline by 72-hours. In contrast, men had the highest HYP concentrations 72-hours after exercise (+607 ng/mL, 95%CI: 246;968, $p=0.002$). For HYP there was also a significant interaction with n-3 PUFA% index ($p=0.035$). Post-hoc analysis revealed that only in men, higher n-3 PUFA% index was associated with HYP response.

Figure 1. Trajectories of a. CK and b. HYP 24, 48, 72-hours post-exercise. 0h indicating baseline/pre-exercise. Repeated measures ANOVA adjusted for baseline concentrations of CK/HYP and plasma omega-3 PUFA index. Data shown as mean and 95%CI, #indicates difference between sexes.

Conclusion: We found significant sex-specific differences in muscle damage response and collagen turnover post-exercise. Plasma n-3 PUFA levels affecting HYP trajectories in men suggests sex-specific PUFA supplementation effects, that at least in men result in enhanced extra-cellular matrix remodeling.



C13

Metabolomic profiling of patients with heart failure and frailty: cross-sectional insights related to inflammation and energy metabolism

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Introduction: Metabolism changes leading to secondary sarcopenia is a hallmark of heart failure (HF). This study aimed to investigate how frailty and muscle weakness are associated with metabolic pathways in patients with HF using high-throughput plasma metabolomics.

Methods: Frailty was defined according to the European Working Group on Sarcopenia in Older People criteria as low physical activity combined with reduced handgrip strength and/or poor performance based on the 30-second chair stand test. Plasma metabolomic profiling was performed using gas chromatography-mass spectrometry to compare metabolic profiles among four groups: HF patients with frailty (HF-Frail), HF patients without frailty (HF-NonFrail), and non-HF controls with and without frailty (NonHF-Frail and NonHF-NonFrail). Circulating biomarkers including growth differentiation factor-15 (GDF-15), myostatin, activin A, follistatin-3, and TNF-α were quantified by enzyme-linked immunosorbent assay (ELISA). Statistical analyses were conducted using MetaboAnalyst and SPSS.

Results: Twenty-five outpatients with HF (mean age 67.9 ± 10.0 years; 19 males, 7 females) and 29 non-HF controls (mean age 67.8 ± 11.1 years; 14 males, 15 females) were included. Among HF patients, 18 were classified as frail (HF-Frail), while 8 of the 29 controls were frail (NonHF-Frail). Compared with NonHF-NonFrail individuals, HF-Frail patients exhibited lower appendicular lean soft tissue index relative to body mass index ($p = 0.03$), reduced functional performance (6-minute walk distance, timed-up-and-go, 30-second chair stand, and handgrip strength; all $p < 0.05$), and elevated GDF-15 levels ($p < 0.01$). Metabolomic analysis revealed that HF-Frail patients had higher levels of glucose, tumour necrosis factor-alpha (TNF-a), GDF-15, amino acids (e.g., alanine, isoleucine, glutamic acid), and carbohydrate metabolites (e.g., galactose, galacturonic acid-1-phosphate) compared with non-HF controls. In contrast, compared with HF-NonFrail patients, HF-Frail showed lower levels of galacturonic acid-1-phosphate, methionine, indole-3-acetamide, and energy-related metabolites (e.g., pyruvic acid, malic acid), but higher concentrations of 2-hydroxy-glutaric acid ($p < 0.05$), isoleucine ($p = 0.02$), and valine ($p < 0.01$).

Conclusions: Patients with HF and frailty display distinct alterations in amino acid and carbohydrate metabolism, accompanied by elevated inflammatory and catabolic markers (TNF-a and GDF-15) compared with both non-frail HF patients and non-HF controls. These findings suggest that metabolic profiling may aid in frailty screening and highlight the potential of targeting catabolic and inflammatory pathways as therapeutic strategies in patients with HF and frailty.

C14

Bolus feeding promotes coordinated temporal phosphorylation patterns in human skeletal muscle compared to continuous feeding

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Background: Specific molecular pathways control the distinct metabolic responses of human skeletal muscle to feeding. While 24-h transcriptomic analyses of skeletal muscle demonstrate robust diurnal rhythmicity, that is sensitive to divergent feeding patterns, phosphorylation signalling across the day is less well defined. This is notable as phosphorylation events are typically more transient in order to govern the rapid changes in cellular metabolism required to respond to changes in nutritional states. It therefore remains unclear how distinct feeding patterns impose structured temporal organisation on the muscle phospho-proteome.

Aims/Objectives: This study is an exploratory analysis to characterise the 24-hour temporal dynamics of the human skeletal muscle phospho-proteome under varying patterns of controlled nasogastric nutrient delivery (continuous *versus* dual-bolus feeding).

Methods: Following 7-days of lifestyle standardisation 18 participants (16M/2F; Age: 26 ± 9 y; BMI: 23.5 ± 2.3 $kg \times m^{-2}$; Resting Metabolic Rate; 1842 ± 229 $kcal \times d^{-1}$) underwent a 24-hour experimental period receiving enteral nutrition either continuously (n = 9) or as two discrete bolus 'meals' (0800 and 2000 h; n = 9), with skeletal muscle biopsies collected every 4 hours for 24 hours (starting at midday). Phospho-proteomic profiling was performed using label-free mass spectrometry, followed by data preprocessing and missingness filtering (≥25 % and ≥70 % presence thresholds). Data were normalised on a per-phospho-site basis, and temporal behaviour was analysed using unsupervised clustering of scaled phospho-site profiles. Cluster assignments were compared between feeding conditions to assess changes in temporal organisation, and pathway enrichment analysis was performed to provide biological context to identified patterns.

Results: Unsupervised clustering identified six distinct temporal phosphorylation profiles per condition across ~10,500 phospho-sites, with the bolus condition characterised by a greater number of transient peaks and troughs relative to the more stable profiles observed under continuous feeding. Phospho-sites that exhibited clear time-specific patterns under bolus feeding became less temporally distinct under continuous feeding. Gene ontology analysis indicated that changes in temporal patterns between bolus and continuous feeding were associated with processes related to muscle development and adaptation, with additional enrichment of glycolysis and NADH regeneration.

Summary: These findings suggest that divergent feeding patterns drive coordinated, time-specific phosphorylation responses in skeletal muscle over a 24-h period, whereas continuous feeding leads to less defined temporal organisation of metabolic signalling. This highlights a potential role for feeding pattern in shaping muscle metabolic and adaptive responses.