Accepted Abstracts

Defining the age-dependent and tissue-specific circadian transcriptome in male mice.

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Aging is characterised by a progressive loss of homeostatic control, leading to functional declines and decreased resilience. Transcriptomic studies have previously examined age-related changes in gene expression across tissues. More recently, RNA-sequencing has been used in rodents to capture age-dependent transcriptomics across multiple organs and ages. However, these studies have not considered the time-of-day impact of aging on the circadian clock, overlooking a critical dimension of aging physiology. Circadian clocks direct a daily transcriptional program that supports homeostasis and resilience, consisting of a network of central and peripheral oscillators giving rise to various rhythmic outputs in a tissue-specific manner. To this aim, our objective was to define age-dependent changes at the systems level, profiling the circadian transcriptome in the hypothalamus, lung, heart, kidney and skeletal muscle in three age groups at multiple time points across the lifespan.

Male C57B6/J-NIA mice were maintained under 12 h:12 h light/dark conditions until 6 (Young), 18 (Aged), or 27 months (Old) of age. Prior to tissue harvest, mice were released into constant darkness (Circadian Time {CT} 0) to study circadian gene expression under free-running conditions. Tissue collections began at CT18 and continued every 4 h for 48 h, with a total of 12 time points. High-quality RNA-sequencing data from all tissues was obtained and sequenced to a depth of at least 40 million reads aligned to the mouse genome. To identify the circadian transcriptome, we deployed the cosinor model implemented in the diffCircadian software (Ding et al, 2021). Specifically, we defined circadian clock output genes as those with 24-h cosine oscillations in transcript abundance based upon a raw p-value <0.01.

We find age-dependent and tissue-specific clock output changes. Overall, the majority of the rhythmically expressed genes (REGs) in Young were tissue-specific, consistent with previous multiorgan genomic studies using young mice. Across all tissues, we found an age- associated

decline in the number of REGs, indicative of weakened circadian control. The largest REGs change was seen in the kidney with ~75 % decline from Young to Old. The heart showed the least change with ~34 % decline. The REGs within each tissue were also age-dependent, with less than 10 % of REGs conserved across ages. In skeletal muscle, the number of REGs decreased from 941 in Young to 699 in Aged and 474 in Old. Across these groups there was a preservation of functional groups including insulin receptor and HIF1 signalling, but pathways such as AMPK, PI3K/AKT, and unfolded protein response lost rhythmicity with age.

Our analyses demonstrates that there is a limited overlap of the circadian transcriptome across ages in all tissues in an age-dependent manner, and that few genes (<3 %) are rhythmically expressed across all ages. Concomitantly, a common feature of aged tissue is an increased variability of gene expression across the day. These data extend the landscape for understanding aging, and highlight the impact of aging on circadian clock function and temporal changes in gene expression, suggesting that the age-related changes in clock output are linked to a loss of resilience.

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Sarcopenia, the loss of muscle mass and strength during ageing, and its definitional components are associated with differential DNA methylation patterns in human primary satellite cells isolated from older individuals

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Sarcopenia, the loss of muscle mass and function during ageing, is the major cause of functional decline and loss of independence in older adults, increasing the risk of falls, frailty, and physical disability (Cruz-Jentoft, 2010). The loss of muscle mass during ageing has been attributed in part to a reduced capacity of ageing muscle to repair itself. Muscle repair and regeneration depends on myogenic stem cells, referred to as satellite cells (SC). However, there is a decline in SC number and function as we age, impairing muscle regeneration and repair (Chakkalakal, 2012). As epigenetic processes have been implicated in the development of many ageing associated human disease, the aim of this study was to examine changes in DNA methylation in relation to sarcopenia in human primary muscle SC.

Using the Infinium Human MethylationEPIC BeadChip, we measured DNA methylation in SC extracted from vastus lateralis muscle biopsies from 119 Hertfordshire Sarcopenia Study extension (HSSe) participants (22 with sarcopenia). Associations with sarcopenia and its components (appendicular lean mass index (ALMi), grip strength and gait speed) were examined to identify differentially methylated CpG (dmCpG) sites. Pathway enrichment of the sarcopenia associated dmCpGs was used to identify potential pathways altered by DNA methylation.

Sarcopenia and its definitional components were associated with differential patterns of DNA methylation. 21 dmCpGs were identified for sarcopenia, 13 for ALMi, 48 for grip strength and 3 for gait speed; the top 100 dmCpG associated genes were enriched for pathways involved with cell TOR signalling, muscle structure development and Wnt signalling. The study demonstrates that sarcopenia is associated with specific DNA methylation alterations in muscle SC, furthering our understanding of the underlying molecular mechanisms.

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Statins Increase Senescence, Reduce Proliferation, and Impair the Differentiation Pathway of Human Myoblasts

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Statins are a class of highly effective low-density lipoprotein (LDL) cholesterol-lowering drugs and are most widely prescribed to reduce the risk of cardiovascular disease and metabolic syndromes. Atorvastatin and simvastatin account for 85% of all statins prescribed (Moon, 2006). Both statins are lipophilic hence, have superior penetration into the muscle which can lead to statin induced myopathy characterised by muscle weakness and pain. There is an increasing level of attention on these muscular side effects as approximately 29% of patients experience them and they are the primary reason for treatment withdrawal (Herghelegiu, et al., 2018). Therefore, we assessed the phenotypic effects of atorvastatin and simvastatin after a 4-day treatment at concentrations of 1μM, 5μM, and 10μM on primary human myoblasts cultured from the Hertfordshire Sarcopenia Study Extension (HSSe) cohort. These myoblasts were extracted from vastus lateralis muscle biopsies from total of 12 female participants. Senescence was measured using the β-galactosidase assay and the percentage of proliferating cells was assessed by the Edu assay. Immunocytochemistry was also undertaken staining for MyoD, MyoG, and MYHC to inform on the effect of statins across the differentiation pathway. Both statins increased the percentage of senescence cells ($P<0.01$) and decreased proliferation in a dose dependent manner ($P<0.001$). MyoD levels decreased at only the highest concentration, 10μM, which was observed with both statins. Atorvastatin decreased MyoG and MYHC across all concentrations ($P<0.001$) unlike simvastatin where significant decreases were only observed at 5μM and 10μM. These results suggest that the increased senescence, reduced proliferation, and reduction in muscle differentiation markers induced by statins alter muscle at the cellular level and may contribute to the common muscle side effects experienced when patients undergo statin treatment.

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Mineral status and its association with musculoskeletal function: The Newcastle 85+ Study

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Background

Data is lacking on nutritional mineral status and its association with musculoskeletal (MSK) function in very old populations (≥85 years). Previous studies have however explored this in younger populations1,2. However, very old adults are important to consider due to their heterogeneity in health and function and greater risk of nutritional deficiencies. Nutritional minerals are involved in a multitude of biological functions such as protecting against oxidative damage which is important in mitigating MSK decline.

Objectives

The aim of this study was to explore the association between serum selenium, iron, zinc and copper and MSK function (hand-grip strength, HGS and Timed-Up-and-Go, TUG) in 85-year-olds living in the Northeast of England.

Methods

Mineral status (selenium, iron, zinc and copper) was assessed using standard laboratory techniques in 757 participants. Hand grip strength was measured using a dynamometer and TUG was measured as the time to rise from a chair, walk 3 m and return. The relationships between the biomarkers of mineral status and MSK function were analysed at baseline using linear regression models and over 5 years follow-up using linear mixed models adjusting for covariates. Covariates included sex, fat-free mass, physical activity, cognition, total calorie, protein and alcohol intake, medications, smoking status, inflammation and either the use of walking aids or presence of hand arthritis.

Results

At baseline, in adjusted models, there was no association with any biomarker of mineral status and MSK function, both HGS and TUG. Similarly, over 5 years, there was no association between any biomarker of mineral status and rate of change in TUG. However, over 5 years serum selenium (β 9.81E-3±3.15E-3 P = 0.02) and iron (β 3.65E-4±1.26E-4 P = 0.004) were positively associated with the rate of change in HGS, whilst serum copper (β-2.09E-3±5.42E-4 P < 0.001) was negatively associated with the rate of change in HGS.

Conclusion

In this UK cohort of very old adults, there were no associations between mineral status and MSK function at baseline and with the rate of change of TUG over 5 years. However, over 5 years, serum selenium and iron were positively associated with the change in HGS whilst serum copper was negatively associated with this change. This could be related to a higher copper/zinc ratio which has been used to indicate inflammation1 and thus induces catabolic effects on muscle leading to reduced strength. Although this explanation warrants further research in other longitudinal studies.

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Human primary fibroblastic cells derived from skeletal muscle have similar marker expression but exhibit adipogenic potential unlike fibroblastic cells of other tissue origins

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Skeletal muscle resident fibroblastic cells are considered to be a potential origin of the intermuscular adiposity that develops in sedentary ageing muscle (Contreras et al 2021). Isolated human skeletal muscle TE7+/collagen VI+/PDGFRα+ fibroblastic cells have been shown to have the potential to differentiate into adipocytes when exposed to media containing fatty acids and/or an adipocyte inducing medium (AIM; Agley et al 2013). However, it is not clear if similar behaviour is apparent in fibroblastic cells from other tissues. The aim of the present study was to compare marker expression and response to an adipogenic stimulus of human primary fibroblastic cells from different tissues (muscle, skin and fat) compared to pre-adipocytes.
Human skeletal muscle fibroblastic cells were isolated from muscle biopsies from the vastus lateralis of young healthy adult volunteers (n=6, aged 22.3±3.2 yrs). Human primary skin and

lung fibroblasts, as well as human primary visceral pre-adipocytes were purchased and cultured as per manufacturer's instructions (n=3 for each, PromoCell). For adipogenic differentiation, all cell types were grown for seven days in skeletal muscle growth medium before applying AIM+oleic treatment. This consisted of a 3-day incubation in a pre-adipocyte differentiation medium followed by a 15-day incubation in an adipocyte nutrition medium (PromoCell) supplemented with 600 µM oleic acid and 15 mg/ml BSA (AIM+oleic). Protein expression and cell morphological changes were analysed using immunocytochemistry. Expression of secreted adipokines, adipisin and adiponectin were analysed from conditioned media collected over 24 hours before and after treatment using a Luminex platform.

Expression of fibroblast marker proteins TE-7, PDGFRα, collagen VI, fibronectin, vimentin and TCF4 were indistinguishable between the four cell types under proliferation conditions. After AIM+oleic treatment three of six skeletal muscle fibroblastic and all pre-adipocyte cell populations showed protein expression of adipocyte marker perilipin (34.3±4.8% and 22.7±3.1% perilipin+ cells respectively, $p<0.01$), exhibited protein expression of other adipocyte protein markers - acetyl-CoA carboxylase and fatty acid synthase, as well as having a classical adipocyte signate ring morphology. None of the skin or lung-derived fibroblasts showed perilipin expression or other adipocyte marker expression. Indeed, although all cell types increased secretion of adipisin (skin:17276±7225 pg/ml, lung:1211±6837 pg/ml, muscle:18504±359 pg/ml, pre-adipocyte: 12183±2592 pg/ml, n=3, $p<0.05$) and showed visible signs of increase in lipid accumulation. Only the groups of perilipin+ skeletal muscle fibroblastic cells and pre-adipocyte populations reached statistical significance for total cellular lipid accumulation as measured by integrated density of oil red O staining (3.3x107±2.0x107 AU and 4.8x107±1.3x107 AU respectively, n=3, $p<0.05$). These cells also showed secretion of adiponectin (1722±318 pg/ml and 748±715 pg/ml for muscle fibroblasts and pre-adipocytes respectively, $p<0.05$). Non perilipin+ cell populations were characterised by accumulating multiple small lipid droplets whereas lipid filled the cytoplasm of perilipin+ cell populations.
These data show that despite being indistinguishable on the basis of their fibroblast marker expression, skin and lung cells differ in their responses to an adipogenic stimuli compared with skeletal muscle origin fibroblastic cells. However, only muscle origin fibroblastic cells derived from some individuals exhibited the full potential for differentiation into adipocytes.

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mTORC1 regulation in senescent muscle cells

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The mechanistic target of rapamycin complex 1 (mTORC1) is dysregulated in aged tissues and senescent cells, and its inhibition can extend lifespan and improve many parameters of health across species. However, there is a very limited understanding of how mTORC1 becomes initially dysregulated. We used etoposide-induced senescent C2C12 myoblasts to investigate the mechanisms underlying hyperactive mTORC1. Existing literature in fibroblasts suggests that hyperactive mTORC1 in senescence is primarily regulated by increased protein breakdown/autophagy. In contrast, we show that inhibition of autophagy has no significant effect on mTORC1. However, inhibition of growth factor signalling intermediate, Akt, restores mTORC1 back to baseline, suggesting that growth factor signalling and Akt are the key mediators of hyperactive mTORC1 in senescent myoblasts. Given the role of stress (ROS, inflammation) in the regulation of Akt activity we further investigated whether alleviating some of this stress could ameliorate the upregulated Akt and subsequently mTORC1 activity. The results showed that inhibition of inflammatory or endoplasmic reticulum stress-relevant signalling pathways did not alter mTORC1, whereas the antioxidant Tiron alleviated Akt but not mTORC1 signalling. To further investigate the role of ROS in mTORC1 dysregulation, senescent myoblasts were treated with antioxidant N-Acetylcysteine and showed that high doses reduced ROS production close to baseline and alleviated both Akt and mTORC1 activity. We provide novel data, indicating that increased ROS in senescent myoblasts can upregulate mTORC1, likely through growth factor signalling.

Passive thigh heating increases muscle temperature and produces reliable torque measurement that increase during moderate and fast velocity knee extensor contractions.

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Background

Evidence supporting the efficacy of local hyperthermia for improving neuromuscular function has primarily focused on isometric contractions with methodology specific outcomes reported. To date a comprehensive assessment of the efficacy and reliability of local hyperthermia on isokinetic force production has yet to be completed.

Objective

This study investigated the effect of local hyperthermia, induced by passive thigh heating, on isokinetic muscle function and reported intra-day reliability. It was hypothesised that heating would provide reliable increases in isokinetic force production.

Methods

Fourteen young, healthy participants (7 female) completed two visits whereby one thigh was heated with a perfusing garment circulating water at 50°C (HEAT) with the contralateral leg as the control (CONT). Following quantification of intramuscular temperature (Tmu) of the vastus lateralis of HEAT and CONT leg, participants performed four repetitions of maximal knee extensions on each leg at 60°/s, 180°/s, and 300°/s on an isokinetic dynamometer (Biodex Medical Systems, USA). Measurements were made at baseline, +30, +60, +90 minutes. Three-way ANOVA determined differences in torque and Tmu across timepoints, between HEAT and CONT, and between visits. Cronbach's alpha was used to identify the intra-day reliability, with coefficient of variance (CV), technical error of measurement (TEM), and minimum detectable change (MDC) also calculated. Statistical significance was set at $p < 0.05$. Data are reported Mean ± SD.

Results

Torque at all speeds was found to be highly reliable, as determined by a Cronbach's alpha of >.99, CV ≤36%, TEM was = 36 Nm (60°/s), 14 Nm (180°/s), and 13 Nm (300°/s). MDC was 11 Nm (60°/s), 8 Nm (180°/s), and 7 Nm (300°/s).

Tmu increased ($p<0.05$) from baseline (32.2 ± 1.7°C) at 30 min in HEAT (36.6 ± 1.1°C) and CONT (33.9 ± 1.5°C), with differences between HEAT and CONT here and thereafter ($p<0.05$). Tmu peaked in HEAT after 90 min (37.3 ± 0.4°C, $p<0.05$) with no further increase in CONT (33.2 ± 2.1°C).

At 60°/s there was no statistical difference in torque ($p>0.05$) at baseline (HEAT 193 ± 18 Nm; CONT 179 ± 15 Nm) or during heating.

Torque at 180°/s was not different at baseline (HEAT 149 ± 14 Nm; CONT 139 ± 12 Nm) however HEAT was greater than CONT at 30 min (+8 ± 17 Nm) at 60 min (+13 ± 14 Nm) and 90 min (+12 ± 15 Nm), all $p < 0.05$.

Torque at 300°/s was not different at baseline (HEAT 114± 39 Nm; CONT 112 ± 36 Nm) but HEAT was greater than CONT at 30 min (+12 ± 12 Nm), 60 min (+10 ± 10 Nm), and 90 min (+8 ± 13 Nm), all $p < 0.05$. No between visit differences were observed for any measurement ($p > 0.05$).

Conclusion

Increasing muscle temperature by ~5°C increased torque 7% at moderate and 8% at fast contractile velocities. Mechanistically it has been proposed that local hyperthermia increases intramuscular fluid aiding force transmission, and calcium handling resulting in faster half relaxation time. These benefits may be especially pronounced in aged muscle with impaired calcium handling.

High-intensity interval training attenuates the loss of proteostasis in obese insulin-resistant muscle.

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Abstract

High-intensity interval training (HIIT) is an attractive training strategy for improving performance and protecting against chronic disease. Changes to muscle function are underpinned by dynamic proteome remodeling but little is known regarding how exercise alters the turnover of individual proteins in humans. We compared individual protein abundance and turnover rates in obese insulin-resistant (OIR; n = 3) and healthy, trained (TR; n = 4) male participants (38 ± 8 years), and conducted longitudinal analysis of proteome changes in OIR after 10-weeks HIIT. Newly synthesised proteins were labelled by oral consumption of deuterium oxide (D2O; 50 ml of 99.8 atom %) taken 4 times per day, for 14 days. Biopsies of vastus lateralis were performed before D2O administration (day 0) and after 4, 9, and 14 days of labeling. The OIR group trained 3 times/ week for 10 weeks (4 x 60 s cycling at 100% maximum power output interspersed by 60 s recovery periods), Training volume was increased by 1 interval bi-week and the experimental protocol was repeated over the final 2 weeks of HIIT. Physiological measurements were taken > 72 h before and after HITT and D2O incorporation and protein abundance data were quantified by liquid chromatography-tandem mass spectrometry. Baseline data were compared by between-subject ANOVA and within-subject ANOVA was used to compare pre- vs post-HIIT responses. By design, the average BMI (kg.m-2) of OIR (34.0 ± 5.8) was greater (P = 0.02) than TR (24.2 ± 2.4 kg.m-2) participant, whereas peak oxygen consumption (VO2peak; ml.kg-1.min-1) of OIR (26.1 ± 4.4) was significantly (P < 0.01) less than TR (45.5 ± 7.9) and OIR had significantly (P < 0.01) lower insulin sensitivity (Matsuda Index; OIR = 1.7 ± 0.6 vs TR = 5.7 ± 1.4). HIIT increased VO2peak by 9 % (P = 0.25) and peak power output by 14 % (P = 0.06) in OIR. Abundance and turnover rate data were quantified for 880 and 301 proteins, respectively, in all OIR and TR participants. In total 352 proteins exhibited significant (p < 0.05, false discovery rate < 5%) differences in abundance at baseline. OIR muscle was enriched with markers of cellular stress and protein unfolding, including small heat shock proteins (HSPB; 1, 2, 3, and 6) and members of the 90 kDa HSP family. Additionally, HSP72, and cytoprotective enzymes such as alcohol dehydrogenases (AK1A1, and ALDH2) exhibited significantly slower turnover rates in OIR. HIIT significantly (p < 0.05) altered the abundance of 53 proteins and increased the turnover rate of 20 proteins in OIR muscle. Four proteasome subunits that were more abundant in OIR muscle compared to TR at baseline,

significantly decreased ($p < 0.05$) in abundance post-exercise. Significant ($p < 0.05$) increases were also observed in the turnover rate of HSP7C, HS90B, as well as the antioxidant and detoxifying enzymes PRDX2 and ALDH1. These data indicate greater proteotoxic stress and impaired proteome dynamics in OIR muscle and provide insight to exercise-induced changes in protein-specific abundance and turnover rates that act to restore muscle proteostasis.

Regulation of Peroxiredoxin Oxidation for the Maintenance of Muscle Mass and Function in Ageing

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It is crucial to determine how skeletal muscle responds and adapts to exercise in order to maintain mass and function during ageing. Despite numerous studies addressing the underlying mechanisms, such as with up/down regulation of specific proteins, a process altered during ageing, little is understood about the initiators of signalling pathways that lead to adaptations in muscle. One described mechanism is the potential interaction of hydrogen peroxide (H_2O_2) with 2-Cys Peroxiredoxins (Prx) whereby it is proposed that increased cellular H_2O_2 leads to oxidation of Prx with the formation of disulphide bridges, causing dimerisation. The dimerised Prx passes the oxidising equivalent on to less reactive proteins, ultimately leading to the activation/de-activation of transcription factors.
We investigated Prx oxidation pre and post-contractile activity in muscle fibres isolated from the flexor digitalis brevis of adult (6–8 months) or old (26 months) C57BL/6J mice, with contractions induced by electrical stimulation. Fibres from adult mice displayed significantly increased dimerisation of Prx2 after two and 15 minutes of contractions (28% increase, $p<0.05$; 34% increase, $p<0.05$ respectively), indicating increased Prx2 oxidation. There was also a significant increase in Prx3 dimerisation post 15min stimulation (96% increase, $p<0.05$). The extent of dimerisation of all Prx in fibres from old mice was diminished following contractile activity.
We have successfully knocked down (up to 90%) gene expression of Prx 1, 2 and 3 in myotubes from a human skeletal muscle cell line. Following this, the cells were exposed to electrical or chemical (H_2O_2) stimulation. Cells were then harvested and RNASeq performed. These data allows us to describe the mRNA responses and adaptations to contractile activity and aberrant H_2O_2 levels in skeletal muscle that are mediated by specific Prx and determine their role in altered adaptive responses in muscle during ageing, leading to development of interventions to maintain muscle as we age.

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Post-Operative Exercise Training in Patients with Metastatic Colorectal Cancer – A Randomized Controlled Trial

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Background

Approximately 50-75% of the patients with colorectal cancer and liver metastases (mCRC) who undergo surgical treatment with curative intent, experience a relapse in less than two years (De Jong MC et al., 2009). These patients undergo multiple rounds of surgery and chemotherapy, which leads to severe physical deconditioning. Physical activity decreases the risk for developing CRC and is associated with increased survival rates in mCRC patients (Friedenreich CM et al., 2019).

Objectives

To test the effects of supervised exercise training, applied in a dose-response manner on:
Primary objective:
Peak oxygen consumption (VO2peak)
Secondary objectives:

- Tumor recurrence rate
- Muscle strength and function
- Immune function and inflammatory response

Methods

In a randomized controlled trial, sixty-six mCRC patients are randomized 1:1:1 to either 150 or 300 min/week of exercise, or standard of care. The duration of the supervised, combined aerobic and resistance exercise training is 6 months and it starts post-surgery for liver metastases resection. Assessments for VO2peak, muscle strength and function, insulin sensitivity, body composition, immune and inflammatory status, tumor recurrence rate take place before the surgery and at 3, 6, 9, 12 months after initiation of exercise training (and at 24 and 36 months for tumor recurrence).

Results

Results are expected beginning of 2025.

Conclusion (and perspectives)

Exercise training is expected to improve VO2peak and overall physical condition and delay tumor recurrence. This project will serve as a steppingstone to further optimize the guidelines for exercise prescription in mCRC patients.

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Age-related differences in skeletal muscle fibre-specific mTOR-mediated signalling via immunofluorescent microscopy.

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Background

Ageing results in a gradual loss of skeletal muscle mass and type II fibre atrophy, through dysregulated muscle protein synthesis (MPS). Traditional immunoblotting techniques have revealed dysregulated mTOR-mediated signalling as a mechanistic driver of impaired MPS in older adults. However, immunoblotting cannot determine fibre-type specific protein abundance, localisation, or translocation of these molecular regulators within cells. Immunofluorescence microscopy (IF) has the capacity to address the shortcomings associated with immunoblotting and uncover mechanisms of age-related MPS impairment and muscle decline. This study aimed to use IF to measure age-related and fibre-specific differences in the abundance and localisation of mTOR-mediated proteins.

Methods

Resting muscle biopsies from eight young males (YM; 24 ± 4 years, BMI; 24 ± 4 kg·m2) and seven older males (OM; 67 ± 5 years, BMI; 25 ± 2 kg·m2) were embedded in OCT compound, frozen in liquid nitrogen-cooled isopentane for IF subsequent analysis. Embedded muscle samples were cut using a microtome blade, and cryosections (7mm) were collected on room-temperature uncoated glass slides. Sections were fixed and incubated in MHC-I, Rheb, TSC2 or WGA, mTOR and Sestrin-2 primary antibodies and incubated in contrasting Alexa Fluor

secondary antibodies. Images were captured at 10x for fibre type distribution and 20x for analysis of fibre-type immunofluorescent abundance. Full ethical approval was granted (18/EM/0004) and procedures were conducted in accordance with the Declaration of Helsinki.

Results

OM displayed a significantly (P=0.0151) lower proportion of type I! fibres than YM (YM, 57.6 ± 0.6% vs OM, 47.6 ± 2.8%). Mean CSA was not different between groups for type I (YM, 4745.8 ± 98.1 mm2 vs OM, 6133.0 ± 408.6 mm2) or type II fibres (YM, 6556.7 ± 196.0 mm2 vs OM, 5752.3 ± 246.8 mm2). Notably, a 2-fold higher abundance of TSC2 in OM compared with YM (YM, 12.71 ± 0.28 A.U. vs OM, 25.45 ± 0.64 A.U.; P<0.001). OM displayed a ~31% (YM, 68.86 ± 3.36 A.U. vs OM, 47.22 ± 2.77 A.U., P=0.020) and a ~63% (YM, 39.21 ± 2.15 A.U. vs OM, 14.49 ± 0.57 A.U., P<0.001) lower abundance in Sestrin-2 and mTOR, respectively, compared with YM. Rheb abundance was ~43% higher in OM compared to YM (YM, 18.66 ± 0.92 A.U. vs OM, 26.71 ± 0.58 A.U., P<0.001). There were no differences in protein target abundance between fibre types within either group.

Discussion

Using IF, it is possible to characterise fibre-specific molecular regulators of skeletal muscle. Whilst the selection of regulatory proteins we investigated were similarly abundant in type I and II fibres, differences in mean fluorescence relative to fibre area of mTOR, TSC2, Sestrin-2 and Rheb may be implicated in age-related MPS impairment and muscle decline. IF could be used to identify dysregulated signalling events that underpin age-related anabolic resistance (e.g., impaired MPS response to amino acids and/or contraction).

Conclusion

IF characterisation of the fibre type-specific abundance of key regulators of mTOR revealed age-related differences that may be linked to dysregulated proteostasis.

Role of altered cortisol production in the development of sarcopenia

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Skeletal muscle mass and function is decreased as we age, a condition known as sarcopenia. Ageing is associated with increases in minimum and mean cortisol plasma levels and shortening of the evening cortisol quiescent period, suggesting impaired circadian function and

higher basal levels with increasing age It has also been shown that the deleterious effects of bed rest on human skeletal muscle are exacerbated by hypercortisolemia (Stamou et al, 2023).

The aim of this study was to examine the direct effects of increasing levels of cortisol on muscle cells. C2C12 myoblasts were fused to myotubes by serum deprivation for up to 7 days. Cellular viability was assessed using light-microscopy to identify any gross changes in cell morphology at 3, 24 and 48 hours following treatment with a range of cortisol concentrations in the physiological range (10 – 0.1uM). Indices of muscle atrophy and evidence of altered levels of inflammatory cytokines was determined by mRNA analyses and release of cytokines/chemokines by myotubes was assessed using bead based multiplex technology (Luminex immunoassay).

Cortisol treatment had no significant effect on C2C12 myotube viability at any concentration or time point, or on gene expression of IL-6, RANTES (CCL5) or MCP-1 (CCL2) compared with control untreated myotubes. No increase in the release of cytokines and chemokines from C2C12 myotubes was seen following treatment with cortisol and in some instances a significant decrease in cytokine/chemokine production was seen, particularly following treatment with higher concentrations of cortisol when compared with control values (eg, 10uM cortisol, 24 hours post-treatment: IL-6: 1.6 +/- 1.6% CXCL5: 17.4 +/- 1.8%; KC (CXCL1): 11.6 +/-10.3%. These data are in contrast to previous work from our group which revealed an increase in the release of IL-6, CXCL-1 (KC), CCL2 (MCP-1) and CCL5 (RANTES) from C2C12 myotubes following treatment of myotubes 25ng/ml TNF-α (Lightfoot et al, 2015).

In summary, increased levels of cortisol do not appear to have any direct deleterious effects on myotube viability or production of pro-inflammatory cytokines. However, this does not exclude any indirect effects of cortisol on muscle including the effects on other organs indirectly affecting muscle function. It may also be that cortisol has a deleterious effect when muscle is under stressful conditions and this possibility will also be examined.

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Targeting Neuromuscular Ageing using Novel Synthetic Retinoids and Chrono-Pharmacological Approaches

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Deterioration in neuromuscular function is one of the main drivers of age-related loss of muscle mass and strength in animals and humans, leading to frailty and a lower quality of life. In addition, muscle atrophy is accelerated in many chronic diseases of ageing and is a key determinant of mortality, thus posing a global health challenge and an unmet clinical need for developing new therapies.

Many promising small molecules have been tested as therapies for age-related muscle atrophy in preclinical models, however, they often fail in clinical trials due to a lack of translational knowledge on drug administration regimens, such as dose, duration and timing of treatment. Retinoic acid derivatives have been shown to have protective effects on muscle and neuronal ageing in preclinical models, however, they have several properties that make them difficult to administer to patients safely and effectively. We, thus, focused on the development of an in vitro drug screening platform using chrono-pharmacological approaches, in order to elucidate the optimal time-of-treatment for several novel retinoid-based compounds.

In order to study the impact of retinoids in a time-of-day manner, we conducted genomic and cellular based assays, namely real-time qPCR and high-throughput real-time bioluminescence imaging, to ascertain retinoid effects on cellular circadian rhythms, a conserved ~24 time-keeping mechanism, governed by positive/negative transcriptional/translational feedback loops. Preliminary data in skeletal muscle cells showed that the treatments with retinoids had profound effects on the clock gene expression, targeting primarily the negative feedback loop of the molecular clock. This result was seen at both gene promoter and mRNA level. Moreover, our data has revealed that chrono-based timing of retinoid treatments had differential effects on clock reporter oscillations. Given that misalignment of circadian rhythms is linked to increased risk of drug toxicity, our findings shed light on the potential of chronotherapy in providing improved understanding of toxicology and efficacy of these novel compounds.
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Turnover rates of human muscle proteins in vivo reported in fractional, mole and absolute units.

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Abstract

Ageing is associated with losses in skeletal muscle mass and function, which may in part may be due to age-related changes in protein synthesis. Past studies have commonly investigated the fractional synthesis rate of mixed proteins from either whole muscle or subfractions such as the myofibrillar fraction, which contain hundreds or thousands of different proteins. More contemporary proteomic studies are now capable of reporting the fractional synthesis rate of individual proteins in human muscle and offer the opportunity to gain new insight into the effects of ageing. However, the use of fractional synthesis rate calculations may not be appropriated when applied at the individual protein level, especially in the context of ageing where age-related differences in the abundance profile of muscle proteins are known to occur. Co-analysis of both the abundance and turnover rate of each protein may be required for an accurate understanding of the effects of ageing. This study investigates the consequences of different units of measurement that incorporate protein molecular weight and abundance data alongside measurements of fractional turnover rate (%/d). Three physically active males (21 ± 1 years) were recruited and underwent a 12-day protocol of daily deuterium oxide (D2O) consumption. Participants underwent 2 muscle biopsies of the vastus lateralis on days 8 and 12. Protein abundances were normalised to yeast alcohol dehydrogenase that was spiked into each sample during sample preparation and FTR was calculated from time-dependent changes in peptide mass isotopomers. FTR and abundance data (fmol/ µg protein) were combined to calculate molar turnover rates (MTR; fmol/ µg protein/ d) and absolute turnover rates (ATR; ng/ d). Abundance data were collected for 1,772 proteins and FTR data were calculated from 3,944 peptides representing 935 proteins (average 3 peptides per protein). The median (M), lower- (Q1) and upper-quartile (Q3) values for protein FTR (%/d) were M = 4.39, Q1 = 0.96, Q3 = 7.84. Our analyses suggest MTR data is preferred over FTR, particularly for studies on multiprotein complexes, wherein MTR takes account of potential differences amongst the molecular weight of the component subunits. ATR data may be preferred over MTR and FTR, particularly when the aim is to compare between samples that may exhibit different abundance profiles.

Dynamic proteome profiling uncovers age-related impairments in proteostasis in human skeletal muscle

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Background

Healthy muscle ageing focuses on maintaining the quality and functional capacity of skeletal muscle. Muscle function is underpinned by the proteome and evidence from model organisms suggests that ageing may lead to a progressive decline of protein quality through losses in protein homeostasis (proteostasis). Mechanisms, including the accumulation of erroneously translated, misfolded, or incomplete proteins contribute to losses in protein quality and present a burden to the proteostasis network. Protein turnover plays a key role in proteostasis (Nishimura et al., 2021), and resistance exercise-induced increases in protein turnover may improve proteostasis in human skeletal muscle.

Objective

In models of muscle diseases such as myopathy and obesity, muscle dysfunction is associated with groups of proteins that are more abundant but exhibit slower rates of turnover compared to younger adults. We tested the hypothesis that the muscle proteome of healthy older adults may also exhibit this characteristic and that resistance exercise would improve muscle protein quality by increasing the turnover rate of select proteins.

Methods

Eight healthy males consisting of four younger (28 ± 5 y) and four older (69 ± 3 y) adults were recruited from the Merseyside area. Participants underwent 5 sessions of unilateral leg press exercise (3 sets of 10 repetitions) at 90% of 10-repetition maximum interspersed by 3-day recovery periods during a period of 14-day intervention. Dynamic proteomic profiling was used to investigate protein-by-protein differences in abundance and synthesis rates. To study protein synthesis, participants orally consumed deuterium oxide, which labels amino acid precursors and enables the fraction of newly synthesized protein to be calculated from time-dependent changes in peptide mass isotopomer profiles before and after the resistance exercise intervention (Nishimura et al., 2023).

Results

The abundance of 1768 proteins and turnover rates of 765 proteins were measured in all muscle samples from each participant (i.e. no missing values from a total n=32 samples). One-way analysis of variance highlighted statistically significant ($P < 0.05$, a false discovery rate <23%) differences in the abundance of 198 proteins between young versus older muscle. Subunits of eukaryotic initiation factor 3 (eIF3), including EIFCL, EIF3F, EIF3A, and EIF3M were more abundant in older muscle, whereas 24 subunits of the 40S and 60S ribosomal proteins were less abundant in older muscle. Two-way analysis of variance between age (Younger versus Older) and intervention (Exercise versus Control) found an interaction effect ($P < 0.05$) on the turnover rate of the cytosolic (EF2) and mitochondrial (EFTU) elongation factors, where older, but not younger muscle specifically responded to resistance exercise training.

Conclusion

Dynamic proteome profiling uncovered an imbalance in eukaryotic initiation factor 3 (eIF3), the largest initiation factor complexes, in the muscle of older adults. An accumulation of eIF3 subunits in older muscle could indicate a stalling of ribosomal translation early during elongation that is known to particularly impact mitochondrial proteins. Importantly, resistance exercise increased the turnover rate of both the cytosolic (EF2) and mitochondrial (EFTU) elongation factors, which may suggest the repressed mitochondrial protein translation was restored in exercised muscle.

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