New Perspectives on the Physiological Basis of Muscle Loss
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Abstract book

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SA01

Unravelling the roles of chronological and biological ageing in the etiology of muscle decline

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Sarcopenia is associated with functional impairments, loss of independence as well as chronic disease risk and poorer prognosis. Sarcopenia is underpinned by dysregulation of muscle protein turnover that results in a net negative muscle protein balance that is the metabolic basis of muscle loss. Age-related alterations in muscle protein turnover are exacerbated by inactivity/disuse and the presence of obesity and lipid excess, highlighting how aspects of biological or lifestyle ageing may accelerate this disease condition. In contrast, those who have participated in long-term exercise training (Master Athletes) generally display an optimal ageing trajectory, underscored by superior skeletal muscle health and performance. Nonetheless, despite these benefits the data from Master Athletes suggests that, to some degree, skeletal muscle deterioration is characteristic to inherent chronological ageing. Specifically, long-term exercise participation may not necessarily prevent/slow sarcopenia, but instead may shift the "set-point" from which deterioration begins. This talk will explore the mechanisms through which chronological and biological ageing processes alter skeletal muscle protein turnover, morphology, and sarcopenia-related outcomes. The conclusion will highlight how sedentarism, obesity and episodic disuse events fuel a vicious cycle of physiological decline, disability and metabolic disease risk that accelerates sarcopenia progression, whereas long-term exercise training and/or physically active living can counteract these detrimental effects to "buy-back" years of good health and function.

SA02

Changing focus from protein mass to proteostasis for healthy muscle aging

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The loss of skeletal muscle function with age often precedes and exceeds loss of mass, indicating that protein quality is as important as protein quantity. Although muscle mass, and hence protein mass, has an impact on overall function, it is equally important to have the correct proteins for cellular tasks and for those proteins to be assembled properly and function well. The matching of well-functioning proteins to the demands of the cell is referred to as protein homeostasis, or proteostasis. The dynamic mechanisms through which proteostasis is maintained is a network of complex interrelated cellular activities such as protein biogenesis, folding, transport and degradation that collectively determine proteome structure and function. This talk will address why it is important to consider proteostasis, rather than just protein mass, in studies of muscle aging. Further, the talk will discuss how to use tracer-based and other methods to address skeletal muscle proteostasis during aging and interventions.

SA03

Adipose-derived extracellular vesicles and their effect on human myotubes

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Background

With obesity rates escalating and an ageing population, the incidence of individuals who are co-morbid with sarcopenia and obesity (termed sarcopenic obesity) is becoming more prevalent. Critically, obesity worsens sarcopenia[1], impairing muscle metabolic function and exacerbating the age-related impairment in the anabolic response of muscle to exercise or nutrition[2, 3]. Currently, the pathogenesis of sarcopenic obesity is poorly elucidated. However, intercellular communication between adipose and muscle appears pivotal. Of significance, we previously reported that in older humans, the secretome from obese (but not lean) adipose tissue impairs myotube thickness and the fusion of muscle cells into multinucleated fibres[4], supporting the notion that cellular cross-talk between adipose and muscle is pathological with age and obesity.

Significantly, extracellular vesicles (EVs) have emerged as mediators of intercellular cross-talk, capable of transporting biomolecules between cell types[5], and mediating multiple biological processes implicated in the development of age-related chronic diseases. We hypothesised therefore that with obesity, adipose-derived EVs drive pathological adipose-muscle cross-talk, accelerating muscle ageing (sarcopenia). The aim of this study was to characterise EVs released from obese and lean human adipose tissue, and determine their effect on the transcriptome of human myotubes.

Methods

Adipose conditioned media (ACM) was generated over 24h from subcutaneous adipose tissue collected from patients undergoing orthopaedic surgery who were either lean (n=4), over-weight (n=5) or obese (n=7) (NRES 16/SS/0172). EVs were isolated from ACM by ultracentrifugation and characterised by nanoparticle tracking analysis (NTA), ExoView and their RNA cargo profiled by small RNA-sequencing. Primary human myoblasts were differentiated into multinucleated myotubes, and either untreated (n=5) or treated for 24h with either obese (n=5), over-weight (n=3) or lean (n=3) EVs, before being analysed by RNA sequencing and qPCR.

Results

NTA and ExoView analysis confirmed the presence of EVs in the resuspended ultracentrifuged ACM pellets. The concentration of EVs was significantly ($p<0.05$) greater from the ACM of lean individuals, compared to ACM from non-lean (BMI>25). RNAseq analysis revealed that human

myotubes treated for 24h with obese EVs exhibited differential expression of 129 genes (86 upregulated, 43 down-regulated), compared to untreated myotubes. In contrast, myotubes treated with over-weight EVs exhibited differential expression of only 6 genes, and no significant differentially expressed genes were exhibited by myotubes treated with normal-weight EVs, compared to untreated (FDR=0.2). IPA and Quaternary Dot Product Scoring Statistic confirmed an atrophic, inflammatory effect of EV treatment, which was exacerbated with increasing BMI, with IL1β a key upstream regulator and p38 mediated MAPK signalling implicated. Investigating this further, we found *via* qPCR analysis that stimulation of myotubes with obese EVs induced the expression of the muscle atrophic genes (MAFBx, FOXO3) and the pro-inflammatory genes IL-6 and IL-1β.

Finally, in attempting to identify potential EV mechanisms, EV cargo analysis using small RNA-sequencing detected a total of 629 miRNAs, of which 14 were differentially expressed in non-lean EVs, compared to lean EVs, including miRNAs implicated in musculoskeletal remodelling and ageing ($p<0.05$, fold-change>1.5).

Conclusion

EVs from non-lean adipose tissue transport miRNA cargo implicated in musculoskeletal ageing and differentially affect the transcriptomic profile of human myotubes *in vitro*.

1. Batsis JA, Villareal DT. Sarcopenic obesity in older adults: aetiology, epidemiology and treatment strategies. Nat Rev Endocrinol 2018; 14: 513-537. 2. Beals JW, Burd NA, Moore DR, van Vliet S. Obesity Alters the Muscle Protein Synthetic Response to Nutrition and Exercise. Front Nutr 2019; 6: 87. 3. Smeuninx B, McKendry J, Wilson D, Martin U, Breen L. Age-Related Anabolic Resistance of Myofibrillar Protein Synthesis Is Exacerbated in Obese Inactive Individuals. J Clin Endocrinol Metab 2017; 102: 3535-3545. 4. O'Leary MF, Wallace GR, Davis ET, Murphy DP, Nicholson T, Bennett AJ, et al. Obese subcutaneous adipose tissue impairs human myogenesis, particularly in old skeletal muscle, via resistin-mediated activation of NFkappaB. Sci Rep 2018; 8: 15360. 5. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 2018; 19: 213-228.

SA04

Disuse as an intervention to study the regulation of skeletal muscle mass: complicating factors.

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Physical inactivity (or muscle disuse) offers a useful laboratory intervention to study the role of muscle contraction in the regulation of skeletal muscle mass and quality. Physical inactivity interventions, such as limb immobilisation or bed-rest, also offer appropriate strategies to study muscle loss and deconditioning in the context of clinical situations such as injury, illness or hospitalisation. Such experimental physical inactivity experiments are commonly carried out in healthy, uninjured volunteers over relatively short periods (1 to 14 days). These 'uncomplicated' disuse models have shown us that the withdrawal of contraction *per se* brings about rapid declines in muscle mass and quality. Mechanistically, such work has shown declines in postabsorptive muscle protein synthesis rates and the development of anabolic resistance to dietary protein likely drive this muscle deconditioning, given human experiments have thus far failed to show changes in muscle protein breakdown. Interestingly, disuse studies encompassing complicating factors that may be beyond only withdrawal of muscle contraction, such as impact on nutritional requirements, presence of muscle damage, inflammation or stress related responses, give a less clear mechanistic picture. This lecture will review the contemporary evidence base for the physiological regulation of skeletal muscle mass during disuse, drawing on data from human studies applying both uncomplicated and more complicated experimental models.

N/A

SA05

Nutritional interventions to support active ageing

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Ageing is associated with an insidious loss of muscle mass, impairing functional and metabolic capacity, and negatively impacting both an individual's lifespan and healthspan. Effectively intervening in this process is a pressing societal and research concern, especially given that the population of older adults is increasing globally. Mechanistically, ageing is associated with a blunted muscle protein synthetic response to protein ingestion. As such, nutritional interventions to combat the anabolic resistance to protein feeding form the bulwark of strategies to mitigate age-related muscle loss. This presentation will review the contemporary evidence base for these protein-centric nutritional interventions, with a particular focus on the source of dietary protein. Additionally, we will explore interactions with exercise and potential novel nutritional interventions.

SA06

Oligonucleotide senotherapeutics for the diseases of ageing

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Common, chronic diseases associated with age have common roots. It is now apparent that many such disorders have their origins in the age-related decline of a number of interlinked basic health maintenance mechanisms, termed the 'hallmarks of ageing'. Our team has discovered a new, and druggable, hallmark of ageing; dysregulation of alternative splicing (AS). Splicing factor expression shows characteristic alterations in normal mammalian ageing, in human primary senescent cells, in cells from individuals with human progeroid syndromes and is predictive of multiple later ageing phenotypes in human populations. Furthermore, targeted restoration of splicing factor expression using small molecules or oligonucleotide therapeutics can attenuate cellular senescence phenotypes. In this presentation, I will outline the background leading to our discovery of dysregulated alternative splicing as a new hallmark of ageing. I will then describe our emerging data demonstrating that it is possible to attenuate splicing factor expression in a partially-senescence specific fashion in human primary cells of multiple lineages using oligonucleotide senotherapeutics, and that by doing so we are able to reverse features of senescence and markers of age-related disease in patient cells from an exemplar ageing disorder IPF and ex vivo in fibrosis-induced living human primary tissues. Drugs that target the regulation of splicing factors may therefore represent promising novel anti-degenerative therapies in the future.

N/A

SA07

Dysregulation of hydrogen peroxide-mediated responses to contractile activity in skeletal muscle loss.

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Attenuated responses to redox stress are a common feature of aged organisms and these appear to present in skeletal muscle as a reduced ability to respond to contractile activity. Contracting skeletal muscle generates superoxide from membrane-localised NADPH oxidases and this is rapidly converted to hydrogen peroxide (H_2O_2) which acts to stimulate specific adaptive responses. The nature of these responses is extensive and includes increased generation of stress proteins and upregulation of mitochondrial biogenesis. Recent data indicate that the concentrations of H_2O_2 generated within muscle fibres are insufficient to directly oxidise redox-sensitive proteins in key response pathways and indicate that effector proteins, such as peroxiredoxins, play a key role in mediating adaptations. Understanding the specific mechanisms involved and how these are modified in ageing and other conditions of skeletal muscle loss provides a potential route for interventions to maintain muscle mass and function.

SA08

Effects of beta2-agonist salbutamol on muscle mass maintenance are mediated by muscle contraction

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Muscle disuse leads to rapid loss of muscle mass and development of insulin resistance, which may be ameliorated by beta2-agonist administration. Here, we tested the impact of the fast-acting beta2-agonist salbutamol during immobilization. In humans, salbutamol enhanced insulin-stimulated glucose disposal on the whole-body level, but not in immobilized muscle. Salbutamol decreased the efflux of amino acids from the immobilized forearm, indicating increased muscle protein synthesis and/or inhibition of breakdown, but did not affect amino acid net balance. In agreement, in mice salbutamol increased cumulative muscle protein synthesis, but did not result in a net gain of muscle mass upon immobilization, due to an accompanying increase in muscle protein turnover. Molecular analyses revealed immobilization inhibited salbutamol's effects on muscle-transcriptome. In conclusion, salbutamol can increase muscle mass and glucose uptake, but not as effectively in inactive muscle, demonstrating that the mechanism of action and efficacy of beta2-adrenoreceptor signaling is muscle contraction dependent.

C01

MicroRNA miR-675 promotes the inflammatory response in muscle cells to promote muscle loss in COPD and following surgery.

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We and others have shown that the microRNA miR-675 and its host gene H19 associate with reduced muscle mass in patients with COPD and in older individuals. To determine the mechanisms by which miR-675 may contribute to muscle loss we compared its expression with that of all genes quantified by microarray in the quadriceps biopsies from 41 COPD patients and 12 controls then defined gene sets associating with H19/miR-675 expression by gene set enrichment analysis (GSEA). In patients, H19 and miR-675 were tightly correlated (r=0.0.67, $p=4x10^{-8}$) and both associated with epithelial mesenchymal transition (EMT) and myc regulated gene sets (both at FDR<0.001). miR-675 but not H19 expression was positively associated with TNFa signalling at an FDR<0.05. We next analysed miR-675 expression in 18 patients undergoing aortic surgery. Pre-surgical miR-675 expression was correlated with post-surgical gene expression quantified by RNAseq and the correlations analysed with GSEA. This analysis showed that genes positively correlated with pre-surgical miR-675 associated were enriched for inflammatory gene sets including the TNFa signalling gene set (2.9 normalised enrichment score (NES), FDR<0.001) and those negatively correlated were enriched for genes associated with oxidative phosphorylation (NES=-5.0, FDR <0.001) and myogenesis (NES=-4.0, FDR<0.001).

To understand this phenomenon, we determined the effect of miR-675 and its antagomiR on TNFa induced expression of MCP-1 in mouse C2C12 and human LHCN myoblasts. miR-675-5p enhanced basal MCP-1 expression in the absence of TNFa and the miR-675 antagomiR suppressed TNF-a induced MCP-1 expression.

To determine the mechanism by which this increase in inflammatory susceptibility occurred we determined the effect of miR-675-5p by RNAseq in LHCN cells in basal and TNFa treated conditions. Determination of differential gene expression followed by GSEA showed that genes elevated by transfection with miR-675 were enriched for those associated with the TNFa signalling both with and without TNFa stimulation (NES =2.0, FDR<0.001in the absence or presence of TNFa). Gene sets suppressed in the presence of miR-675 were enriched for those associated with cell proliferation in particular E2F and myc targets.

The genes showing the increased expression in the presence of miR-675 included both SAA1 and SAA2 as well as a number of members of the TNF receptor superfamily (including TNFSFR1B, TNFRSF9, TNFSFR10B, TNFSFR10D, TNFSFR11B). Future work will be designed to determine the mechanism by which miR-675 promotes expression of these genes.

Together these data indicate that the inflammatory response of muscle cells is increased by miR-675 providing a mechanism by which this miRNA enhances muscle atrophy.

C02

Post-exercise myofibrillar protein synthesis correlates with serum total and free testosterone, but not oestradiol or progesterone, across the menstrual cycle in young females

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Optimal adaptation to resistance exercise likely requires a maximal rate of post-exercise myofibrillar protein synthesis (MyoPS), which is thought to be partly regulated by sex hormones. In postmenopausal women, supplementation with testosterone and progesterone but not oestrogen has been shown to increase postabsorptive muscle protein synthesis. However, limited data are available on how naturally fluctuating sex hormones across the menstrual cycle regulate post-exercise MyoPS in younger females. This study investigated the relationship between sex hormone concentrations and MyoPS following resistance exercise and protein ingestion in young females.

Seventeen, healthy, eumenorrheic females (age: 27±7 y; BMI: 24±3 kg/m^2) participated in this randomised cross-over study during the early follicular (4±1d following menses) and late follicular (2±2 d before luteinising hormone surge) phases of the menstrual cycle. On each visit, a blood sample was collected to measure serum sex hormone concentrations (oestradiol, free oestradiol index, progesterone, oestrogen to progesterone ratio, testosterone, and free testosterone), participants then received a primed continuous infusion of L-[*ring*-$^2H_5$]phenylalanine for 7.5 h. Following a bout of resistance exercise, participants ingested a protein beverage. Muscle biopsies were collected before and during a 4 h post-exercise postprandial period to assess MyoPS. This study was approved by the Sport and Health Sciences Ethics Committee of the University of Exeter, in accordance with the standards for human research as outlined in the Declaration of Helsinki. Sex hormone concentrations between menstrual cycle phases were analysed using a paired t test. The relationship between sex hormones and MyoPS were assessed using Pearson's product moment correlation. Data are expressed as means±SD.

Serum oestradiol (855±571 vs. 183±78 pmol/L; P<0.001), progesterone (4.0±5.1 vs. 1.1±0.7 nmol/L; P=0.041), free oestradiol index (13.1±7.1 vs. 3.3±2.7 pmol/nmol; P<0.0001), oestradiol to progesterone ratio (659±866 vs. 225±154 pmol/nmol; P=0.046), total testosterone (1.3±0.6 vs. 1.2±0.6 nmol/L; P=0.023), and free testosterone (18±15 vs. 16±15 pmol/L; P=0.097) were all greater during the late follicular phase compared to during the early follicular phase. A moderate correlation was observed between free testosterone and postexercise MyoPS from 0-4 h (r=0.475, P=0.008), and this was trending to be correlated under the basal and early postexercise 0-2 h period (r=0.334, P=0.072; r=0.355, P=0.054; respectively). Total testosterone also moderately correlated with postexercise MyoPS from 0-4 h (r=0.418, P=0.022), but the remainder of the sex hormones (oestradiol, r=0.003, P=0.987; free oestradiol index, r=0.275, P=0.141; progesterone r=-0.078, P=0.682; oestrogen to progesterone ratio

r=0.105, P=0.583) did not significantly correlate with MyoPS over 0-4 h or at any other time point (*P*>0.050).

Post-exercise MyoPS correlated with total and free testosterone yet did not correlate with any other sex hormone, despite a wide range of concentrations measured across the early and late follicular phases. These findings suggest that testosterone, both total and free, may play an important role in the regulation of muscle mass in females.

C04

The contribution of whole-food and supplemental derived dietary protein, from animal and non-animal origins, to daily protein intakes in young adults: a cross-sectional analysis.

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To maximise the skeletal muscle adaptive response to resistance exercise, dietary protein amount and distribution are typically optimised, though less consideration is given to the origin of that protein. We characterised dietary protein intakes throughout the day, with the focus being on protein source (i.e. animal vs. non-animal) and form (i.e. whole-foods vs. isolated supplemental forms) from a cohort of young (18-40 y) resistance trained (training ≥3x/week for ≥6months; TRA; m, *n*=30; f, *n*=14) and recreationally active (active but not engaged in structured training; REC; m, *n*=30; f, *n*=30) individuals. Using 3-day weighed food diaries obtained from 10 previously conducted human nutritional physiology studies, we retrospectively assessed daily macro-nutrient intakes in a standardised manner using dietary analysis software (Nutritics Dublin, Ireland). Energy intakes tended to be greater in TRA compared with REC (10.2±2.9 vs. 8.6±2.8 MJ·d^{-1}, respectively; *P*=0.056) and were greater in males compared with females (10.0±1.6 vs. 8.3±0.0 MJ·d^{-1}, respectively*; P*=0.006). TRA consumed a greater (*P*=0.002) proportion of daily energy intake as protein compared with REC (23±6 vs. 19±5 %TotMJ) which also tended to be greater in males compared with females (22±3 vs. 20±2 %TotMJ; *P*=0.06). Absolute (*P*<0.0001) and relative (to body mass [bm]; *P*<0.001) protein intakes were greater in TRA (males, 159±54 g·d^{-1} or 1.6±0.7 g·kg^{-1} bm·d^{-1}; females, 105±40 g·d^{-1} or 2.0±0.6 g·kg^{-1}bm·d^{-1}: *P*<0.001) compared with REC (males, 101±37 g·d^{-1} or 1.3±0.5 g·kg^{-1} bm·d^{-1}; females, 85±23 g·d^{-1} or 1.3±0.4 g·kg^{-1} bm·d^{-1}: *P*<0.001), with absolute (*P*=0.025) but not relative (*P*=0.129) intakes being greater in males. Daily protein distribution followed a skewed pattern in both TRA (dinner, 49±25 > lunch, 39±25 > breakfast, 27±14 g·d^{-1}; *P*=0.001) and REC (dinner, 39±17 > lunch, 26±13 > breakfast, 15±10 g·d^{-1}; *P*=0.001), with TRA consuming more protein at breakfast (*P*<0.0001) and lunch (*P*=0.012). A greater proportion of total protein was consumed from animal- compared with non-animal- derived sources in TRA (68 vs. 32%, respectively; *P*<0.0001) and REC (64 vs. 36%, respectively; *P*<0.001), but that skew being present in males (72 vs. 28%, respectively; *P*<0.0001) and not females (56 vs. 44%, respectively; *P*=0.288). To a similar extent in both training statuses and sexes, a greater proportion (~88%) of total protein was consumed as whole-foods compared with supplemental protein (*P*<0.0001). We show that animal- and wholefood- derived proteins contribute the majority to daily dietary protein intakes in trained and recreationally active young men and women. Given current (sports nutrition) dietary protein guidelines are underpinned primarily from studies investigating isolated animal derived proteins, more mechanistic studies to investigate protein-rich whole-foods from a range of sources are warranted.

C05

The acute effects of low-intensity interval cycling with blood flow restriction in healthy middle-aged adults: potential applications for COPD patients.

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Muscle loss in chronic obstructive pulmonary disease (COPD) impairs patients' exercise capacity and health related quality of life (HRQoL). Exercise training, as part of a programme of pulmonary rehabilitation (PR), is important in the treatment and demonstrates clinically important improvements in symptoms burden, exercise capacity and HRQoL. However, weakness and fatigability of the ambulatory muscles hinder patients' ability to achieve optimal exercise intensity. This study explores a novel rehabilitation intervention, low-intensity interval cycling with blood flow restriction (IC-BFR), ultimately aimed at mitigating muscle loss and improving uptake and adherence in PR programmes designed for COPD patients. However, its application in COPD patients has been limited. We investigated the acute physiological responses of IC-BFR in healthy age-equivalent adults.

Fifteen healthy participants (male: n = 6, female: n = 9, age: 58.5 ± 5.7 y, BMI: 25.8 ± 2.9 kg/m2, $\dot{V}O_{2peak}$ 33.7 ± 10.9 ml/kg/min) volunteered for this study which had local ethics approval. Participants initially performed a ramp-incremental cycling test to determine $\dot{V}O_{2peak}$ and gas exchange threshold (GET) followed by a familiarisation session. In a randomised, crossover design, participants performed two experimental trials: interval cycling exercise with BFR and without (CON). Exercise involved three sets of three 2-minute low-intensity intervals at a power output equivalent to 90% of GET. Intervals were separated by 2-minute recovery. Each set was separated by a 5-minute inactive period. BFR was applied during each interval only. Heart rate (HR), oxygen saturation ($\%O_{2sat}$), blood pressure (BP) and rate perceived exertion (RPE) were monitored throughout exercise. Central BP and arterial stiffness (AIx) were assessed pre- and immediately post-exercise. Blood samples were obtained pre-exercise and at 24-, 48-, and 72-hours post-exercise and assessed for biomarkers of muscle damage (creatine kinase, CK) and inflammation (C-reactive protein, CRP; interleukin-6, IL-6). Data were analysed using a Linear Mixed Model test which accounted for the repeated measures design and missing data. Data are presented as mean ± SD. Significance was accepted at $p < 0.05$.

HR increased ($p < 0.001$) throughout exercise in both conditions but was not different between conditions ($p = 0.518$). $\%O_{2sat}$ remained unchanged ($p = 0.190$) throughout exercise and was not different between conditions ($p = 0.194$). Systolic ($p < 0.001$) and diastolic ($p < 0.012$) BP were greater during the interval phases of exercise in BFR compared to CON. However, during the recovery phases systolic ($p < 0.125$) and diastolic ($p < 0.626$) BP were the same. Central systolic ($p = 0.073$) and diastolic ($p = 0.064$) BP and AIx ($p = 0.485$) did not change. RPE was higher ($p = 0.033$) throughout exercise in BFR compared to CON. There were no changes in CRP ($p = 0.073$), IL-6 ($p = 0.091$), or CK ($p = 0.160$) in either condition.

BFR applied during interval exercise increased BP and RPE to a greater extent compared to CON. There were no other effects of BFR or muscle damage and inflammation. IC-BFR is well-tolerated in healthy adults, paving the way for its potential implementation in COPD PR programmes.

C06

Effects of acute caloric restriction on skeletal muscle homeostasis in ageing mice

Harrison Gallagher[1], Peter Tickle[2], Paul Hendrickse[3], Emily Storey[1], Marcelo Gomes Pereira[1], Nadira Yusupovna Yuldasheva[4], Lee Roberts[4], Thomas Scott Bowen[1]

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Background: Ageing is associated with a progressive decline in skeletal muscle mass and strength termed sarcopenia, which can influence both limb and respiratory muscles. However, established pharmacological interventions to treat sarcopenia remain limited. Lifelong caloric restriction (CR) of moderate deficits (~40%) can reduce sarcopenia in ageing rodents, but in humans extended CR periods are limited by both desire and commitment.

Objectives: To characterise the effects of an acute and mild caloric restriction intervention on limb and respiratory muscle properties in ageing mice.

Methods: Older male C57BL/6 mice (23 months; *n*=9) were subject to 4 weeks of caloric restriction (10-25% reduction in food intake) and were compared to *ad libitum* chow-fed control mice including age-matched (n=7) or young (10 months; n=7). Following euthanasia, a limb muscle (EDL) was evaluated for wet-mass and fibre cross-sectional area (via immunohistochemistry), whereas diaphragm bundles were isolated and directly stimulated *in vitro* across the force-frequency relationship to examine contractile function.

Results: Ageing was associated with an 18% reduction in EDL mass vs young controls (P=0.08), with calorie-restriction further decreasing wet-mass by 24% (P<0.01) and myofiber size by 14% (P=0.079) vs control younger mice. However, twitch specific force increased by 32% (P<0.01) following caloric restriction in aged mice (5.5 vs. 7.2 N/cm^2).

Conclusion: Acute caloric restriction in ageing mice was associated with atrophy in the limb muscle but improved contractile function in respiratory muscle. Mild and acute caloric restriction during ageing may have divergent structural and functional effects between limb and respiratory muscles.

C07

Anthropometric variables and cardiovascular profiles of participants in different sporting activities at the University of Ibadan

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Introduction: Regular sports participation offers significant health benefits, enhancing cardiovascular fitness, body composition, and overall well-being. Understanding the fitness profiles of student-athletes is crucial for optimizing athletic performance and promoting health among university populations. This study addresses the lack of comprehensive assessments by evaluating anthropometric characteristics and cardiovascular parameters across various sports disciplines at the University of Ibadan.

Methods: A total of 135 (male = 95, female = 40) student-athletes from the University of Ibadan participated in this cross-sectional study. Participants engaged in swimming, football, combat sports, basketball, athletics, and volleyball were recruited based on regular sports participation and no pre-existing cardiovascular conditions. Structured questionnaires were administered to collect demographic, sporting history, and other health-related information. Anthropometric measurements (height, weight, BMI, body fat percentage, hip circumference) and cardiovascular parameters (resting heart rate, blood pressure, VO2 max) were assessed using standardized techniques. Data obtained were analysed using descriptive statistics and ANOVA at $p<0.05$.

Results: Significant variations were observed in anthropometric characteristics and cardiovascular parameters across different sporting activities. Volleyball players exhibited the highest weight ($p<0.05$), while basketball players had the tallest stature. Additionally, specific sports showed distinct cardiovascular demands, with basketball and swimming showing higher VO_{2max} ($p<0.05$) compared to other sports. Blood pressure showed significant reductions ($p<0.05$) in systolic blood pressure among student-athletes.

Conclusion - The study highlights significant differences in anthropometric and cardiovascular profiles across different sporting activities. The findings provide valuable insights for optimizing training strategies, injury prevention measures, and health promotion initiatives within the university sporting community.

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C08

Novel Therapeutic Multiratio Lipid Profile Indices with chronic inflammatory biomarker for Predictive Detection of Early Muscle loss In Young Obese Adults

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Novel Therapeutic Multiratio Lipid Profile Indices with chronic inflammatory biomarker for Predictive Detection of Early Muscle loss In Young Obese Adults

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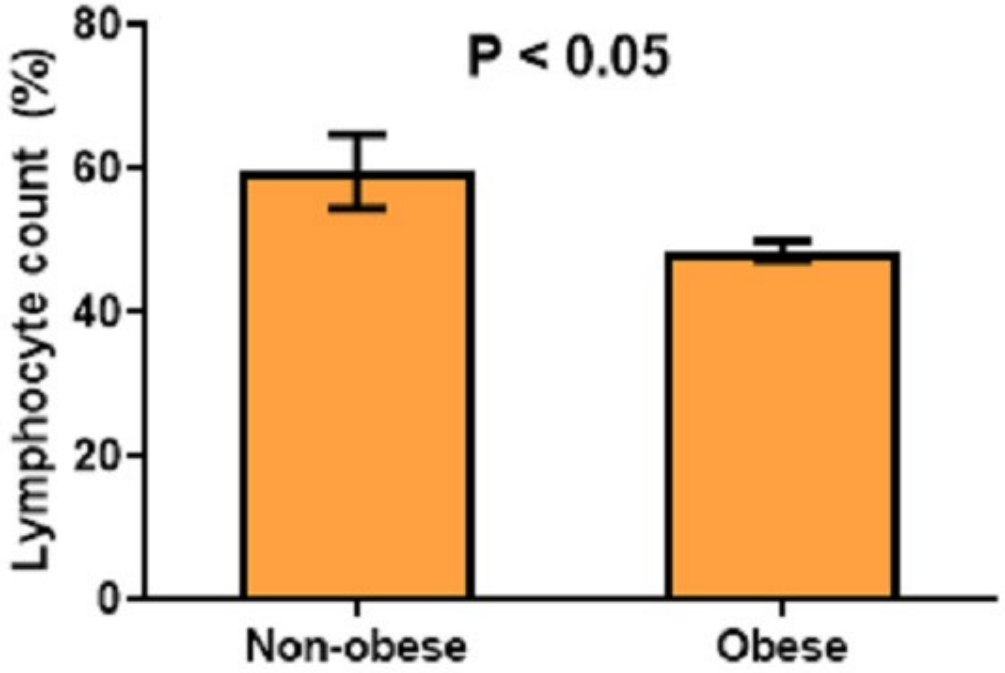
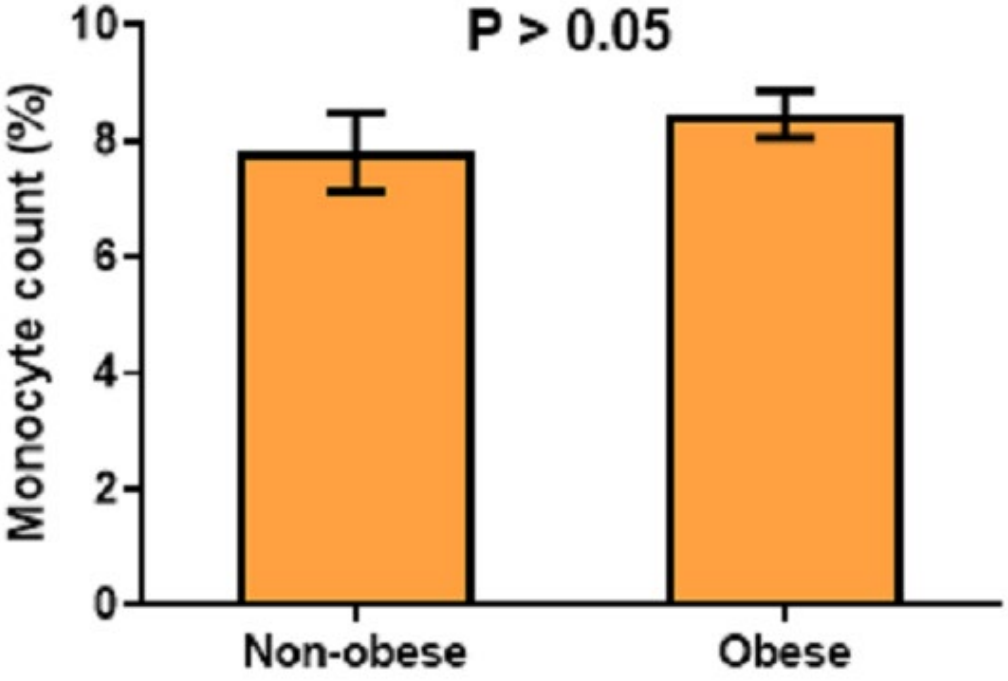
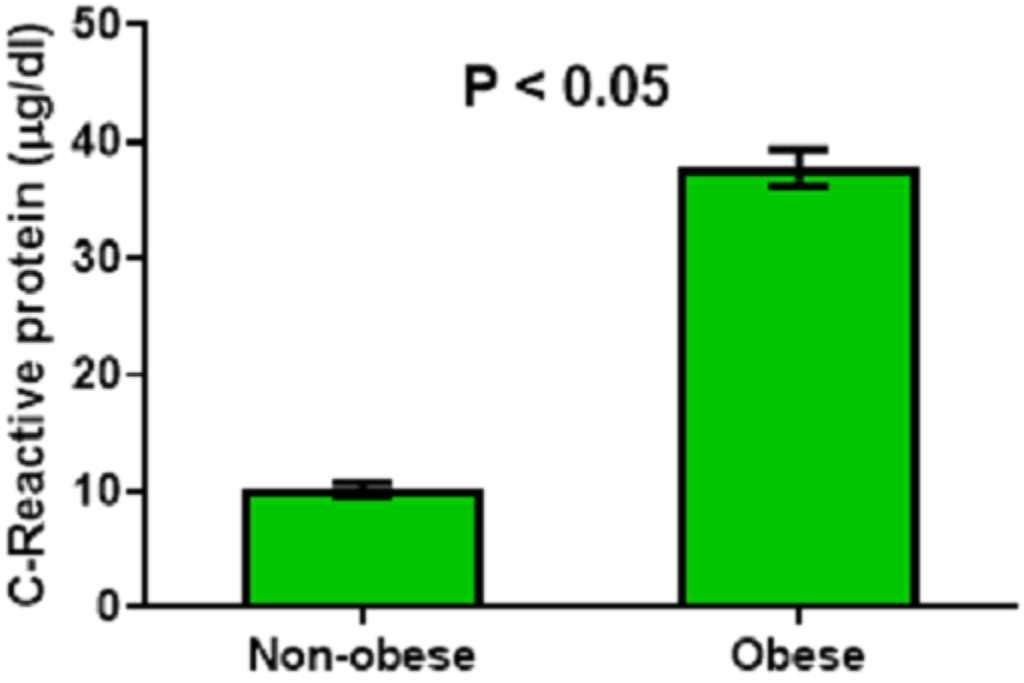
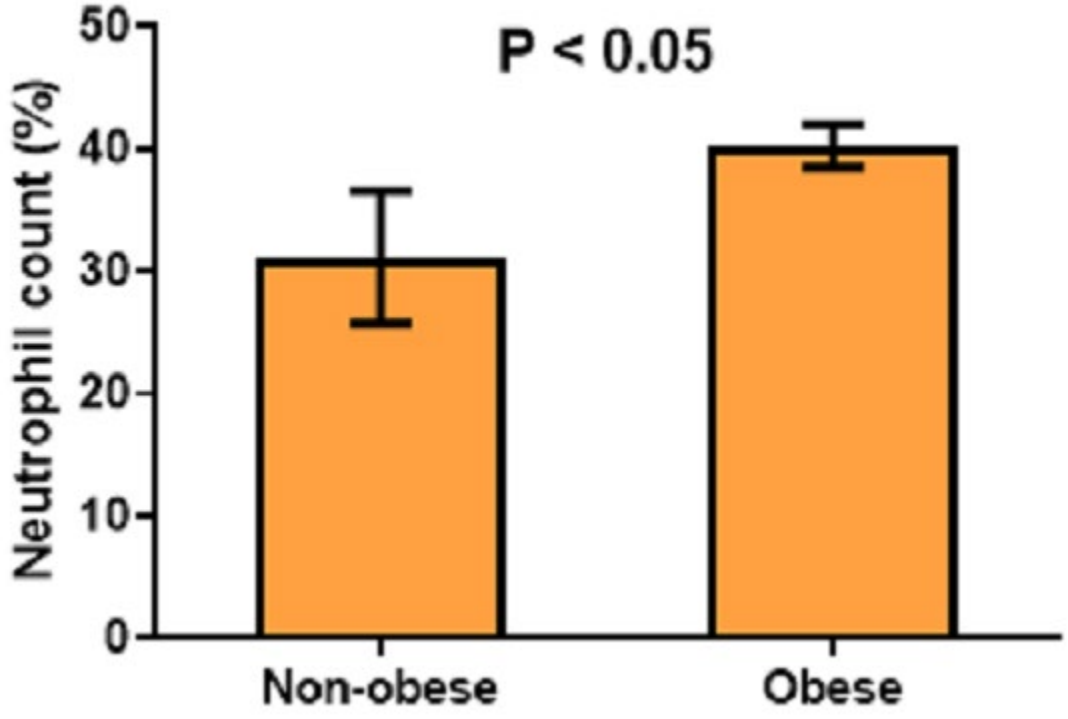
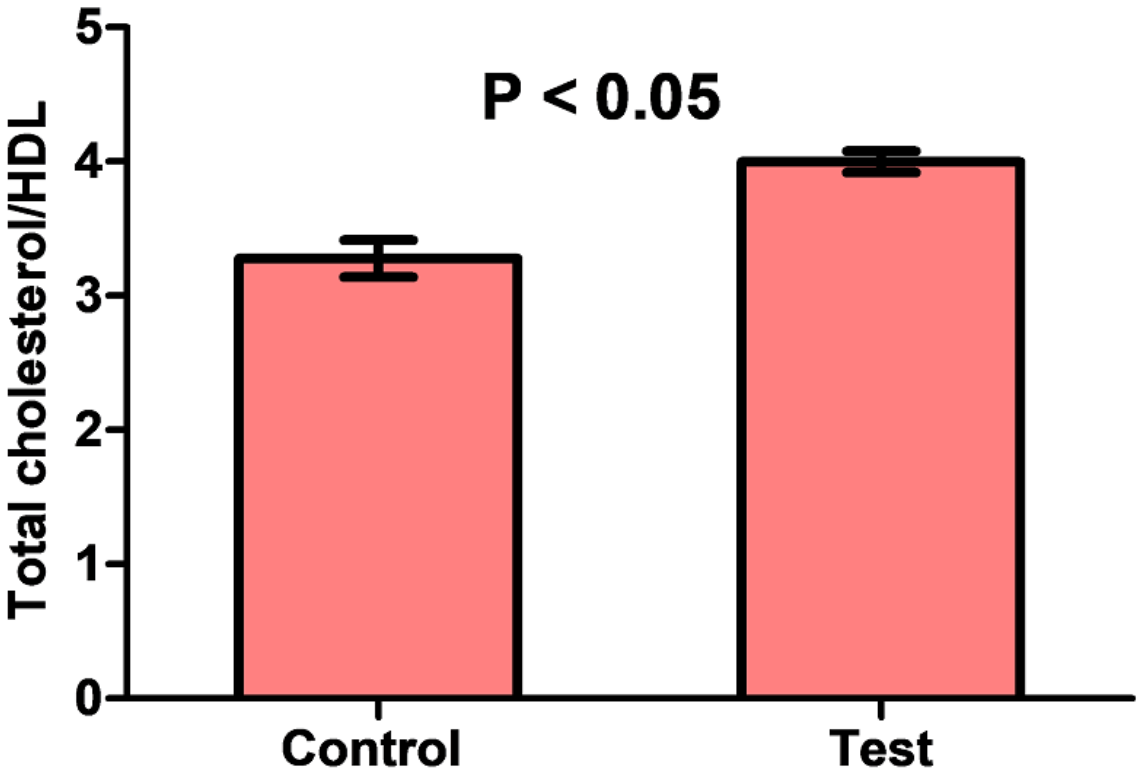
ABSTRACT
The prevalence of overweight and obesity is increasing at an alarming rate in some developing countries and in the developed world. BMI correlates well with the percentage body fat in the young and middle aged where obesity is most prevalent. Obesity is often accompanied by chronic low-grade inflammation. Adipose tissue, especially visceral fat, secretes pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). This study was aimed at employing a novel multiratio lipid profile indices for predictive detection of gradual muscle loss by examining the castelli risk index-1, castelli risk index-2, chronic inflammatory biomarker CRP and lipid profile variation amongst a population of young obese females in Benin City, Nigeria. The study was carried out with ethical permision from the College of medical sciences, University of Benin, employing 120 participants with 60 obese subjects as test subjects and 60 slim subjects as control. 5mls of blood were collected into lithium heparin bottles and centrifuged for 15mins. The supernatant was separated and placed in plain bottles and refrigerated at -20oC for lipid profile analysis, Castelli Risk Index 1 and 2 , and Atherogenic Index of Plasma were then derived by calculation. Our results revealed that there was no significant difference in triglyceride, LDL, and total cholesterol concentration between the control and test subjects ($p>0.05$) however there was significant reduction in HDL concentration and a significantly elevated value of C- Reactive protein, castelli risk index-1 , castelli risk index-2 and artherogenic index in obese participants compared to the non obese participants ($p<0.05$). In conclusion, High Castelli Risk Index values often correlate with insulin resistance and insulin is crucial for muscle protein synthesis. Insulin resistance can impair this process, leading to muscle atrophy hence considered an effective and prognostic tool for early muscle loss in young obese adults. In individuals with obesity, chronic low-grade inflammation is common. Elevated CRP and associated inflammatory markers are linked to insulin resistance. Insulin resistance impairs glucose uptake in muscle cells, which is crucial for muscle maintenance and growth. Reduced glucose uptake leads to decreased muscle protein synthesis and increased muscle breakdown.
Key words: Castelli index, Artherogenic index, lipoproteins, C-Reactive proteins


P < 0.05
Lymphocyte count (%)
80
60
40
20
0
Non-obese
Obese


P > 0.05
Monocyte count (%)
10
8
6
4
2
0
Non-obese
Obese


P < 0.05
C-Reactive protein (µg/dl)
50
40
30
20
10
0
Non-obese
Obese


P < 0.05
Neutrophil count (%)
50
40
30
20
10
0
Non-obese
Obese



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C09

Effect of myocardial infarction on skeletal muscle stem cell properties

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Background

Myocardial infarction (MI) is a leading cause of morbidity and mortality globally, and is a primary risk factor for chronic heart failure. The main symptom in patients with heart failure is exercise intolerance, but this is poorly correlated with cardiac dysfunction. In contrast, heart failure is commonly associated with skeletal muscle pathology that is closely linked to worse symptoms. However, the underlying mechanisms remain poorly defined. Skeletal muscle health is maintained by a population of quiescent muscle stem cells (MuSCs), but their role in heart failure-induced muscle pathology remains poorly explored.

Methods

We studied a mouse model of heart failure 4 weeks following MI surgery after ligation of the left coronary artery (n=7) compared to sham controls (n=4) in 15-week-old C57Bl6 females. To assess whether MuSCs were dysregulated post MI, fluorescent activated cell sorting (FACS) was used to isolate VCAM1$^+$/α7-Integrin$^+$/CD31$^-$/CD45$^-$/Sca1$^-$ MuSCs from hindlimb muscles. Populations of CD31$^+$ endothelial cells, CD45$^+$ hematopoietic cells, and Sca1$^+$ fibro/adipogenic progenitors were also characterised.

Results

Pathological cardiac remodelling was confirmed via *in vivo* echocardiography (left ventricular ejection fraction <40 %) and stained ventricular cryosections (infarction size >20%). Hindlimb muscle mass was 10 % lower (p=0.019) in mice with heart failure compared to controls (308±23 vs 344±13 mg, respectively). Moreover, isolated muscle fibre bundles showed overt weakness when stimulated maximally *in vitro* (27±3 vs. 16±2 N/cm^2) (p<0.001). Total isolated MuSCs were higher (p=0.002) in mice with heart failure compared to controls (3.4±0.5 vs. 2.3±0.4 %, respectively). The percentage of endothelial, haematopoietic and fibro/adipogenic progenitor cells were not different between groups (p>0.05)

Conclusion

These preliminary data indicate that heart failure may influence MuSC properties, but whether they contribute towards to the observed muscle pathology requires further investigation.

C10

The role of nicotinamide nucleotide transhydrogenase (Nnt) in energy metabolism and muscle metabolic health in B6JRccHsd mice during aging and hypoxia conditions

Jinmeng Yang[1], Jingyi Song[2], Desiree Bos[3], Marcel Jaklofsky[4], Melissa Bekkenkamp-Grovenstein[1], Claudia Carmone[1], Jaap Keijer[1], Sander Grefte[1]

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Introduction:

Age-related decline in skeletal muscle mass and function, leading to sarcopenia, significantly impacts the quality of life among the elderly, which becomes increasingly prevalent in modern society. Aging correlates with decreased mitochondrial energy production and increased production of reactive oxygen species (ROS). Healthy cells actively balances ROS production and detoxification using NADPH-dependent antioxidant defence systems. A major mitochondrial source of NADPH is nicotinamide nucleotide transhydrogenase (*Nnt*)(Chortis, Taylor et al. 2018). *Nnt* expression level decrease with age and this study focus on the role of *Nnt* in energy metabolism and muscle physiology during aging.

Objectives:

1. To investigate the role of *Nnt* on the lifespan of B6JRccHsd mice and the link between longevity and muscle transcriptome changes at early life.
2. To investigate the role of *Nnt* in glucose tolerance and muscle insulin signalling in B6JRccHsd mice during aging.
3. To reveal the effect of *Nnt* on the muscle molecular metabolic and bioenergetic signatures in young, adult and old B6JRccHsd mice exposed to an acute (6 hour) hypoxia exposure.

Method:

The experimental protocol for animal handling in accordance with the EU Directive 2010/63/EU for animal experimentation and approved by the Animal Welfare Committee of Wageningen University, Wageningen, the Netherlands (2020.W-0019.001 and 2020.W-0019.004).

Our study employs a unique mouse model, C57BL/6JRccHsd, with Nnt^{wt} (wild type) and Nnt^{mut} (mutation) in an identical genetic background. For objective 1, all mice (Female-Nnt^{wt}: n=37; Female- Nnt^{mut}: n=37; Male-Nnt^{wt}: n=45; Male- Nnt^{mut}: n=44) received *ad libitum* water and food until human end point criteria (HEP) was reached. Transcriptome analysis will be performed on *M. gastrocnemius* from mice used for objective 2. In objective 2, all mice (n=13) will be analysed for functional (EchoMRI, oral glucose tolerance test(OGTT), Rotarod, and Inverted Mesh Hanging Test), histological and molecular analysis at multiple time points (24 days, 3, 6, 12, 18, 21 and 24 months). Additionally, mice will undergo a 6-hour hypoxic exposure via our Indirect

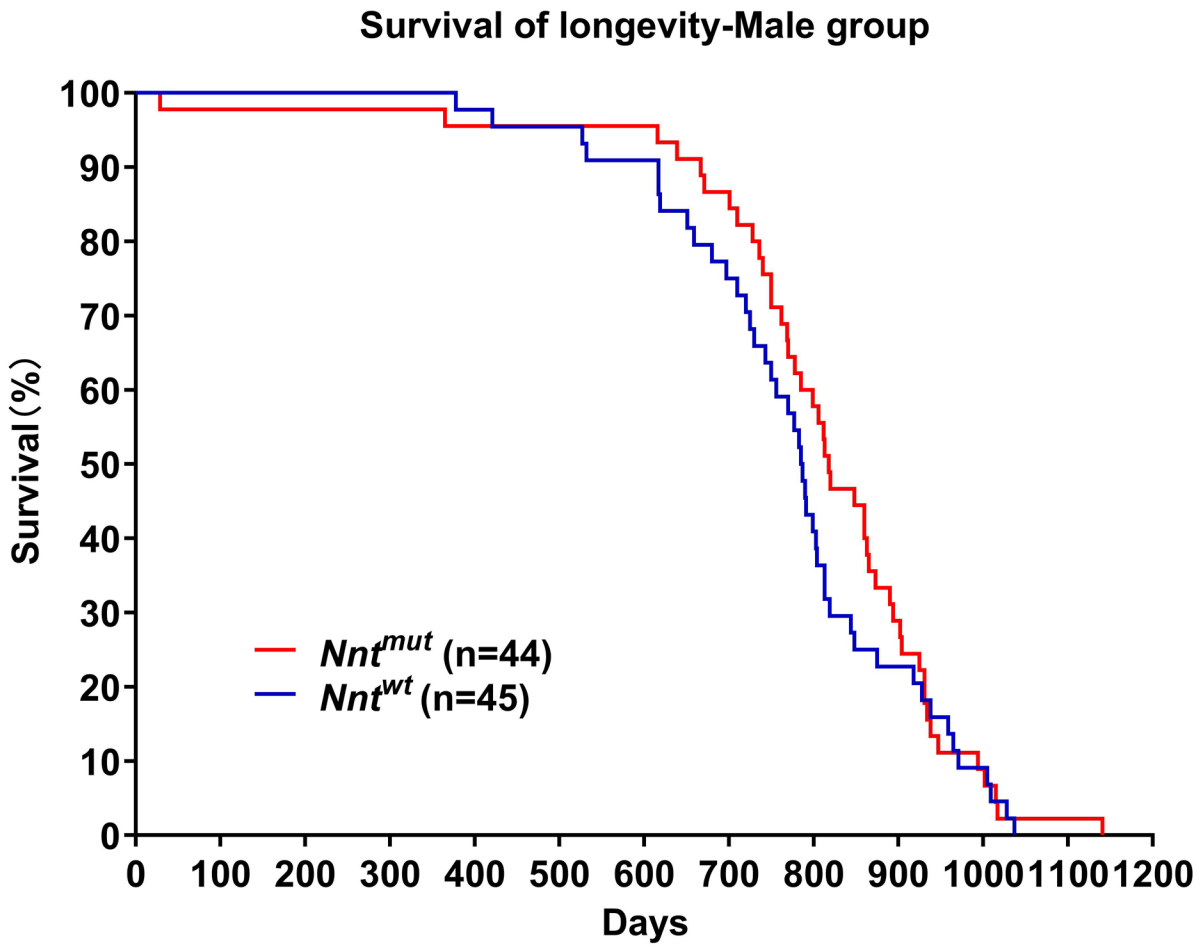
Calorimetry (InCa) setup for objective 3 and to calculate the respiratory exchange ratios (RER) (van der Stelt, Shi et al. 2022).

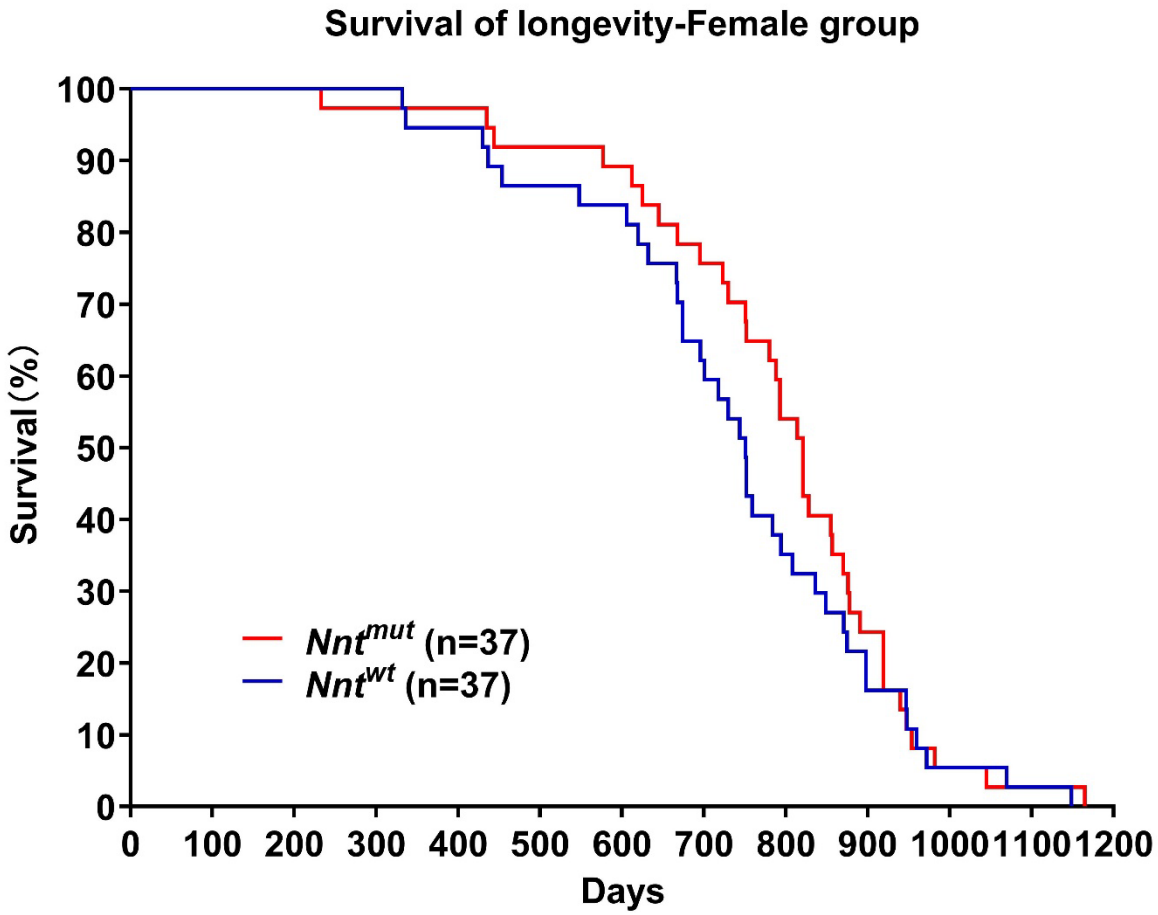
Results:

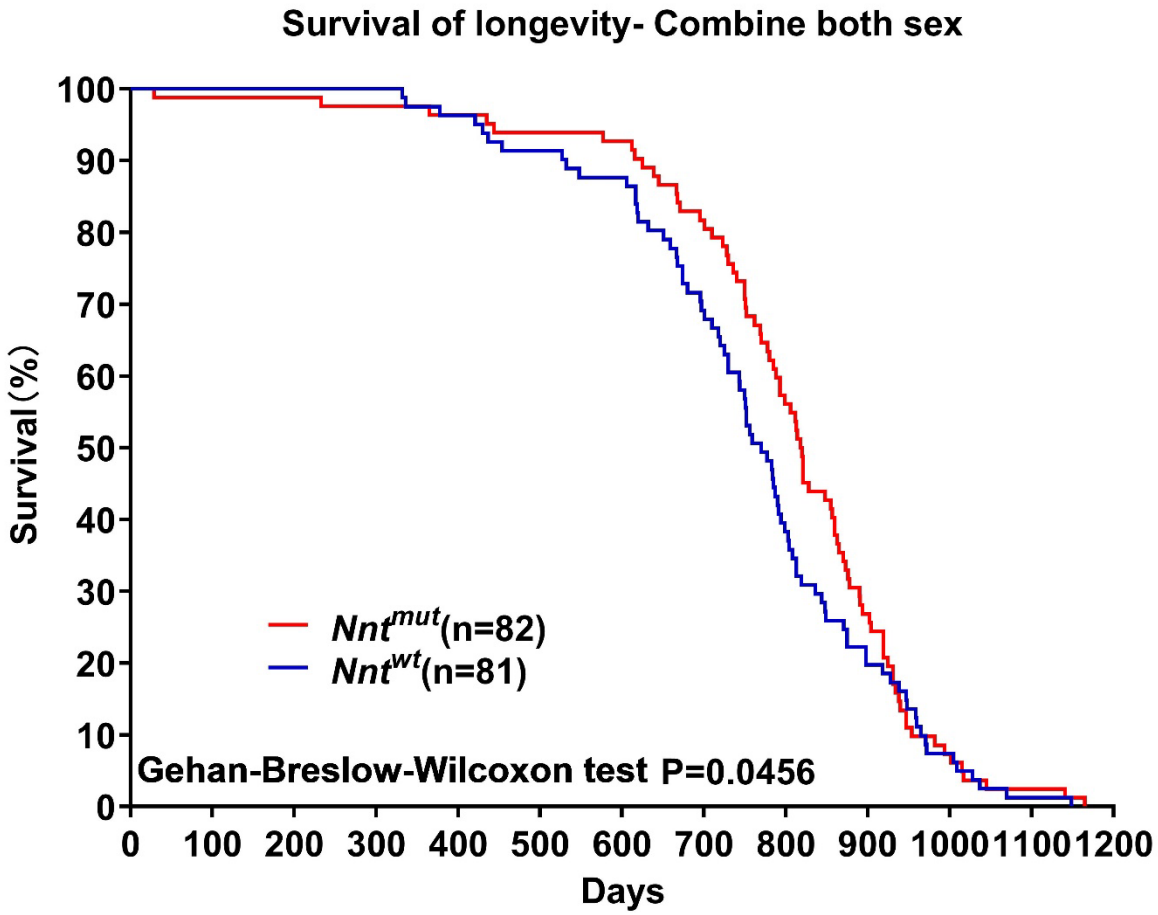
Longevity study did not reveal significant changes in the medium and maximum lifespan(Fig.1). However, when focusing on the early life survival of both female and male mice as a single group(*c*: n=81; Nnt^{mut}: n=82), the Gehan-Breslow-Wilcoxon test showed a significant improved early survival with the Nnt^{mut} (Fig.2). Three months old female Nnt^{mut} mice showed significantly higher glucose level compare to Nnt^{wt} mice ($p<0.05$) (Fig.3). Future steps involve confirming insulin insensitivity and defective glucose handling in other age groups, exploring skeletal muscle metabolism in mice of different ages after a 6-hour hypoxia treatment and exploring associations between longevity and muscle transcriptome changes by RNAseq and other molecular techniques.

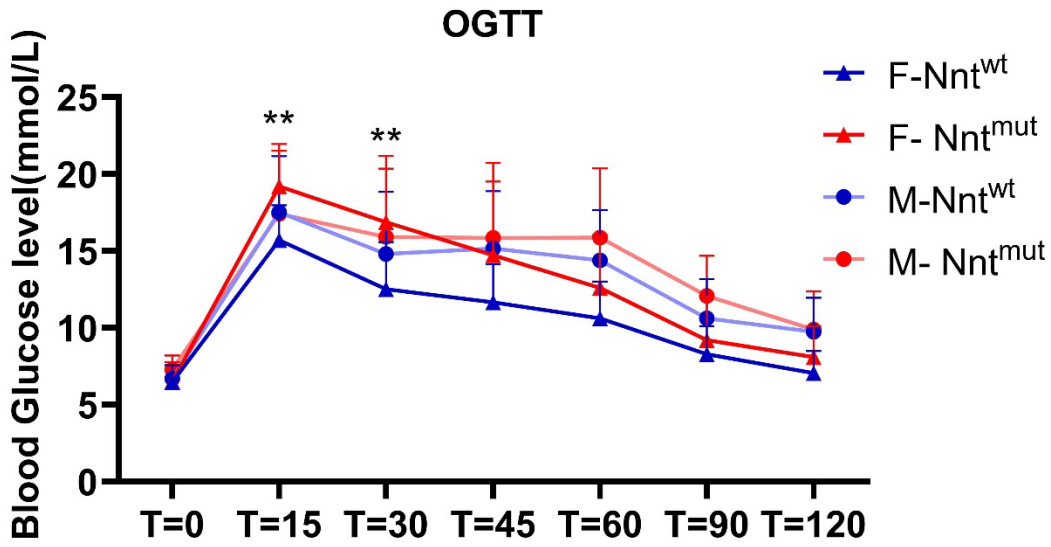
Conclusions:

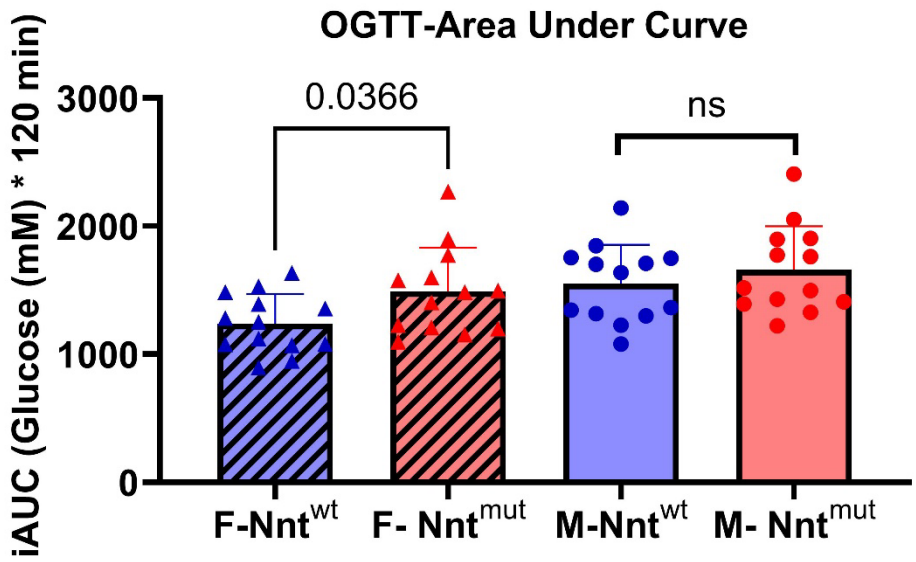
Nnt^{mut} positively affects the early life survival of both female and male mice as a single group, without an effect on maximum life span. Female Nnt^{mut} mice exhibits impaired glucose tolerance at the age of 3 months. Overall, this research aims to study the role of *Nnt* in mice energy metabolism and muscle metabolic health during aging.


Survival of longevity-Male group
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Days
Nnt^mut (n=44)
Nnt^wt (n=45)


Survival of longevity-Female group
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Days
Nnt^mut (n=37)
Nnt^wt (n=37)


Survival of longevity- Combine both sex
Survival (%)
Days
Nnt^mut (n=82)
Nnt^wt (n=81)
Gehan-Breslow-Wilcoxon test P=0.0456


OGTT
Blood Glucose level(mmol/L)
F-Nnt^wt
F- Nnt^mut
M-Nnt^wt
M- Nnt^mut
T=0
T=15
T=30
T=45
T=60
T=90
T=120



Reference Chortis, V., et al. (2018). "Nicotinamide Nucleotide Transhydrogenase as a Novel Treatment Target in Adrenocortical Carcinoma." Endocrinology 159(8): 2836-2849. van der Stelt, I., et al. (2022). "The female mouse is resistant to mild vitamin B deficiency." European Journal of Nutrition 61(1): 329-340.

C11

Enhancing appetite in ageing: the role of fat-free mass and exercise

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Ageing is associated with reduced appetite and energy intake (EI), termed the anorexia of ageing. In older adults, both low body weight and weight loss are strong predictors of poor outcomes, including pathological undernutrition, sarcopenia, reduced functional capacity, and frailty Rolland *et al*., (2011). However, the mechanisms underlying the anorexia of ageing are not fully understood. Cross-sectional studies in young adults demonstrate positive associations between EI and fat-free mass (FFM), seemingly mediated by resting metabolic rate (RMR) (Hopkins *et al*., (2016). Longitudinally establishing a causal effect is crucial for older adults experiencing loss of appetite due to the viscous cycle between appetite, EI, and weight reduction, predominately in FFM. This work aimed to 1) quantify differences in appetite and EI between healthy older and younger adults, 2) assess the acute effects of resistance exercise on appetite and EI, and 3) manipulate body composition through resistance exercise and protein supplementation to assess the effect on appetite, EI and, RMR in older adults.

A meta-analysis quantified differences in concentrations of appetite-related hormones, subjective appetite, and EI between healthy older and younger adults. Data from 713 young (28±7 years) and 710 older adults (73±5 years) were included based on inclusion criteria. All work followed PRISMA guidelines. Analysis revealed significantly higher concentrations of leptin [Fasted: SMD 1.23 (0.15, 2.30), p=0.025; Postprandial: SMD 0.62 (0.23, 1.01), p=0.002], insulin [Fasted: SMD 0.24 (−0.02, 0.50), p=0.073; Postprandial: SMD 0.16 (0.01, 0.32), p=0.043], CCK (Fasted: SMD 0.41 (95% CI 0.24, 0.57); p<0.001); Postprandial: SMD 0.41 (0.20, 0.62); p<0.001)and postprandial PYY [SMD 0.31 (−0.03, 0.65); p=0.075] in older adults compared to young adults. In accord, subjective hunger [Fasted: SMD −1.00 (−1.54, −0.46), p<0.001; Postprandial [SMD −0.31, (−0.64, 0.02), p=0.064] and EI were significantly lower in older adults [SMD −0.98 (−1.74, −0.22), p=0.011]. In subsequent studies, 54 individuals (23 males and 31 females) over 60 years volunteered to participate. Both studies received ethical approval. Resistance exercise led to significant reductions in subjective appetite (49 ± 8 mm h^{-1} vs. 52 ± 9 mm h^{-1}, p=0.007, d=0.27) however appetite profiles converged with the control condition within one hour. Additionally, *ad-libitum* EI was unaffected two-hours post-exercise (RE = 681 ± 246 kcal; CON = 673 ± 235 kcal; p=0.865). Finally, 12-weeks resistance exercise and protein supplementation in older adults resulted in significant increases in FFM (+1.2 kg; p=0.002), postprandial subjective appetite (+8 mm; p=0.027), *ad libitum* EI (+119 kcal; p=0.012) and daily EI (+133 kcal; p=0.010) compared to the control. The increases in *ad libitum* EI correlated with increases in FFM (r=0.527, p=0.001), attributing 54% of the change in EI to FFM changes. In conclusion, FFM increases were associated with increased *ad libitum* EI and postprandial appetite in older adults, although no differences were observed in RMR, leptin, insulin or PYY concentrations.

Older adults experience dysregulated appetite leading to suppressed EI, which can be attenuated with a combined diet and exercise lifestyle approach. More work is required to identify the underlying mechanisms.

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C12

Proteomics and ubiquitylomics profiling reveal the effect of age on mitochondria, sarcomere integrity and proteostasis in skeletal muscle

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Skeletal muscle mass and function progressively decline with ageing. This continual decline results in the development of sarcopenia and is a leading cause of mortality in older individuals. However, the molecular mechanisms driving this decline are not fully understood which prevents the development of pharmaceutical interventions. Proteins are key regulators of muscle function, driving both molecular and physical properties. Ubiquitylation is a post-transcriptional modification which has a widespread impact on the function of the proteome, regulating most biological processes in cells and tissues but well known for its role in protein degradation. We were interested in obtaining a comprehensive insight into age-related changes of proteins and their ubiquitin modifications to identify potential biomarkers of muscle decline. To do this we took the gastrocnemius complex muscle from young (6 month) and old (21-22 month) C57BL/6 male and female mice (n=3 from each sex), fractionated the lysate into soluble and insoluble proteins and analysed changes in the total proteome and ubiquitin-enriched proteome using quantitative mass spectrometry. Bioinformatics analysis of the proteomic dataset highlighted profound changes to the mitochondrial and sarcomere, along with a general decline in proteostasis in older muscle. Notably, we found sarcomeric proteins enriched in the soluble fraction of old muscle, many of which were ubiquitylated. We hypothesise that these proteins have been damaged and released from the sarcomere for ubiquitin-mediated degradation. Overall, this global protein profiling has provided new insight into age-related changes in skeletal muscle that likely contribute towards the loss of muscle mass and function. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

C13

Circadian clock modulation by bear serum in mouse skeletal muscle explants

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Introduction: Damped amplitude of circadian rhythm in locomotor activity and clock gene expression is strongly suggested to initiate or exacerbate skeletal muscle catabolism in, e.g., aging, obesity and diabetes [1]. However, functional drugs or nutrients to counteract/reverse circadian rhythm disruption have not been identified yet. A bio-inspired approach could help fill this gap, by taking advantage of the peculiar ability of hibernating brown bears (*Ursus arctos*) not to develop muscle atrophy and metabolic diseases although they stay torpid (inactive and fasting with a reduced metabolic rate) for 5-7 months. We have shown previously that the hibernating bear serum contains components able to inhibit proteolysis in human muscle cells *in vitro* [2]. We therefore assessed the potential of bear serum to modulate muscular circadian rhythms and metabolic efficiency.

Method: To monitor the molecular clock oscillation, soleus and extensor digitorum longus (EDL) muscles, and the hypothalamic suprachiasmatic nucleus (SCN) from *Period2-luciferase* knockin mice [3] were dissected and cultured with 5% bear serum (BS, pool from 15 females and 4 males, see [4] for details about bear immobilization) collected either during summer (SBS, n=10) or winter (WBS, n=11). 5% fetal bovine serum (FBS, n=15) was used as a control. In parallel, mouse skeletal muscle C2C12 cells were cultured with SBS (n=6), WBS (n=6), or horse serum (HS, n=6) to evaluate mitochondrial oxygen consumption rate. All data was analyzed using a one-way ANOVA followed by Tukey-Kramer multiple comparison tests.

Results: We observed that both SBS and WBS increased the amplitude of *Per2* independently of muscle type (202% increase $p=0.043$ in SBS-soleus, 231% increase $p=0.003$ in WBS-soleus, 680% increase $p<0.001$ in SBS-EDL, and 690% increase $p<0.001$ in WBS-EDL). The circadian period was shortened in soleus cultured with SBS ($p=0.031$), but not in EDL. The robustness of the rhythm was higher in soleus cultured with WBS ($p=0.019$) and in EDL cultured with SBS ($p<0.001$) or WBS ($p=0.011$) than in FBS-treated muscles. Neither SBS nor WBS affected any of the circadian parameters tested in the central clock, i.e. SCN. On the other hand, mitochondrial oxygen consumption in mouse skeletal muscle cells was not affected by either SBS or WBS ($p=0.688$ and $p=0.664$, respectively).

Conclusions: Previous reports showed that the amplitude of clock gene oscillation was higher in skeletal muscle of healthy people than in diabetic patients, suggesting that an ample oscillation is linked to the maintenance of proper metabolic function [5]. In our study, both SBS and WBS increased the amplitude of *Per2* oscillation in skeletal muscles, but not in the SCN. However, bear serum had no effect on energy metabolism in myocytes. The effect of bear serum on skeletal muscle clock therefore appears to be independent of energy metabolism, at

least in *in vitro* conditions. Further studies are needed to determine (i) whether bear serum coordinates the circadian clock and energy metabolism under specific culture conditions, such as low temperature, and (ii) to what extent bear serum modifies the circadian transcriptome/proteome of exposed cells.

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C14

Skeletal muscle dysregulation in end-stage liver disease: a role for pro-inflammatory cytokines?

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Introduction

Patients with end-stage liver disease (ESLD) often present with sarcopenia, defined as loss of skeletal muscle mass and quality, which is associated with reduced quality of life and increased mortality. However, the molecular mechanisms driving sarcopenia in ESLD are not fully understood and there are currently no therapeutic interventions.

Aims and Objectives

This study aimed to identify potential circulating factors that may contribute to sarcopenia progression in ESLD, through driving transcriptomic changes in the skeletal muscle. To achieve this, we had 3 main objectives:

1.Profile the skeletal muscle transcriptome of patients with ESLD and age and sex matched healthy controls (HC) to identify potential mechanism of muscle dysfunctions in ESLD.

2 Determine whether the altered skeletal muscle transcriptome in ESLD may be driven by circulating factors, by stimulating primary human myotubes with ESLD patient plasma and performing RNAseq.

3. Profile patient serum to identify differential cytokines in ESLD and cross-reference with skeletal muscle transcriptomic data to identify potential candidates driving altered gene expression.

Methods

Quadriceps muscle tissue, plasma and serum was obtained from ESLD patients (n=24) and age/sex-matched HC (n=18). ESLD patients were recruited to a larger prospective observational study, The Evaluation of Sarcopenia in Inflammatory Disease (clinical trial ID: NCT04734496, ethical approval 18/WM/0167). Local ethical approval was granted for recruitment of HC (ERN_19-0831). This study was conducted in accordance with the Declaration of Helsinki. Total RNA was isolated from snap frozen muscle tissue, obtained via muscle biopsy of the vastus lateralis, and subjected to RNAseq (Illumina). Serum levels of 62

cytokines were profiled by Luminex and ELISA. *In vitro,* primary human myotubes were cultured with media containing 10% ESLD, or HC plasma (24h, n=6) followed by RNAseq (BGI genomics). Differentially expressed genes ($p<0.05$, fold-change >1.5) were determined using Qlucore and DESeq2, with subsequent pathway analysis performed utilising Ingenuity (IPA, Qiagen). Statistically significant cytokines were determined by either Student's t tests or Mann-Whitney U tests as appropriate.

Results
387 and 225 genes were significantly up- and downregulated respectively in ESLD muscle compared to HC, with cellular senescence identified as a top dysregulated cellular function by IPA. Upstream regulators predicted to drive these transcriptomic changes in ESLD included hepatocyte growth factor (HGF) and interleukin-1 signalling. Serum levels of 16 cytokines were significantly ($p<0.05$) greater and 4 significantly lower ($p<0.05$) in ESLD, including HGF and interleukin-1 receptor antagonist respectively. Treatment of myotubes with ESLD plasma partly replicated the transcriptomic phenotype of ESLD muscle, with significant activation of cellular senescence pathways observed and interleukin-1 again a purported upstream regulator.

Conclusions
In conclusion, skeletal muscle of ESLD patients exhibits an altered transcriptome associated with increased cellular senescence, which may be partly driven by circulating inflammatory mediators, including HGF and IL-1. Targeting such mediators may provide a novel therapeutic intervention to limit sarcopenia progression in ESLD patients.