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Short report

Mitochondrial respiratory chain function and content are preserved in the skeletal muscle of active very old men and women

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ABSTRACT

Introduction: The loss of mitochondrial function and content have been implicated in sarcopenia although they have been little studied in the very old, the group in which sarcopenia is most common. In this pilot study, our aim was to determine if mitochondrial respiratory chain function and content are preserved among healthy 85-year-olds.

Methods: We recruited 19 participants (11 female) through their general practitioner and assessed their medical history, functional status and self-reported physical activity. We identified sarcopenia using grip strength, Timed Up-and-Go and bioimpedance analysis. We assessed mitochondrial respiratory chain function using phosphorous magnetic resonance spectroscopy, estimating $\tau_{1/2}$ PCr, the recovery half-time of phosphocreatine in the calf muscles following a bout of aerobic exercise. We performed a biopsy of the vastus lateralis muscle and assessed mitochondrial respiratory chain content by measuring levels of subunits of complex I and IV of the respiratory chain, expressed as *Z*-scores relative to that in young controls.

Results: Participants had a median (IQR) of 2 (1,3) long-term conditions, reported regular aerobic physical activity, and one participant (5.3%) had sarcopenia. Sixteen participants completed the magnetic resonance protocol and the mean (SD) $\tau_{1/2}$ PCr of 35.6 (11.3) seconds was in keeping with preserved mitochondrial function. Seven participants underwent muscle biopsy and the mean fibre *Z*-scores were −0.7 (0.7) and −0.2 (0.4) for complexes I and IV, respectively, suggesting preserved content of mitochondrial respiratory chain enzymes.

Conclusion: Muscle mitochondrial respiratory chain function and content are preserved in a sample of active, well-functioning 85-year-olds, among whom sarcopenia was uncommon. The results from this study will help inform future work examining the association between muscle mitochondrial deficiency and sarcopenia.

1. Introduction

Impairments in skeletal muscle mitochondrial function and content have been implicated in the development of sarcopenia, the age-related loss of muscle mass and performance (Brierley et al., 1996; St-Jean-Pelletier et al., 2017; Joseph et al., 2012). The assessment of skeletal muscle mitochondria presents challenges. Assessment of content, such as staining for cytochrome oxidase, requires the collection of muscle tissue, as do *in vitro* measures of function such as respirometry of isolated mitochondria (Hepple, 2014). *In vivo* measurement of function is possible using phosphorous magnetic resonance spectroscopy (^{31}P-MRS), requiring participants to undertake controlled exercise sufficient to deplete muscle reserves of phosphocreatine (Hollingsworth et al., 2008).

As expected for an age-related condition, sarcopenia is most common among the very old (Cruz-Jentoft et al., 2014), with a prevalence of 21% in a sample of 85-year-olds (Dodds et al., 2017). There have been few studies of mitochondrial function and content in this age

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group, with relevant studies typically having a mean age below 85 (Coen et al., 2013; Choi et al., 2016; Spendiff et al., 2016; Rygiel et al., 2017; Distefano et al., 2017). The opportunity to collect muscle samples during hip fracture surgery has been used to investigate whether impaired mitochondrial homeostasis is associated with sarcopenia among the very old (Marzetti et al., 2016). Older patients with hip fracture are recognised to have not only high levels of sarcopenia but also disability and multimorbidity (Di Monaco et al., 2011; Krishnan et al., 2014); in this setting, the influences of ageing *per se* and those of acute illness and overall frailty may be difficult to disentangle.

A complementary approach is to study community-dwelling very old individuals, including those with few medical and functional problems, who may provide important insights into factors that promote healthy ageing (Ferrucci, 2008). We therefore undertook a pilot study in which we assessed the feasibility of recruiting community-dwelling 85-year-old people to attend for detailed phenotyping including ^{31}P-MRS and muscle biopsy. The aim of the present study was to determine if skeletal muscle mitochondrial respiratory chain function and content are preserved among healthy 85-year-olds.

2. Methods

2.1. Participants

We recruited participants aged 85 years, born in 1931, who were registered with a general practice within the North East & North Cumbria Clinical Research Network, England. We excluded those with a cardiac pacemaker or any other metallic or programmable device (*e.g.* cochlear implants or surgical clips) or those who were taking anticoagulant drugs. We also excluded individuals considered unsuitable for approach by their general practitioner (GP). All participants needed to have capacity to provide written informed consent.

Potential participants were identified through their GPs and were sent a letter of invitation, a study information pack and a letter of support from their GP. Individuals expressing an interest in the study were then contacted by the research team and an appointment made to visit them in their own home. At this visit, the requirements of the study were discussed in detail and initial informed consent obtained. Endurance of consent was verified at each contact and prior to any research procedure throughout the research process. The study was approved in the UK by the Tyne & Wear South Research Ethics Committee (15/NE/0382). Fieldwork took place between May and August 2016.

We asked participants whether they had ever been diagnosed by a doctor with 11 common conditions (heart attack, congestive heart failure, angina, stroke/mini-stroke/TIA, hypertension, diabetes, asthma, depression, chronic lung disease, kidney disease or cancer) and recorded their regular prescribed medications. We used the 15-item geriatric depression scale (GDS) and the mini-mental state examination (MMSE) to assess mood and cognition, respectively. We enquired about difficulty or needing help across 17 activities of daily living such as dressing/undressing, cutting toenails, shopping and managing finances. We used the Short Form 36 (SF-36) Health Survey Questionnaire to derive general health and physical functioning scores (Ware and Gandek, 1998). We assessed physical activity using the rapid assessment of physical activity (RAPA), deriving scores for aerobic activity (1–7, with 7 being most active) and strength and flexibility activity (0–3, with 3 most being active) (Topolski et al., 2006).

2.2. Identification of sarcopenia

We measured grip strength (kg) with a Jamar handheld hydraulic dynamometer (Promedics, UK) using three trials in both hands following a standard protocol (Roberts et al., 2011) and using the maximum value obtained for analyses. Participants completed the Timed Up-and-Go (TUG) test: a stopwatch was used to measure the time taken to get up from a chair and walk as quickly and safely as possible up to and around a marker placed 3 m away, walk back to the chair and sit back down. We converted this time to an estimate of gait speed (m/s) using the formula (6 / [TUG time]) * 1.62 (Cooper et al., 2011; Cooper et al., 2015). We measured total body weight (kg) and estimated appendicular lean mass (kg) using a Tanita MC-780MA body composition analyser (Tanita Corporation, Arlington Heights, IL.). We estimated height based on demi-span, measured twice to the nearest millimetre. We calculated skeletal muscle index (SMI) (kg/m^2) from appendicular lean mass divided by height-squared. We applied the European Working Group sarcopenia definition to our results, using recognised cut-points for grip strength of < 30 kg in men and < 20 kg in women, for gait speed of ≤ 0.8 m/s and for SMI of < 7.26 kg/m^2 in men and < 5.45 kg/m^2 in women (Cruz-Jentoft et al., 2010). We considered participants with weak grip and/or slow gait speed, in combination with low SMI, to have sarcopenia.

2.3. Phosphorous magnetic resonance spectroscopy

Participants attended for ^{31}P-MRS scanning and were requested to perform a low-intensity plantar flexion exercise in the scanner with incremental loading, until the phosphocreatine in the gastrocnemius and soleus muscles was depleted by approximately 50%. Measurements were taken every 10 s during exercise and recovery. We fitted an exponential recovery curve to the area under the phosphocreatine peak from which we modelled the time taken, $\tau_{1/2}$ PCr (seconds), for recovery halfway to baseline, as a measure of mitochondrial oxidative function, with shorter times implying higher function (Hollingsworth et al., 2008) (Fig. 1).

2.4. Muscle biopsy

We obtained biopsy of the vastus lateralis muscle under local anaesthesia from seven participants using a Weil Blakesley conchotome. The samples were snap frozen in isopentane cooled in liquid nitrogen. We telephone participants the following day to check their wellbeing and visited them at home one week after their biopsy to check the wound had healed and that there were no signs of infection present. We also enquired about any pain at the site, rated on a scale of 0 (no pain) – 10 (worst pain).

2.5. Quadruple immunofluorescence

Two 10 μm sections from each biopsy were used for the quadruple immunofluorescence with antibodies to laminin, NDUFB8 (subunit of complex I), MTCOI (subunit of complex IV) and porin, as described previously (Rygiel et al., 2017; Rocha et al., 2015) (Fig. 1). Control samples were biopsies obtained from five younger patients undergoing orthopaedic surgery (see Supplementary Table 1 for full details). The control and participant sections were reacted the same day with the same batch of antibody and identical concentrations. All exposure times were set and maintained throughout the imaging.

The immunofluorescence data from the fibres in the control samples were used to produce linear regression models for the relationships between levels of complex I and porin, and between complex IV and porin. The regression findings were then used to predict the expected levels of complex I and IV per fibre among study participants based on the fibres' measured porin levels. The measured values in complex I and IV were then expressed as *Z*-scores (the number of standard deviations the measured values were above that predicted by the linear regression models). We classified fibres with $Z \geq -3$ (so measured values no lower than 3 standard deviations below that predicted from the relationships seen in young controls) as positive.