ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2023; **14**: 1482–1494
Published online 4 May 2023 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.13220

Evidence for inefficient contraction and abnormal mitochondrial activity in sarcopenia using magnetic resonance spectroscopy

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Abstract

Background Mitochondrial dysfunction has been implicated in sarcopenia. ^{31}P magnetic resonance spectroscopy (MRS) enables non-invasive measurement of adenosine triphosphate (ATP) synthesis rates to probe mitochondrial function. Here, we assessed muscle energetics in older sarcopenic and non-sarcopenic men and compared with muscle biopsy-derived markers of mitochondrial function.

Methods Twenty Chinese men with sarcopenia (SARC, age = 73.1 ± 4.1 years) and 19 healthy aged and sex-matched controls (CON, age = 70.3 ± 4.2 years) underwent assessment of strength, physical performance, and magnetic resonance imaging. Concentrations of phosphocreatine (PCr), ATP and inorganic phosphate (Pi) as well as muscle pH were measured at rest and during an interleaved rest–exercise protocol to probe muscle mitochondrial function. Results were compared to biopsy-derived mitochondrial complex activity and expression to understand underlying metabolic perturbations.

Results Despite matched muscle contractile power (strength/cross-sectional area), the ATP contractile cost was higher in SARC compared with CON (low-intensity exercise: 1.06 ± 0.59 vs. 0.57 ± 0.22, moderate: 0.93 ± 0.43 vs. 0.58 ± 0.68, high: 0.70 ± 0.57 vs. 0.43 ± 0.51 mmol L^{-1} min^{-1} bar^{-1} cm^{-2}, $P = 0.003$, <0.0001 and <0.0001, respectively). Post-exercise mitochondrial oxidative synthesis rates (a marker of mitochondrial function) tended to be longer in SARC but did not reach significance (17.3 ± 6.4 vs. 14.6 ± 6.5 mmol L^{-1} min^{-1}, $P = 0.2$). However, relative increases in end-exercise ADP in SARC (31.8 ± 9.9 vs. 24.0 ± 7.3 mmol L^{-1}, $P = 0.008$) may have been a compensatory mechanism. Mitochondrial complex activity was found to be associated with exercise-induced drops in PCr [citrate synthetase activity (CS), Spearman correlation rho = −0.42, $P = 0.03$] and end-exercise ADP (complex III, rho = −0.52, $P = 0.01$; CS rho = −0.45, $P = 0.02$; SDH rho = −0.45, $P = 0.03$), with CS also being strongly associated with the PCr recovery rate following low intensity exercise (rho = −0.47, $P = 0.02$), and the cost of contraction at high intensity (rho = −0.54, $P = 0.02$). Interestingly, at high intensity, the fractional contribution of oxidative phosphorylation to exercise was correlated with activity in complex II (rho = 0.5, $P = 0.03$), CS (rho = 0.47, $P = 0.02$) and SDH (rho = 0.46, $P = 0.03$), linking increased mitochondrial complex activity with increased ability to generate energy through oxidative pathways.

Conclusions This study used ^{31}P MRS to assess ATP utilization and resynthesis in sarcopenic muscle and demonstrated abnormal increases in the energy cost during exercise and perturbed mitochondrial energetics in recovery. Associations

between mitochondrial complex activity and the fractional contribution to energy requirement during exercise indicate increased ability to generate energy oxidatively in those with better mitochondrial complex activity.

Keywords Sarcopenia; Mitochondrial function; Spectroscopy; Muscle; Mitochondrial complex activity

Received: 1 July 2022; Revised: 16 January 2023; Accepted: 28 February 2023
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Introduction

Sarcopenia, characterized by progressive loss of muscle mass, quality and/or strength, is associated with increased muscle breakdown[1] and reduced muscle protein synthesis.[2,3] It has been linked with reduced physical function[4,5] and reduced quality of life and has been shown to predict loss of independence in older adults.[6,7] With a 2015 report from the WHO[8] predicting that the world's population above 60 will have increased from 900 million in 2015 to over 2 billion in 2050, sarcopenia poses an increasing public health burden. Estimated costs from the United States indicate excess costs of approximately $900 for every person with sarcopenia and that a 10% reduction in prevalence would result in annual savings of over one billion US dollars in the United States alone.[9] As such, the importance of developing preventative and therapeutic strategies for sarcopenia is clear. Identification of the mechanisms leading to sarcopenia and non-invasive methods for monitoring potential interventions are required to accomplish this.

The problem with diagnosis and treatment of sarcopenia lies in its multifactorial nature, with many potential triggers implicated including reduced muscle activity, changes in nutritional intake, increases in oxidative stress and pro-inflammatory cytokines, changes in hormonal levels, reduced satellite cell numbers and loss of neuromuscular junctions, among others.[7] Recent evidence has implicated mitochondrial dysfunction, degradation of energy production through the respiratory chain (respiratory complex activity)[10] and associated impaired proteostasis as major contributors to sarcopenia.[11] The mitochondrial free-radical theory of ageing (MFRTA)[12] suggests that oxidative damage to mitochondrial DNA (mtDNA) could trigger dysfunction in the electron transport chain (ETC) and impair oxidative phosphorylation, resulting in reduced energy production and further generation of reactive oxygen species (ROS). This then leads to more damage.[12] Evidence supporting the MFRTA was provided by Bua *et al*.[13] in a study in which ETC abnormalities were assessed in vastus lateralis muscle and were found to increase from 6% at 49 years to 31% at 92 years of age. However, although the exact mechanism is unclear, it has been shown that there exists an age-related decline in muscle mitochondrial mass,[14] TCA cycle activity,[15] ATP production[16,17] and oxygen consumption[18] consistent with mitochondrial dysfunction or loss. In vivo measurements using ^{31}P magnetic resonance spectroscopy (MRS) to measure oxidative phosphorylation indicate mixed findings, with many showing large reductions in rates of oxidative phosphorylation in older versus younger subjects,[19,20] with more recent studies indicating that, at least in *active* healthy ageing, mitochondrial respiratory chain function is preserved.[21] However, although much work has been carried out to understand the changes in healthy ageing, it is unclear how mitochondrial function is further perturbed in sarcopenia. There is initial evidence for perturbed phospholipid profiles (likely glycerophosphoethanolamine) and decreases in phosphocreatine in sarcopenia.[22] In addition, our group has previously shown differences in mitochondrial oxidative capacity and NAD+ biosynthesis in a cohort of patients with sarcopenia[10] whose respiratory chain complex expression for complexes I, II, III and V (NDUFA9, SDHa, UQCR2 and ATP5a) was downregulated 44–51% in sarcopenic muscle and who had reduced mitochondrial complex activity and TCA cycle enzymes citrate synthetase and succinate dehydrogenase levels. Similarly, NAD+ has been shown to be markedly reduced with ageing and further reduced in aged-impaired muscle, although exercise in aged adults can improve NAD + levels towards those of younger controls.[23] Here, we utilized ^{31}P MRS to non-invasively assess characteristics of ATP synthesis and utilization in exercising muscle in order to probe muscle mitochondrial function and energy demand in older subjects with and without sarcopenia. We also assessed and compared in vivo measures of bioenergetics status from ^{31}P MRS with clinical parameters and mitochondrial complex expression and activity from biopsy.

Research design and methods

Subjects

Twenty male subjects of Chinese ethnicity ≥65 years with a diagnosis of sarcopenia (SARC) and 19 sex and aged matched controls without a diagnosis of sarcopenia (CON) were recruited from two studies on healthy community-dwelling older men in Singapore [Singapore Sarcopenia Group (SSG)[24] and Aging in a Community Environment Study (ACES)[25]].

Clinical parameters

Clinical testing included measurements of height, weight, total lean body mass [assessed using dual-energy X-ray absorptiometry (DXA), APEX software version 4.0.1, Discovery Wi DXA system], grip strength (kg), 6 m gait speed (m/s), chair rise time (s) and time-up-and-go (TUG, s). Diagnosis of sarcopenia was based on the Asian Working Group for Sarcopenia 2014 definition[26]: total appendicular lean mass normalized for height less than 7.00 kg/m^2 with evidence of either low physical performance based on gait speed of $<$0.8 m/s OR low muscle strength based on hand grip $<$26 kg.

Magnetic resonance imaging and magnetic resonance spectroscopy acquisition and analysis

Magnetic resonance imaging and spectroscopy were performed in the calf using a Siemens Tim Trio 3T system (Siemens Healthineers, Erlangen, Germany). ^{1}H transmission was performed using the Q-Body coil with reception using a 12-channel flex body coil. ^{31}P MRS spectroscopy data were performed using a double-tuned ^{1}H–^{31}P MRS rigid quadrature coil (Rapid Biomedical GmBH, Rimpar, Germany). Imaging included a T_1-weighted turbo spin echo (TSE) image (matrix = 704 × 496 × 25, voxel size = 0.54 × 0.54 × 7 mm^3, TI/TE/TR = 100/12/800 ms). T_1-weighted images were manually segmented using the Medical Imaging Toolkit (MITK version 2015.5.2, German Cancer Research Centre, Heidelberg, Germany), generating segmentations and calculating cross-sectional areas (CSA) for single-slice whole muscle, as well as gastrocnemius and soleus, at the point of the largest CSA within the dominant calf.

A fully relaxed ^{31}P MR spectrum was acquired with the following parameters: pulse-acquire sequence, TR = 12 000 ms, BW = 3000 Hz; 2048 points, 16 averages. The coil was centred on the back of the calf over the region with the largest CSA. Padding was positioned between the coil and leg to limit localization to (predominantly) gastrocnemius and soleus. Pulse duration was optimized to give 90° flip angle at the gastrocnemius (set constant for all subjects). The subsequent ^{31}P data acquired during the exercise paradigm used the same conditions but with TR = 2000 ms and 840 acquisitions (time ~28 min).

Fully relaxed ^{31}P MRS spectra were truncated to 512 points, zerofilled back to 2048 points, before phase and frequency correction and averaging in jMRUI (V 6.0, jMRUI consortium, EU). Exercise paradigm spectra were first filtered using a k-rank singular value decomposition (SVD, k = 5) to improve signal-to-noise (SNR) characteristics using an in-house built Matlab (MathWorks, Natick, MA, USA) program based on literature[27] and assessed for validity using simulated data with SNR matched to the real data. Filtered data were then treated as for fully relaxed spectra. Spectral analysis was performed using the advanced method for accurate, robust and efficient spectral fitting (AMARES)[28] in jMRUI, fitting singlet peaks to PCr, GPC, GPE, PC, PE, (NADH + NAD) and two peaks to Pi (to allow for two pools at different pH), ATP was fitted using two doublets (γ- and α-ATP) and a triplet (β-ATP). For fully relaxed data, results are presented as peak area ratios to ATP. Exercise data were converted to concentrations assuming ATP to be 8.2 mmol/L and applying correction for short TR saturation effects calculated from mean fully relaxed data. Initial results indicated higher than expected PCr concentrations (40 mmol/L); however, further analysis revealed reduced relative contribution from β-ATP peaks relative to γ due to reduced relative excitation or under-fitting. A correction factor (C) for ATP was therefore calculated based on all subjects $C = \gamma + \alpha + \beta/(3\gamma) = 1.12$. This correction resulted in resting PCr concentrations (concentrations are denoted by square brackets), [PCr], of 33 ± 2 and [Pi] of 5.1 ± 1.3 mmol/L, which is matched to expected values.[29] pH was calculated from the shift (δ) between the PCr and Pi peaks: $pH = 6.75 + log\left(\frac{\delta - 3.27}{5.63 - \delta}\right)$, [30] taking a weighted sum of the two pH values.[31]

In vivo assessment of bioenergetics status

The collection of ^{31}P MRS during an exercise task enables measurement of real-time exercise-induced increases in ATP demand, oxidative mitochondrial synthesis and the fractional oxidative contribution to exercise and has the potential to allow estimation of proton buffering capacity and efflux rate. A detailed description of the theory with details of how calculations are performed to allow parameter extraction can be found in the Supporting Information.

Measurement of maximum voluntary contraction

Prior to scanning, subjects were positioned on the scanner bed with their dominant leg positioned on an MR-compatible plantarflexion pedal ergometer system (Lode B. V. Groningen, The Netherlands). For assessment of maximum voluntary contraction (MVC), the ergometer pressure was set to maximum. Subjects were then requested to maximally push the pedal for 2 s. Pressure was reduced in 0.2 bar increments and subjects repeated plantar flexion until a *full* compression of the pedal was achieved. Pressure was then increased by 0.8 bar and repeated until the full compression was achieved three times. The average of the resulting values was then taken as each individual's MVC.

Exercise paradigm

For assessment of metabolic rates, exercise was carried out via a 0.5-Hz repeated plantar flexion using the MR-compatible ergometer. The paradigm consisted of 1-min rest, 3-min exercise at *low intensity* (44% MVC), 6-min rest, 3-min *moderate intensity* exercise (66% MVC), 6-min rest, 3 min at *high intensity* (88% MVC) and final 6-min rest. Subjects who could not perform the full 3-min exercise were requested to maintain activity as long as possible (at full intensity) before stopping when performance could not be continued and alerting operators using the call bell.

Near-infrared spectroscopy acquisition and analysis

A near-infrared spectroscopy (NIRS) probe (OxiplexTS) was positioned on the muscle to allow measurement of muscle oxy-haemoglobin concentration [HbO], deoxy-haemoglobin concentration [Hb] and oxygen saturation (OS %). Calibrated NIRS data could not be acquired from seven subjects (4 CON, 3 SARC) due to problems with the system calibration. [Hb] were fitted with SigmaPlot 14.0 (Systat Software Inc, San Jose, USA) using a modified exponential function described by

$$[\mathrm{Hb}]_t = [\mathrm{Hb}]_0 + \mathrm{A}\left(1 - e^{\left(\frac{-t-\tau}{k}\right)}\right)$$

to obtain A, the change in [Hb], τ, the temporal delay in [Hb] changes, and k, the time constant for changes in [Hb].

Mitochondrial activity from muscle biopsies

Muscle biopsies were collected from the vastus lateralis muscle using a BioPince™ (Angiotech) 16-G full-core biopsy needle. These were snap frozen in liquid nitrogen and stored at −80°C prior to further analysis. Analysis was performed, as previously described.[10] Mitochondrial enzyme and respiratory chain complex activities were measured on mitochondrial fractions isolated from 15 to 30 mg of frozen muscle biopsies.[32,33] More details are provided in the Supporting Information.

Statistical analysis

Group differences for ^{31}P MRS data and clinical parameters are presented as mean ± standard deviation (SD), and significance values were assessed using independent samples *t*-tests in SPSS 25. Contractile efficiencies and NIRS data were assessed to be non-parametric based on skew (>2) and kurtosis (>7) measures, and as such, results are presented as median ± inter-quartile range with statistical significance assessed using a Mann–Whitney *U* test in SPSS. The significance level was set at 0.05. No corrections were made for multiple comparisons for measured parameters.

Time course data were downsampled (factor 2) and corrected for multiple comparisons in time using cluster correction with individual threshold and cluster threshold (assessed from cluster size and permutation) set at $P < 0.05$.

Spearman's correlation coefficient (rho) was used to measure the strength of the monotonic relationship between MRS measures and measures from biopsies. 5% Benjamini–Hochberg false discovery rate (FDR) was set as significant.[34]

Results

Participant characteristics

Twenty community-dwelling sarcopenic men of Chinese ethnicity and 19 age-matched controls were recruited. Sarcopenia was defined based on consensus clinical definitions of the Asian Working Group for Sarcopenia using skeletal muscle mass evaluation by DXA measurement of appendicular lean body mass (ALM/height^2), grip strength and gait speed (*Table* 1). Despite age, ethnicity and sex matching, SARC had significantly lower BMI compared with CON (70.3 ± 4.2 vs. 73.1 ± 4.1 kg m^{-2}, $P = 0.003$). This difference was driven by loss in lean body mass (38.6 ± 3.0 vs. 44.5 kg, $P = 0.00001$), with groups showing similar fat mass (15.3 ± 4.0 vs. 15.5 ± 3.8 kg, $P = 0.9$). Despite similar fasting glucose levels (5.6 ± 0.5 vs. 5.4 ± 0.3 mmol/L, $P = 0.3$), HbA1c was significantly elevated in the SARC group (6.1 ± 0.4 vs. 5.7 ± 0.3, $P = 0.002$). Functional measures tended to be poorer in the SARC group, with these reaching significance for 6-m walk gait speed (1.1 ± 0.3 vs. 1.4 ± 0.2 ms^{-1}, $P = 0.001$) and grip strength (25.0 ± 2.7 vs. 34.9 ± 6.0, $P = 2E\text{-}7$).

Muscle volume and strength

Measured MVC was significantly reduced in SARC compared with CON (2.9 ± 0.7 vs. 3.6 ± 0.7 bar, $P = 0.002$; *Table* 2, top). Similarly, the maximum CSA for the gastrocnemius and soleus muscles (assumed to be active during the plantarflexion task based on previous studies) was significantly reduced (17.5 ± 2.3 vs. 22.5 ± 1.9 cm^2, $P = 1E\text{-}5$). Calculation of the measured muscle power per unit CSA, referred to as the maximum contractile power, showed similar values between the two groups (0.17 ± 0.05 vs 0.16 ± 0.04, $P = 0.7$).

In vivo assessment of bioenergetics status

Resting state measures

Resting levels of Pi/PCr, (Pi+PCr)/ATP and pH (*Table* 2) were not significantly different between the SARC and CON groups

Table 1 Clinical characteristics of study population

Measure	CON (n = 19)	SARC (n = 20)	P-value
Age (years)	70.3 ± 4.2	73.1 ± 4.1	0.09
BMI (kg m^{-2})	22.7 ± 1.0	21.3 ± 1.5	0.003*
HbA1c	5.7 ± 0.3	6.1 ± 0.4	0.002*
Fasting glucose (mmol/L)	5.4 ± 0.3	5.6 ± 0.5	0.3
Lean body mass (kg)	44.5 ± 4.0	38.6 ± 3.0	1E-5*
ALM (kg)	20.6 ± 2.0	16.7 ± 1.	7E-8*
ALM/height (kg m^{-2})	7.4 ± 0.4	6.2 ± 0.5	1E-9*
Fat mass (kg)	15.5 ± 3.8	15.3 ± 4.0	0.9
Weight (kg)	63.0 ± 6.2	57.5 ± 5.7	0.008*
Gait speed 6-m walk (m s^{-1})	1.4 ± 0.2	1.1 ± 0.3	0.001*
Grip strength (kg)	34.9 ± 6.0	25.0 ± 2.7	2E-7*
Chair rise time (s)	7.9 ± 1.9	9.0 ± 1.8	0.08
TUG time (s)	7.2 ± 1.1	8.0 ± 1.0	0.05

Data are presented as mean ± SD. Significant results were determined using an independent Student's t-test and are indicated by *.
Abbreviations: ALM, appendicular lean mass; BMI, body mass index; CON, control (without sarcopenia); SARC, with sarcopenia; HbA1c, glycated haemoglobin; TUG, time-up-and-go test.

Table 2 Resting state metabolic measures

Measure	CON (n = 19)	SARC (n = 20)	P-value
MVC (bar)	3.6 ± 0.7	2.9 ± 0.7	0.002*
Muscle CSA (Soleus + Gastroc. cm^2)	22.5 ± 1.9	17.5 ± 2.3	1E-5*
Max. contractile power (bar/cm^2)	0.16 ± 0.04	0.17 ± 0.05	0.7
(PCr + Pi)/ATP	1.82 ± 0.12	1.78 ± 0.16	0.4
Pi/PCr	0.11 ± 0.02	0.14 ± 0.06	0.1
pH	6.94 ± 0.03	6.92 ± 0.04	0.6
Oxy %	79.2 (66.9–81.8)	72.8 (63.6–77.1)	0.3
[HbO] (μM)	57.1 (41.5–77.8)	54.8 (35.0–94.9)	0.9
[Hb] (μM)	22.5 (13.9–27.6)	24.6 (18.3–30.2)	0.3

TOP: Muscle measures and ^{31}P MRS. Data are presented as mean ± SD for CON (non-sarcopenic control group) and SARC (sarcopenic group). Significant results were calculated using an independent samples t-test and are indicated by *. Bottom: NIRS data are presented as median (interquartile range). Significant results were determined using a Mann–Whitney U test and are indicated by #.
Abbreviations: CSA, cross-sectional area; Hb, deoxyhaemoglobin; HbO, oxyhaemoglobin; MVC, maximum voluntary contraction; Oxy %, blood oxygen %; PCr, phosphocreatine; Pi, inorganic phosphate.

(Pi/PCr 0.14 ± 0.06 vs. 0.11 ± 0.02, P = 0.1; (Pi+PCr)/ATP 1.78 ± 0.16 vs. 1.82 ± 0.12, P = 0.4; pH 6.92 ± 0.04 vs. 6.94 ± 0.03, P = 0.6). No differences were measured in resting muscle perfusion parameters or blood oxygenation (Oxy 72.8 vs. 79.2%, P = 0.3; [HbO] 54.8 vs. 57.1 μM, P = 0.9; [Hb] 24.6 vs. 22.5 μM, P = 0.3).

Changes in muscle metabolites during exercise and recovery

An example fully relaxed spectrum (acquired at rest) and spectral time course during low-intensity exercise and recovery is included in *Figure* S1.

The mean group time-course data for PCr, Pi, ATP, ADP, pH and Hb are shown in *Figure* 1 (4 s time resolution).

Despite similar initial concentrations, PCr concentrations decreased more in the SARC group compared with CON at both low and moderate exercise intensities ($P < 0.05$). Shortfall in PCr was similar for exercise at high intensity. The significant reduction in PCr in SARC relative to CON during exercise at low and moderate intensity is suggestive of either increased energy demand or delayed/reduced increases in oxidative or glycolytic phosphorylation.

Changes in Pi and pH during exercise and in recovery were slightly more prominent in SARC; however, the difference did not reach significance. Despite this, a significant increase in ADP (relative to CON and estimated from ATP, PCr and pH) was found towards the end of the first exercise period, but not thereafter, suggesting potential upregulation of compensatory mechanisms. No differences are seen in blood deoxygenation either during exercise or in recovery.

Bioenergetic parameters calculated during exercise and recovery are outlined in *Tables* 3 and 4 respectively. During exercise (*Table* 3), increased PCr shortfall was seen in SARC at both low (11.5 ± 3.3 vs. 8.0 ± 3.4 mmol/L, P = 0.003) and moderate intensity (16.1 ± 5.1 vs. 12.4 ± 3.8 mmol/L, P = 0.007) but not high (17.6 ± 5.5 vs. 15.8 ± 4.4 mmol/L, P = 0.1, in agreement with findings from time-course cluster analysis). The end-exercise ADP concentration was higher (31.8 ± 9.9 vs. 24.0 ± 7.3 μmol/L, P = 0.008), and the rate of drop in PCr (0.56 ± 0.16 vs. 0.43 ± 0.18 min, P = 002) was longer at low, but not at moderate or high intensity. However, calculated ATP demand was not significantly different between SARC and CON at any intensity (data not shown). Despite this, and despite matched contractile power,

DOI: 10.1002/jcsm.13220

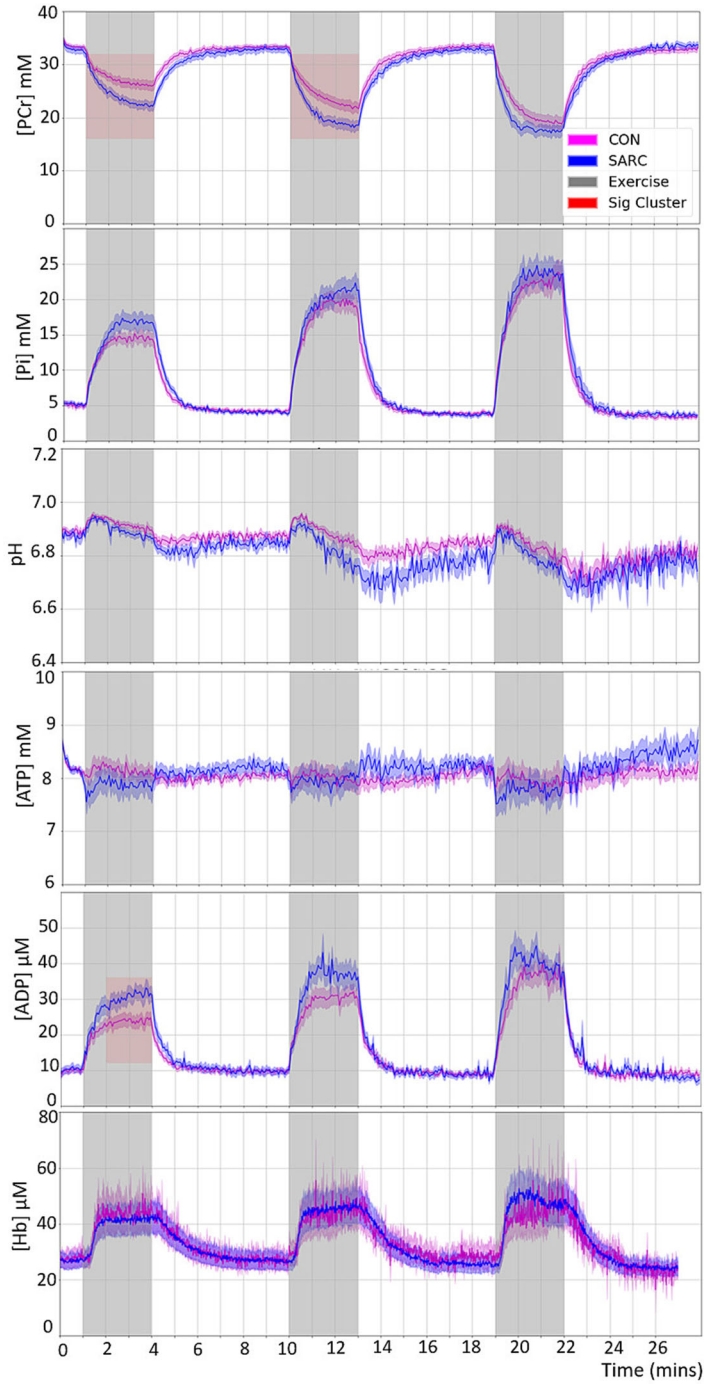

Figure 1 Mean ± standard error time-course data for top to bottom: PCr, Pi, pH, ATP, ADP and Hb. Clusters of between group significance are indicated in red. When exercise begins, rapid increases in muscle ATP consumption cannot be met by increases in oxidative or glycolytic phosphorylation. Instead, the *creatine kinase* pathway is utilized (described by PCr + ADP + $H^+ \rightarrow$ Cr + ATP), resulting in a decrease in PCr and an increase of Pi. Gradually, rates of oxidative and non-oxidative phosphorylation increase to meet the energy demand with the initial rate of drop in PCr providing a measure of the ATP demand. Conversely, when exercise ceases and muscle ATP requirement falls, glycolytic phosphorylation stops, and excess energy generated via oxidative phosphorylation is used to recover levels of PCr. The initial rate of PCr recovery is a measure of the end-exercise rate of mitochondrial oxidative ATP synthesis and is suggested to be a marker of the mitochondrial function. Figure generated using matplotlib, compiled in MSOffice.

calculation of the contractile cost (ATP demand/exercise force/muscle CSA) showed significant increases at all intensities in SARC relative to CON (low 0.51 vs. 0.28, P = 0.0002; moderate 0.45 vs. 0.24, p = 0.0008; high 0.40 vs. 0.21, P = 0.0001). No differences were seen in the deoxyhaemoglobin parameters at any time point ($P > 0.05$).

DOI: 10.1002/jcsm.13220

Table 3 Measured exercise-induced metabolic measures

Measure	CON (n = 19)	SARC (n = 20)	P-value
PCr shortfall (A, mmol L^{-1})			
Low	8.0 ± 3.4	11.5 ± 3.3	0.003*
Moderate	12.4 ± 3.8	16.1 ± 5.1	0.007*
High	15.8 ± 4.4	17.6 ± 5.5	0.1
Rate of change in PCr (k, min)			
Low	0.43 ± 0.18	0.56 ± 0.16	0.02*
Moderate	0.55 ± 0.20	0.56 ± 0.17	0.7
High	0.53 ± 0.13	0.56 ± 0.36	0.9
Contractile cost ((A/k)/Force/CSA)			
Low	0.28 (0.23–0.42)	0.51 (0.35–0.67)	0.0002#
Moderate	0.24 (0.18–0.28)	0.45 (0.36–0.56)	0.0008#
High	0.21 (0.18–0.27)	0.40 (0.28–0.57)	0.0001#
End-Ex [ADP, μmol L^{-1}]			
Low	24.0 ± 7.3	31.8 ± 9.9	0.008*
Moderate	31.5 ± 9.5	36.4 ± 13.3	0.2
High	35.2 ± 15.3	38.0 ± 10.4	0.5
[Hb] (A)			
Low	5.3 (3.3–13.2)	6.2 (5.0–14.8)	0.7
Moderate	9.9 (5.3–15.3)	12.2 (9.7–24.5)	0.3
High	11.4 (7.6–18.2)	15.3 (9.0–36.8)	0.4
Hb time constant (k, min)			
Low	0.17 (0.10–0.31)	0.25 (0.15–0.71)	0.1
Moderate	0.22 (0.17–0.27)	0.24 (0.17–#0.61)	0.7
High	0.19 (0.13–0.26)	0.17 (0.11–0.30)	0.9
Hb delay (τ, min)			
Low	0.41 (0.27–0.48)	0.34 (0.21–0.40)	0.1
Moderate	0.32 (0.25–0.38)	0.26 (0.20–0.28)	0.09
High	0.24 (0.22–0.33)	0.26 (0.22–0.29)	0.9

Measured metabolic parameters during exercise from ^{31}P MRS for CON (non-sarcopenic muscle) and SARC (sarcopenic muscle). Significant results are indicated by * for independent samples t-test and # for Mann–Whitney U.
Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; CSA, cross-sectional area; Hb, deoxyhaemoglobin; PCr, phosphocreatine.

Following exercise (*Table* 4), akin to the drops in PCr during exercise, the recovered PCr was significantly higher in SARC than CON, at *all* intensities (low 11.7 ± 3.8 vs. 8.0 ± 3.1 mmol/L, P = 0.002; moderate 14.5 ± 4.4 vs. 12.2 ± 3.1 mmol/L, P = 0.02; high 16.3 ± 4.9 vs. 13.2 ± 5.4 mmol/L, P = 0.008). However, no change was seen in the blood deoxyhaemoglobin parameters or PCr recovery rate or in the rate of oxidative ATP synthesis, indicating normal mitochondrial oxidative ATP synthesis rates in SARC ($P > 0.05$). *Figure* 2 shows a plot of $R/Q_{max,CON}$, the relative rate of mitochondrial oxidative ATP synthesis, R, as compared with the mean maximum rate calculated in the control group, $Q_{max\ CON}$, against end-exercise [ADP] (calculated from low-intensity exercise).

Figure 3 shows plots of change in PCr (ΔPCr) against change in pH (ΔpH) from baseline during exercise (left) and recovery (right). Data were downsampled by a factor of 3 (6 s temporal resolution). These plots are useful for understanding the potential mechanisms for differences in ATP generation between groups as well as probing proton clearance. In particular, at low intensity, we see that at end exercise (~bottom most point on each plot), the pH for SARC and CON are similar despite different PCr values. These demonstrate the relative increase in pH driving the ADP concentrations and oxidative synthesis faster. However, the source of this potential pH increase is unclear. Possible sources could be decreased contribution from glycolytic phosphorylation, increased proton efflux, increasing basal metabolic demand or a combination of these. Estimation of the fractional contribution of oxidation to exercise energy requirement (*Table* 4, calculated from fraction of end-exercise oxidative ATP synthesis relative to the exercise ATP demand) indicates no increase in glycolytic phosphorylation, at any exercise intensity. Analysis of the proton efflux rate and apparent efflux rate indicates no difference between SARC and CON, although there was a tendency for increased apparent efflux in SARC at low and moderate intensities (P = 0.1 and 0.0,6 respectively).

Associations of magnetic resonance spectroscopy mitochondrial dysfunction markers and mitochondrial complex expression and activity

Previously, we have shown downregulation of mitochondrial complex genes (complexes I, II, III, IV and V) in sarcopenic muscle, with reductions in protein expression of active subunits (NDUFA9, SDHa, UQCR2 and ATP5a), reduced mitochondrial enzymatic activity of complexes I–IV and markers of TCA cycle enzyme activity of citrate synthase (CS) and succinate dehydrogenase (SDH). Here, the following MRS measures were assessed for association with measures of mitochondrial complex expression and activity in muscle biopsies of the

Table 4 Measured metabolic measures from post-exercise recovery

Measure	CON (n = 19)	SARC (n = 20)	P-value
PCr recovery (mmol L^{-1})			
Low	8.0 ± 3.1	11.7 ± 3.8	0.002*
Moderate	12.2 ± 3.1	14.5 ± 4.4	0.02*
High	13.2 ± 5.4	16.3 ± 4.9	0.008*
Rate of PCr recovery (min)			
Low	0.62 ± 0.28	0.71 ± 0.21	0.3
Moderate	0.70 ± 0.27	0.77 ± 0.22	0.4
High	0.71 ± 0.29	0.72 ± 0.26	0.9
End-exercise Ox. ATP synthesis (mmol L^{-1} min^{-1})			
Low	14.6 ± 6.6	17.3 ± 6.5	0.2
Moderate	19.6 ± 8.1	20.5 ± 8.2	0.8
High	22.4 ± 12.8	25.0 ± 10.2	0.2
Fractional oxidation (%)			
Low	0.75 ± 0.23	0.87 ± 0.36	0.2
Moderate	0.93 ± 0.52	0.72 ± 0.30	0.2
High	0.80 ± 0.49	0.77 ± 0.57	0.9
Proton efflux (E, mmol L^{-1} min^{-1})			
Low	3.7 ± 3.7	2.4 ± 1.7	0.2
Moderate	3.5 ± 10.4	2.9 ± 4.8	0.8
High	3.7 ± 10.1	2.5 ± 5.2	0.7
Initial apparent efflux rate (λ, min^{-1})			
Low	−84 ± 311	47 ± 160	0.1
Moderate	−75 ± 395	240 ± 567	0.06
High	143 ± 302	167 ± 388	0.8
Hb (A)			
Low	8.1 (3.0–10.9)	11.5 (6.8–23.7)	0.1
Moderate	11.9 (5.6–18.4)	15.1 (11.3–33.0)	0.2
High	15.4 (7.3–21.3)	17.1 (10.6–37.6)	0.1
Hb time constant (k, min)			
Low	0.69 (0.52–0.72)	1.08 (0.47–1.51)	0.4
Moderate	0.75 (0.57–0.97)	0.92 (0.65–1.25)	0.2
High	0.75 (0.51–1.24)	0.70 (0.59–1.04)	0.7
Hb delay (τ min)			
Low	0.52 (0.26–0.72)	0.32 (0.28–0.82)	0.7
Moderate	0.42 (0.35–0.68)	0.43 (0.27–0.76)	1.0
High	0.46 (0.28–0.70)	0.36 (0.2–0.58)	0.5

Note: Measured metabolic parameters from ^{31}P MRS during post-exercise recovery for CON (non-sarcopenic muscle) and SARC (sarcopenic muscle). Significant results are indicated by * for independent samples t-test and # for Mann–Whitney.
Abbreviations: ATP, adenosine triphosphate; CSA, cross-sectional area; Hb, deoxyhaemoglobin; Ox, oxidative; PCr, phosphocreatine.

same participants: resting Pi/PCr, exercise-induced shortfall in PCr (A), end-exercise ADP concentrations and the PCr recovery rate (each at 44% only, assuming this to be predominantly oxidative exercise and closely related to mitochondrial function). Correlations were also assessed for the contractile cost and the fractional oxidative contributions to exercise (at all intensities).

Significant correlations were found between ^{31}P MRS measures of bioenergetic and mitochondrial enzyme activity (complex II, complex III, CS and SDH; *Table* 5). However, no correlation was found between ^{31}P MRS measures of bioenergetic and mitochondrial function with those of mitochondrial mRNA expression (complex I, complex II, complex III and complex IV) or protein complexes (NDUFA9, SDHa, UDQCR2, ATP5a; *results not shown)*.

No correlation was seen for resting Pi/PCr levels with measures of mitochondrial complex activity. However, lower levels of mitochondrial activity in CS (a marker of mitochondrial content in skeletal muscle) were associated with larger exercise-induced decreases in PCr and higher post-exercise PCr recovery rate, suggesting that lower mitochondrial enzyme activity was associated with either increased energy demand or perturbed upregulation of oxidative and/or glycolytic phosphorylation as well as reduced mitochondrial oxidative ATP synthesis. Similarly, higher levels of ADP at end-exercise were negatively correlated with activity in complex III, CS and SDH during exercise at low intensity. Here, inter-individual differences in pH kinetics (and their contribution to ADP concentrations) likely strengthen the correlation with complex III, CS and SDH, indicating differences in pathways for energy generation (PCr recharge, oxidative and glycolytic phosphorylation) are being reflected in mitochondrial complex activity.

Discussion

Phosphorus (^{31}P) MRS techniques provide a unique non-invasive window into oxidative metabolism by characterizing ATP, ADP and creatine phosphate (PCr) in skeletal

DOI: 10.1002/jcsm.13220

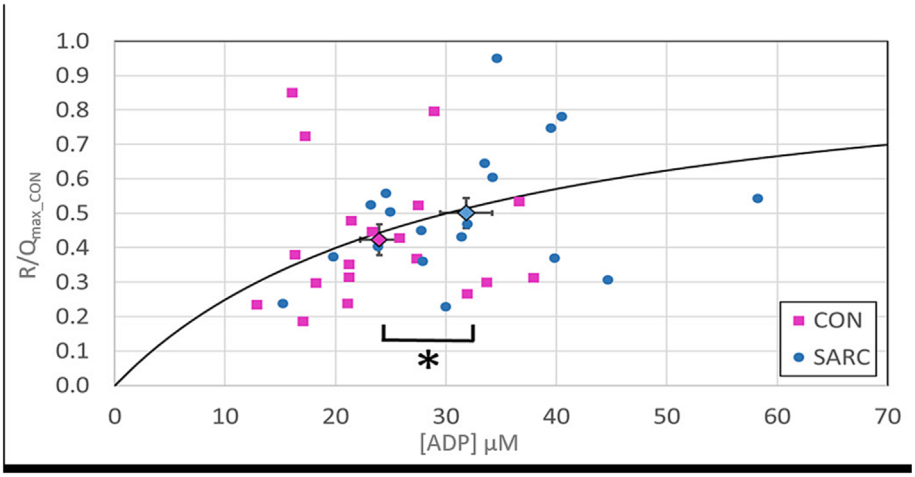

Figure 2 A plot showing the relationship between the initial rate of post-exercise PCr recovery and the end-exercise concentration of ADP relative to the mean CON Q_{max} taken from exercise at low intensity. Mean group values are plotted as diamond with error bars and are given as mean ± standard error. Significance is indicated for $*P < 0.05$. Here, abnormalities in mitochondrial oxidative ATP synthesis rates can be identified by divergence from the black line in the y-direction (defined by $R/Q_{max,CON} = 1 = (1 + K_m/[ADP])$). Differences in [ADP] are indicated by shift along x, as seen in SARC. Mean R/Q_{max} values lie along the expected curve for both SARC and CON groups; however, the [ADP] can be seen to be significantly higher in SARC. Increased [ADP] drives R, the mitochondrial ATP synthesis rate, higher, and therefore it is likely that relative increases in [ADP] in SARC compensate for underlying mitochondrial dysfunction, driving oxidative ATP synthesis towards normal levels. Figure generated using Matlab.

muscles. Measurement of PCr resynthesis following exercise has been well reported and is a reliable measure of mitochondrial oxidative metabolism. Here, we use non-invasive ^{31}P MRS to investigate muscle energetics in sarcopenia.

Results of a plantarflexion exercise paradigm show an increased contractile cost during exercise indicating an inefficiency in muscle contraction energetics and possible mitochondrial dysfunction. Although mitochondrial oxidative synthesis rates appear unchanged in sarcopenia, these rates appear to be normalized through compensatory increase of ADP via relatively increased pH. This could be due to either decreased glycolytic phosphorylation, increased proton efflux, increasing basal metabolic demand or a combination of these. Although we find a tendency for increased apparent efflux with no decrease in the contribution of glycolytic phosphorylation, more work is required to confirm this.

This study is the first to document correlations between mitochondrial enzyme activity from muscle biopsies with MRS bioenergetic markers in patients with sarcopenia and healthy aged matched controls, finding strong correlations with complex II and complex III activity as well as CS and SDH. ^{31}P MRS is a highly specialized technique that is not readily available in many clinical settings but may be a useful non-invasive tool in the research setting to evaluate the impact of various factors besides exercise on muscle function, such as weight, diet and specific clinical or pharmacological interventions.

Resting state measures

No changes in muscle metabolite levels nor blood flow or oxygenation are indicated when at rest. However, significant reductions were seen in the muscle CSA, as expected in sarcopenia. Reductions in strength in SARC are proportional to the reductions in CSA, resulting in no difference in cross-sectional power (strength/CSA) between groups, in contrast to those seen in healthy ageing, where reductions in power are further reduced relative to CSA.[35] This may indicate a different mechanism beyond accelerated ageing that induce excess muscle loss, such as selective fibre-type loss, leaving the remaining muscle functioning normally.

Energy demand during exercise

Increased exercise-induced changes in PCr (*Table* 3 and *Figure* 1, top) indicate an increased shortfall in PCr during exercise at both low and moderate, but not high-intensity exercise in sarcopenic subjects. However, the ATP demand was suggested to be similar between the groups. Despite this, the contractile costs (ATP cost per bar of pressure per cm^2 muscle) were significantly higher in the SARC group compared with CON at all exercise intensities, suggesting decreased energy efficiency in sarcopenia.

Assessment of mitochondrial function and proton efflux

At the onset of exercise, PCr acts as a rapid source of energy, generating ATP through creatine kinase activity. This pathway consumes a proton (see *Table* S1) leading to an increase in pH (seen here in *Figures* 1 and 3, and previously reported by Kemp *et al.*[29,36,37]). As exercise continues, pH then declines

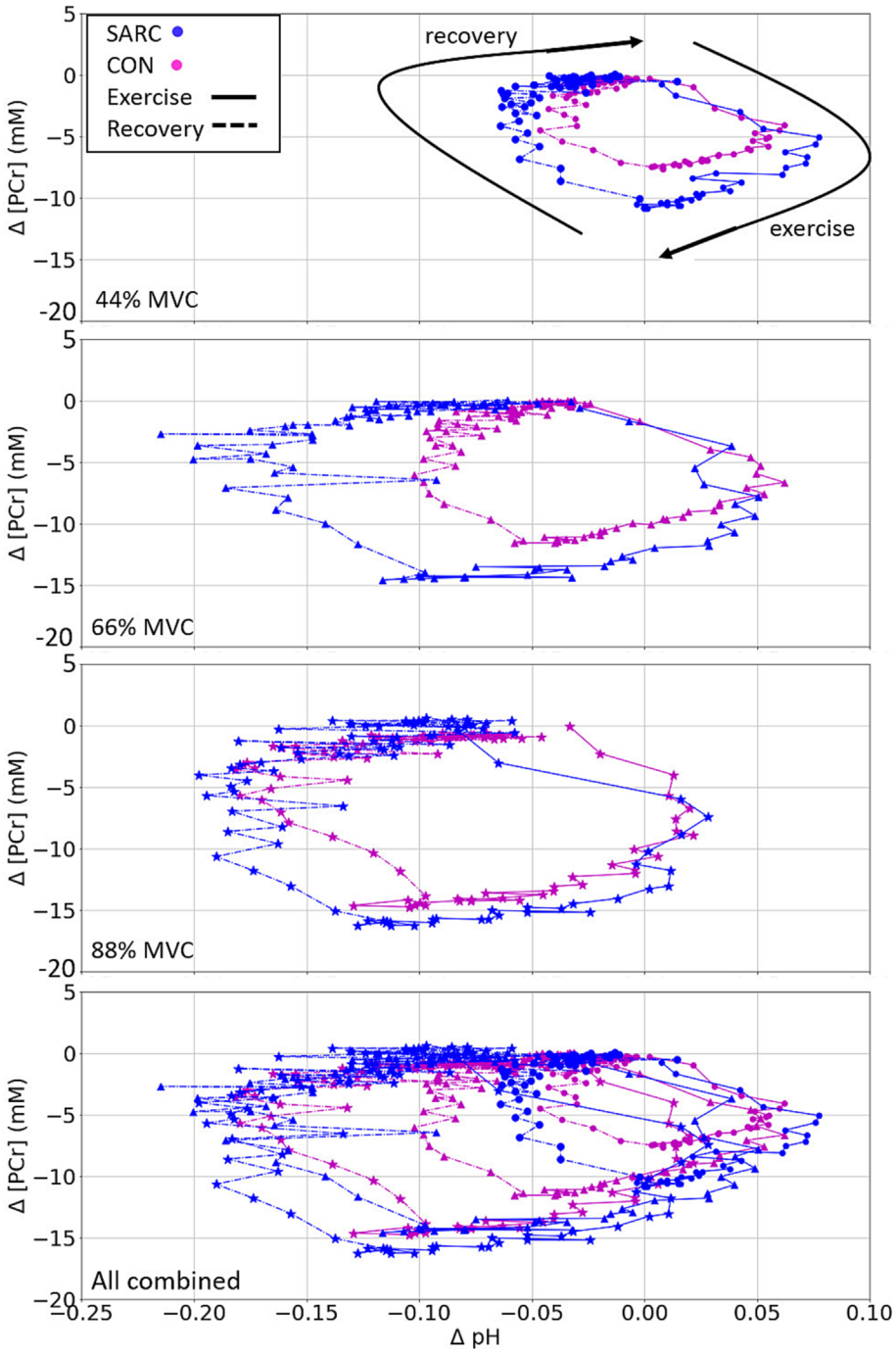

Figure 3 Group mean plots of PCr-pH kinetics during exercise (solid line) and recovery (dotted line) for SARC (blue) and CON (pink). During exercise (see exercise arrow, top), initial trajectories from baseline are approximately linear along $-\Delta PCr/+\Delta pH$, at all intensities. This is driven by recharge from PCr with consumption of a proton to generate ATP (see Supporting Information). Movement along this trajectory is further for SARC at low intensity due to increased ATP demand, but the change of these trajectories happens at the same time in both groups (~18 s) and appears to occur earlier in subsequent exercise trials. At this point, they then follow the same curve along $-\Delta[PCr]/-\Delta pH$, running approximately parallel to each other, until end exercise. Notably, for the low intensity exercise, a distinct difference in PCr can be observed at end exercise; however, the end-exercise pH appears to be the same, demonstrating the increased relative pH in SARC. Following exercise, despite different initial start points, both SARC and CON data follow parallel trajectories along $+\Delta PCr/+\Delta pH$ driven by regeneration of PCr before returning towards baseline. Although PCr returns towards baseline within the 6-min recovery period, the mean group pH shows a tendency for decreasing with subsequent exercise. This and the shortening of time for exercise-induced trajectory change may indicate adaptive processes due to previous activations. Figure generated using matplotlib.

DOI: 10.1002/jcsm.13220

Table 5 Correlation between mitochondrial enzymatic activity and 31P MRS markers of mitochondrial and bioenergetic function

	Complex I	Complex II	Complex III	Complex IV	CS	SDH
Pi/PCr	0.1	0.13	0.05	0.11	0.08	0.10
PCr shortfall (A, low)	−0.29	−0.34	−0.40	−0.40	**−0.42****	−0.37
End-exercise ADP (low intensity)	−0.36	−0.37	**−0.52****	−0.39	**−0.44****	**−0.45****
PCr recovery rate (low)	−0.35	−0.29	−0.05	−0.37	**−0.47****	−0.28
Contractile cost (low)	−0.17	−0.21	−0.27	−0.11	−0.16	−0.28
Contractile cost (mod)	−0.25	−0.38	−0.26	−0.25	−0.35	−0.40
Contractile cost (high)	−0.46	−0.44	−0.03	−0.37	**−0.54****	−0.42
Fractional Ox (low)	0.24	0.29	0.10	0.22	0.30	0.29
Fractional Ox (med)	0.37	**0.48****	**0.53****	0.44	**0.47****	**0.47****
Fractional Ox (high)	0.30	**0.50****	0.29	0.36	**0.46****	**0.47****

Note: Spearman's rho correlation between mitochondrial complex activity and ^{31}P MRS markers of mitochondrial and bioenergetic function. Exercise intensity of the measure is indicated in brackets (low, moderate, high). Significant correlations are indicated with (**) $P < 0.05$ false discovery rate (FDR) corrected.

Abbreviations: CS, citrate synthase; fractional Ox, fractional oxidation; PCr, phosphocreatine; Pi, inorganic phosphate; SDH, succinate dehydrogenase.

as oxidative ATP synthesis increases and protons are effluxed from the system, and/or as proton generating glycolytic phosphorylation increases, driving down the pH. In contrast, when exercise ceases, energy is generated by oxidative phosphorylation, with the excess energy utilized to regenerate PCr. This process generates a proton, driving pH down further. However, pH gradually returns to baseline via proton efflux. As such, the pH of the system at any point is a function of the amount of energy generated by PCr (increases pH) and glycolytic phosphorylation (decreases pH), passive buffering (through inorganic phosphate), post-exercise generation of PCr (decreases pH) and proton efflux (increases system pH).

Assuming exercise to be largely oxidative at low intensities (where no significant drop in pH is observed), relative decreases in [PCr] and increases in [ADP] during exercise (as seen here in SARC) would be indicative of mitochondrial dysfunction. However, the end-exercise rate of oxidative ATP synthesis (calculated from post-exercise recovery kinetics) was found to be normal at all exercise intensities. These findings were unexpected considering abnormal transcriptomic and proteomic changes in mitochondrial oxidative pathways in these same patients.[10] However, increases in ADP in SARC at end exercise (*Figure* 1, *Table* 3) would accelerate post-exercise PCr resynthesis, compensating for underlying dysfunction, as seen in *Figure* 2. Similar findings have been reported previously in patients with hypertension and Duchenne muscular dystrophy carriers and in myotonic dystrophy.[36] Since [ADP] is dependent on both [PCr] and pH, a relative increase in pH may drive this increase in [ADP]. Indeed, this relative pH increase in SARC can be seen in the PCr–pH curves (*Figure* 3), where mean end-exercise pH at 44% is matched across the two groups despite different PCr concentrations. Possible mechanisms for increased relative pH in SARC are increased proton efflux and/or decreased glycolytic phosphorylation. Estimated fractional oxidation rates (calculated from end-exercise mitochondrial oxidation rates and total ATP demand) do not appear to be different in CON and SARC groups. Although there is a tendency for increased efflux, ΔpH values in this study are equivalent to the lowest values provided in previous studies[36,37] even at 88% MVC. These previous studies tended to increase intensity until exercise fatigue was reached, driving the pH much lower. Estimation of efflux here, therefore, has poor sensitivity, and more work is required to elucidate whether this compensatory mechanism is, indeed, upregulated but initial calculations indicate this is likely (*Table* 4).

Interestingly, metabolic responses due to exercise at moderate and high intensity do not show the same response as at low intensity (increased PCr shortfall and end-exercise ADP). This may be due to differences in energy generation (at low intensity generation is largely oxidative, whereas higher intensities are increasingly anaerobic; perturbations may be largely oxidative) or could be due to upregulation of responses in previous stimuli. In the brain, for example, upregulation of lactate efflux during an intense visual stimulus has been proposed as a mechanism for lack of stimulus-induced increases due to subsequent stimuli[38] and is indicated in studies of ischaemic preconditioning.[39] 'Metabolic priming' here could involve upregulation of proton efflux or shifting of the baseline prior to subsequent exercise.

Associations with mitochondrial complex activity and expression

Significant associations were found between mitochondrial complex activity and metabolic measures from ^{31}P MRS, and notably, directionality of the measures was matched across the different complexes for the individual measures/groups of measures (*Table* 5). We had previously shown that mitochondrial activity in complex I–IV and oxidative energy production are reduced in sarcopenic muscle compared with healthy aged muscle.[10] Increased complex activity (in complex II, III, CS and SDH) is associated with smaller drops in PCr and smaller increases in ADP at low intensity, as well as increasing fractional oxidation during exercise at high intensity. These

DOI: 10.1002/jcsm.13220

are suggested to be due to improved oxidative energy generation and increased efficiency. Similarly, higher contractile costs at higher levels of intensity are associated with lower activity in CS, and a tendency for association with complex II and SDH (P = 0.053 FDR corrected), indicating that poorer mitochondrial complex activity is associated with inefficient muscle contraction; however, no association was found for exercise at low intensity. Similarly, higher levels of fractional oxidation during exercise at higher intensities (66 and 88%) are associated with higher activity of mitochondrial complexes II and III (moderate intensity only) and activity of CS and SDH. Notably, higher exercise intensities are associated with increasing levels of anaerobic energy generation. Where oxidative energy generation is perturbed, larger differences between subjects might be expected at higher intensities compared with low intensities.

Conclusions

Our results are the first, to our knowledge, to relate ^{31}P MRS measures of bioenergetic function to measures of muscle mitochondrial expression and activity. Increases in muscle contractile costs and decreases in fractional oxidation during moderate and high intensity are shown to be linked to decreases in activity of mitochondrial complex II and III activity, as well as CS and SDH enzyme activity. These likely indicate a reduction in ability to generate energy through oxidative pathways and may be linked with reduced mitochondrial activity in these complexes, but not in reduced expression across a group of patients with sarcopenia and healthy age-matched controls.

^{31}P MRS results indicate reductions of strength in sarcopenia are a consequence of reductions in muscle volume, resulting in matched contractile power when compared with healthy age-matched controls. However, results of a plantar-flexion task indicate increased contractile costs during exercise and potential mitochondrial dysfunction. Although mitochondrial oxidative ATP synthesis rates appeared normal, these rates are driven by increased end-exercise ADP levels, which could be a consequence of either decreased glycolytic phosphorylation, increased proton efflux, increasing basal energy demand or a combination of these. Although we find a tendency for increased efflux, further studies are needed to elucidate these mechanisms.

Acknowledgements

The authors would like to thank Graham J. Kemp for advice regarding exercise protocols and kinetic analysis. This study was funded by Nestlé. The authors of this manuscript certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[40]

Conflict of interest

M.C.S., J.X.M.H., M.K., J.J.T., N.K., S.B., R.A.M. and S.K.H.T. declare that they have no conflict of interest. This study was part of the MEMOSA research project funded by Société des Produits Nestlé S.A. E.M. and J.N.F. are employees of Société des Produits Nestlé S.A.

Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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