ORIGINAL ARTICLE

Journal of Cachexia, Sarcopenia and Muscle 2018; **9**: 279–294
Published online 24 January 2018 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12272

Physical activity unveils the relationship between mitochondrial energetics, muscle quality, and physical function in older adults

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Abstract

Background The concept of mitochondrial dysfunction in ageing muscle is highly controversial. In addition, emerging evidence suggests that reduced muscle oxidative capacity and efficiency underlie the aetiology of mobility loss in older adults. Here, we hypothesized that studying well-phenotyped older cohorts across a wide range of physical activity would unveil a range of mitochondrial function in skeletal muscle and in turn allow us to more clearly examine the impact of age *per se* on mitochondrial energetics. This also enabled us to more clearly define the relationships between mitochondrial energetics and muscle lipid content with clinically relevant assessments of muscle and physical function.

Methods Thirty-nine volunteers were recruited to the following study groups: young active (YA, *n* = 2 women/8 men, age = 31.2 ± 5.4 years), older active (OA, *n* = 2 women/8 men, age = 67.5 ± 2.7 years), and older sedentary (OS, *n* = 8 women/11 men, age = 70.7 ± 4.7 years). Participants completed a graded exercise test to determine fitness (VO_2peak), a submaximal exercise test to determine exercise efficiency, and daily physical activity was recorded using a tri-axial armband accelerometer. Mitochondrial energetics were determined by (i) ^{31}P magnetic resonance spectroscopy and (ii) respirometry of fibre bundles from *vastus lateralis* biopsies. Quadriceps function was assessed by isokinetic dynamometry and physical function by the short physical performance battery and stair climb test.

Results Daily physical activity energy expenditure was significantly lower in OS, compared with YA and OA groups. Despite fitness being higher in YA compared with OA and OS, mitochondrial respiration, maximum mitochondrial capacity, Maximal ATP production/Oxygen consumption (P/O) ratio, and exercise efficiency were similar in YA and OA groups and were significantly lower in OS. P/O ratio was correlated with exercise efficiency. Time to complete the stair climb and repeated chair stand tests were significantly greater for OS. Interestingly, maximum mitochondrial capacity was related to muscle contractile performance and physical function.

Conclusions Older adults who maintain a high amount of physical activity have better mitochondrial capacity, similar to highly active younger adults, and this is related to their better muscle quality, exercise efficiency, and physical performance. This suggests that mitochondria could be an important therapeutic target for sedentary ageing associated conditions including sarcopenia, dynapenia, and loss of physical function.

Keywords Ageing; Cardiovascular fitness; Skeletal muscle; Mitochondria; Physical function

Received: 16 May 2017; Revised: 1 September 2017; Accepted: 24 October 2017
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Introduction

The progressive loss of muscle mass, strength, and physical function that occurs with ageing is known as sarcopenia.[1,2] This condition is prevalent in adults over 70 years of age and predisposes an individual to mobility disability, nursing home admission, and early mortality.[3] With the rapid increase in the elderly demographic in the US, sarcopenia is likely to become a major burden on the healthcare system. Apart from resistance training, there are very few effective therapeutic strategies to treat sarcopenia,[4] in part because the underlying aetiology is multifactorial, complex, and still needs to be completely delineated. However, there should be increased focus on developing new therapeutics for sarcopenia given its recent code designation in the tenth revision of the International Classification of Diseases.[5]

Physical function requires the integration of multiple physiological systems, including cardiopulmonary, vestibular, sensory, and muscular, and is a key aspect of contemporary clinical definitions of sarcopenia.[2] Physical function can be assessed by a wide range of tests including the short physical performance battery (SPPB), usual gait speed, 6-min walk test, and the stair climb test.[2] Limitations in physical function of older adults, such as inability to ambulate and impaired mobility, are precursors to disability, dependency, and institutionalization.[6] For example, performance in an extended walking test is a significant prognostic factor for total mortality, cardiovascular disease, mobility limitation, and mobility disability in older adults in their eighth decade.[7]

A decline in skeletal muscle mitochondrial capacity is also characteristic of ageing and experimental evidence suggest that mitochondria are also key in the aetiology of sarcopenia.[8] Defective mitochondrial quality control is linked to elevated reactive oxygen species emission, and activation of apoptotic pathways thought to contribute to the pathophysiology of sarcopenia.[8] Mitochondrial dysfunction has recently been studied as an underlying factor for low physical function,[9] slower walking speed,[10–12] fatigability,[13] and loss of exercise efficiency with ageing.[14] However, it should be noted that there is still considerable debate regarding the true nature of ageing-associated mitochondrial dysfunction. While many studies have supported an age-related decrease in mitochondrial capacity,[15–20] several others have failed to find these associations.[21–25] These contradictory results may be explained by the fact that the majority of these studies have not considered important covariates that also affect mitochondria, such as participant physical activity (PA) levels,[18] cardiovascular fitness, and adiposity,[20] all of which likely confound the relationship between mitochondrial capacity and age.[19,22,26–28] In addition, the various analytical approaches employed to assess mitochondrial function,[29] including the use of isolated mitochondria[15,18,20] that has been shown to exaggerate the observed deficit in mitochondrial function in ageing,[30] may contribute to the lack of clarity.

Intramyocellular lipid (IMCL) accumulation has been reported in the *vastus lateralis* of older adults, as determined by both *in vivo*[31] proton magnetic resonance spectrometry (MRS)[31] and *ex vivo* analysis of muscle biopsies.[32,33] IMCL accumulation appears to be specific to type I fibres[32,33] and may occur secondary to a sedentary lifestyle.[33] Accumulation of IMCL occurs along with mitochondrial dysfunction and insulin resistance in sedentary ageing and obesity; however, the general consensus is that IMCL *per se* does not directly mediate these effects. Although the implications of IMCL accumulation in ageing have yet to be clearly defined, IMCL has been recently linked to the pre-frail phenotype.[34] Collectively, these findings suggest that rigorously conducted investigations in well-phenotyped individuals are needed to clarify the nature of age-related declines in mitochondrial function and IMCL. This is critical to further understand the relevance of mitochondrial dysfunction and IMCL in ageing to clinically relevant endpoints, including those that characterize physical function.

Reduced exercise efficiency (increased oxygen consumption per work performed) for physical activities such as walking is also apparent in the elderly.[35] This in turn could impede activities of daily living in older adults,[36,37] potentially contributing to future risk for mobility disability. Although the underlying cause of low exercise efficiency with ageing is unknown, mitochondrial energetics may be involved.[14,38] Furthermore, the influence of physical activity status, cardiovascular fitness, and adiposity on exercise efficiency in older adults has not been thoroughly examined.

The goal of this study was to investigate the role of chronological age and physical activity on a comprehensive profile of skeletal muscle mitochondrial energetics, IMCL, and intermuscular adipose tissue (IMAT) content. A second goal was to examine the relationship between mitochondrial energetics and IMCL with exercise efficiency, muscle quality (strength per unit mass), and physical function. The benefits of resistance exercise in preserving muscle and physical function in older adults has been relatively well documented. Here, we generated evidence from well-phenotyped cohorts indicating that physical activity, predominantly endurance exercise, largely mitigates the negative effects of a sedentary lifestyle on mitochondrial energetics, exercise efficiency, and certain aspects of physical function in older adults.

Materials and methods

Study design and participants

Thirty-nine men and women from the Orlando, FL, area volunteered to participate in this study. Volunteers were eligible to participate if they were weight stable (±4.5 kg in preceding 6 months), had a body mass index (BMI) between 20 and 35 kg/m^2, and were in good general health.

Volunteers were excluded if they were taking medications known to influence muscle metabolism, had a chronic medical condition, had any contraindications to exercise, were pregnant or breast-feeding, or had high resting blood pressure (≤150 mmHg systolic, ≤ 90 mmHg diastolic). A note from the participant's primary care physician/cardiologist for exercise clearance was needed if positive stress test symptoms were observed from exercise test. All participants provided written informed consent, and the study protocol was reviewed and approved by the institutional review board at Florida Hospital, Orlando. Thus, the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants were assigned to the following study groups: young active (YA; 21–40 yrs.), older active (OA) and older sedentary (OS, 65–90 yrs.). As our primary focus was on the negative effects of sedentary lifestyle in older adults, a young sedentary group was not enrolled. We chose to enroll physically active groups of young and old individuals to permit the study of ageing *per se*, independent of sedentary behaviour. An examination of the effects of physical activity, and sedentary behaviours were facilitated by the comparison of OA and OS groups. Participants were considered active if they engaged in aerobic exercise (running, cycling, and swimming) at least 3 days a week without extensive lay off over the previous 6 months. Participants were considered sedentary if they completed one or less structured exercise sessions a week.

All testing was completed over four study visits at the Translational Research Institute for Metabolism and Diabetes at Florida Hospital, Orlando. Visit #1 consisted of a fasting blood draw, physical measurements, medical history/physical activity questionnaires, and resting electrocardiography (ECG). On Visit #2, participants completed a VO_2 peak test with ECG to determine cardiorespiratory fitness. On Visit #3, participants completed magnetic resonance imaging (MRI)/magnetic resonance spectroscopy (MRS), quadriceps contractile and physical function testing, and dual energy X-ray absorptiometry (DXA) scan and were given a triaxial accelerometer to determine free living physical activity over a 1-week period. On study Visit #4, fasting blood samples were drawn, and resting energy expenditure was determined by indirect calorimetry. Participants then consumed a small low glycemic index meal (200 kcal, 15% protein, 35% fat, and 50% carbohydrate), and 15 min later, the muscle biopsy procedure was conducted. Participants then completed the acute exercise bout on a cycle ergometer, all as described in the next sections.

Daily physical activity

The monitor used for this study was the SenseWear® Pro Armband (BodyMedia Inc., Pittsburgh, PA). This activity monitor uses accelerometry, galvanic skin response, and skin temperature to estimate energy expenditure at a 1-min resolution.[39] The SenseWear® Armband has been demonstrated to be a valid, accurate, and reliable method when compared with indirect calorimetry[39] and doubly labelled water.[40] Participants were instructed to wear activity monitors on their upper left arm for at least seven consecutive days. Only days with a wear time of at least 85% were considered for further analysis.

Cardiorespiratory fitness

VO_2 peak was determined as peak aerobic capacity measured during a graded exercise protocol on an electronically braked cycle ergometer, as previously described.[10,41] Heart rate, blood pressure, and ECG (12-lead) was recorded throughout this test. The test was terminated according to the criteria outlined in the American College of Sports Medicine exercise testing guidelines.[42]

Assessment of body composition

DXA scans were performed using a GE Lunar iDXA whole-body scanner and analysed with enCORE software. Coefficients of variation (CV) vary by tissue type and range from 0.5% to 1.1%. MRI was performed on a 3 T Philips Acheiva magnet and was used to assess of IMAT and subcutaneous fat (SAT). Volumetric measurement of fat was performed under standardized conditions with subjects in a supine position. Longitudinal relaxation time (T1) weighted imaging sequences were performed across the thighs. The resultant images were analysed using Analyze 11.0 (Biomedical Imaging Resource, Mayo Clinic, Rochester MN) to segment IMAT and SAT depots and to measure the muscle volume. CVs based on same day measurements with repositioning between scans are 1.1%, 4.2%, and 0.4% for SAT, IMAT, and muscle, respectively.

Magnetic resonance spectroscopy (MRS)

All MRS measurements were acquired on a 3 T Philips Acheiva magnet. Assessment of IMCL was by proton (^{1}H) spectroscopy using multiple single voxel acquisitions. Ratios of IMCL to water were determined using the advances method for accurate, robust, and efficient spectral fitting algorithm within the java magnetic resonance user interface (jMRUI) software. CVs based on same day measurements with repositioning between scans are 7.6% in the *soleus* and 18.3% in the *tibialis anterior*. Assessment of maximal mitochondrial phosphorylation capacity (ATP_{max}) was performed as previously described.[43,44] The phosphocreatine (PCr) recovery time constant (τ) and the PCr level in oxygenated muscle at rest $(PCr)_{rest}$ were used to calculate ATP_{max}. After the fully relaxed spectrum (repetition time = 15 s, echo time = 0.17 s, and number of signal averages = 48) was obtained, the ATP_{max}

experiment was performed by obtaining ^{31}P spectra, with one summed spectrum acquired every 6 s for the duration of the ATP_{max} experiment. Briefly, a ^{31}P surface coil was used to measure phosphorous containing species including PCr, ATP, and Inorganic phosphate (P_i) using a standard one pulse acquisition experiment. For the first minute, the participant remained still to obtain baseline measurements. After the baseline was established, the volunteer was asked to perform isometric contractions of the quadriceps (by slight kicking) for up to 45 s. After kicking stopped, the volunteer remained still for an additional ~8 min to allow the PCr peak to return to baseline. The entire ATP_{max} acquisition took 10 min. The experimental spectra (6 s each) consisted of four (number of signal averages) partially saturated free induction decays (repetition time = 1.5 s and echo time = 0.13 s). The areas/heights of the peaks in each ^{31}P MRS spectrum during the experiment were determined using the advances method for accurate, robust, and efficient spectral fitting algorithm within the jMRUI software. Established formulas were used to determine the intracellular pH based on the PCr and P_i chemical shifts and to calculate τ.[45] The CV for the ATP_{max} measured in the same participant in 1 day repositioned or with less than a week between measurements was on average 3.6%.

Muscle contractile performance

Quadriceps contractile performance of the left thigh was determined during a leg extension exercise using an isokinetic pneumatic-driven dynamometer equipped with load cells and potentiometers (Biodex Medical Systems, Inc., Shirley, NY). One repetition maximum for left leg knee extension was determined using a standard weight stack. The initial weight was determined as a predicted one repetition maximum (1-RM), where the starting weight was 90% and 60% of body weight for men and women, respectively. For each additional 10 years of age, the 1-RM decreased 10%.

Physical function testing

Lower extremity function was assessed by short physical performance battery (SPPB), which consisted of three tasks: five repeated timed chair stands, timed standing balance (with feet in parallel, semi-tandem, and tandem positions), and a 4-meter walk to determine usual gait speed.[46] Additionally, each participant completed a timed stair climb test and a grip strength test using a Jamar® hand-held digital dynamometer.

Percutaneous skeletal muscle biopsy

Percutaneous muscle biopsies were obtained in the morning and participants were instructed not to perform physical exercise 48 hr prior to the biopsy procedure. Prior to the biopsy resting metabolic rate was determined while resting in the supine position over 30 min by indirect calorimetry using a canopy system (Parvo Medics, Sandy, UT). Biopsy samples were obtained from the middle region of the *vastus lateralis* under local anaesthesia (2% buffered lidocaine) as described previously.[47] A portion of the biopsy specimen was placed in ice-cold preservation buffer (BIOPS) for analysis of mitochondrial respiration.[10]

Submaximal exercise test

Participants performed a 6-min warm-up consisting of light cycling on an electronically braked cycle ergometer. After the warm-up, the participants cycled for 40 min at approximately 70% of heart rate reserve that was calculated as heart rate max during the VO_2peak test minus supine resting heart rate. Heart rate, perceived exertion, and blood pressure data were collected every 5 min during the test. Indirect calorimetry measurements were recorded using an open-circuit spirometry metabolic monitor system (Parvo Medics, Sandy, UT). Respiratory variables were collected to verify that the participant was exercising at the correct intensity, which was achieved within 5 min of starting the test and to calculate exercise efficiency parameters.

High-resolution respirometry and ROS emission

Permeabilized myofiber bundles (~1–3 mg each) were prepared immediately after the biopsy procedure**,** as previously described.[48] Briefly, individual fibres were gently teased apart in a petri dish containing ice-cold BIOPS media. The fibre bundles were then permeabilized with saponin (2 mL of 50 μg/mL) for 20 min and washed twice (10 min each) in Buffer Z (105 mM K-MES, 30 mM KCl, 10 mM KH_2PO4, 5mM$MgCl_2$-$6H_2O$, 5 mg/mL bovine serum albumin, 1 mM egtazic acid, and pH 7.4 with KOH). Mitochondrial respiration was evaluated by high-resolution respirometry (Oxygraph-2 k, Oroboros Instruments, Innsbruck, Austria). Measurements were performed at 37 °C, in the range of 400–200 nmol O_2/mL. Two assay protocols were run in duplicate using Buffer Z with blebbistatin (25 μM). In protocol 1, complex I supported LEAK (L_I) respiration was determined through the addition of malate (2 mM) and glutamate (5 mM). Adenosine diphosphate (ADP; 4 mM) was added to elicit complex I supported oxidative phosphorylation (OXPHOS; P_I) respiration. Succinate (10 mM) was then added to elicit complex I and II supported OXPHOS (P_{I+II}) respiration. Cytochrome c (10 μM) was added to assess the integrity of the outer mitochondrial membrane. Finally, a titre of carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (2 μM steps)

was performed to determine the maximal electron transfer system (E_{I+II}) capacity or maximal uncoupled respiration. In protocol 2, Fatty Acid beta Oxidation (FAO) supported LEAK (L_{FAO}) respiration was determined through the addition of palmitoylcarnitine (25 μM) and malate (2 mM). ADP (4 mM) was added to elicit FAO supported OXPHOS (P_{FAO}). Cytochrome c (10 μM) was added to access mitochondrial integrity. Glutamate (5 mM) was then added to elicit complex I and FAO ($P_{CI\&FAO}$) respiration. Succinate was added (10 mM) to stimulate complex I and II and FAO ($P_{CI+II\&FAO}$). Steady state O_2 flux for each respiratory state was determined and normalized to myofiber bundle wet weight (ww) using Datlab 5 software (Oroboros Instruments, Innsbruck, Austria).

Measurement of H_2O_2 emission by mitochondria was measured in permeabilized muscle fibre bundles by real-time monitoring of Amplex Red oxidation using a SPEX Fluoromax 3 (HORIBA Jobin Yvon) spectrofluorometer with temperature control and magnetic stirring as previously described.[49] Mitochondrial H_2O_2 emission rate was expressed as pmol/min/mg wet weight.

Exercise efficiency calculations

Exercise efficiency was calculated from calorimetry data generated during the submaximal test, as previously described.[50] Energy expenditure (EE) during steady-state exercise (20 to 40 min) was calculated using the Brouwer equation as previously described.[51] Gross efficiency (GE) was calculated as the ratio of work output to exercise energy expenditure during the submaximal exercise bout, as follows: GE (%) = ($Work_{(kcal/min)}/EE_{(kcal/min)}$)*100. Net Efficiency (NE) was determined as the ratio of work output to exercise minus resting energy expenditure, as follows: NE (%) = ($Work_{(kcal/min)}/EE_{(kcal/min)}$ – Resting Energy Expenditure $(REE)_{(kcal/min)}$) * 100.

Statistics

Group differences were determined using a one-way analysis of variance with a Tukey's post-hoc test for individual group comparisons. The distribution of sex and race across groups were determined by χ^2 test. To measure associations between variables while adjusting for age and gender, partial correlation analyses were conducted. Forward stepwise and multiple regression models were conducted to explore the best combination of variables to explain muscle and physical function. Statistical significance was set at $P < 0.05$. Statistical analyses were performed using SAS 9.4 and JMP v9.0 (SAS, Cary, NC).

Results

Participants characteristics

Thirty-nine participants were screened and enrolled to one of three groups: YA (n = 10), OA (n = 10), or OS (n = 19). The characteristics of the study groups are shown in Table 1. As per study design, the OS group had lower objectively measured daily EE, activity EE, and steps per day, compared with the active groups. The OS group had lower

Table 1 Participant fitness, physical activity, and body composition characteristics

	YA (n = 10)	OA (n = 10)	OS (n = 19)	ANOVA P-Value
Age (years)	31.2 ± 5.4 [b]	67.5 ± 2.7 [a]	70.7 ± 4.7 [a]	<0.0001
Sex, Ratio	2 women/8 men	2 women/8 men	8 women/11 men	0.327*
Weight (Kg)	67.7 ± 9.7	69.9 ± 15.1	79.1 ± 3.1	0.075
BMI (Kg/m^2)	21.3 ± 1.0 [b]	24.2 ± 1.0 [b]	27.7 ± 0.7 [a]	<0.0001
Waist (cm)	74.6 ± 5.1 [b]	88.2 ± 12.3 [a]	97.4 ± 11.9 [a]	<0.0001
VO_2 peak (mL/kgBW/min)	56.7 ± 9.6 [a]	34.9 ± 4.3 [b]	18.58 ± 3.8 [c]	<0.0001
VO_2 peak (mL/kgFFM/min)	65.8 ± 3.4 [a]	45.3 ± 1.8 [b]	29.6 ± 0.8 [c]	<0.0001
Daily EE (kcal/24 h)	3108 ± 270 [a]	2565 ± 555 [b]	2117 ± 404 [c]	<0.0001
Daily AEE (kcal/24 h)	1833 ± 369 [a]	1248 ± 545 [b]	488 ± 335 [c]	<0.0001
Steps per day (steps/24 h)	10560 ± 4242 [a]	8459 ± 2991 [a]	4883 ± 2683 [b]	0.0003
PASE score	—	215 ± 25	105 ± 18	0.0017
Total fat mass (Kg)	10.1 ± 0.9 [b]	17.3 ± 2.8 [b]	29.9 ± 1.7 [a]	<0.0001
Total lean mass (Kg)	55.4 ± 2.6	51.5 ± 2.9	46.6 ± 2.3	0.065
Left leg lean mass (Kg)	9.5 ± 0.50 [a]	8.3 ± 0.46 [a,b]	7.7 ± 0.43 [b]	0.033
SMI (ALM/h^2)	8.23 ± 0.3	7.89 ± 0.3	7.43 ± 6.9	0.1859
Hand grip strength (Kg)	44.3 ± 2.8 [a]	35.6 ± 2.5 [b]	31.9 ± 1.7 [b]	0.001

Data are presented as mean ± standard deviation. ANOVA, analysis of variance; BMI, body mass index; BW, body weight; FFM, fat-free mass; EE, energy expenditure; AEE, activity energy expenditure; PASE, physical activity scale for the elderly; SMI, skeletal muscle index; ALM, appendicular lean mass; YA, young active; OA, old active; OS, old sedentary.
[a], [b], and [c]indicate significant differences between groups ($P < 0.05$).
*Sex distribution across groups were determined by χ^2 test.

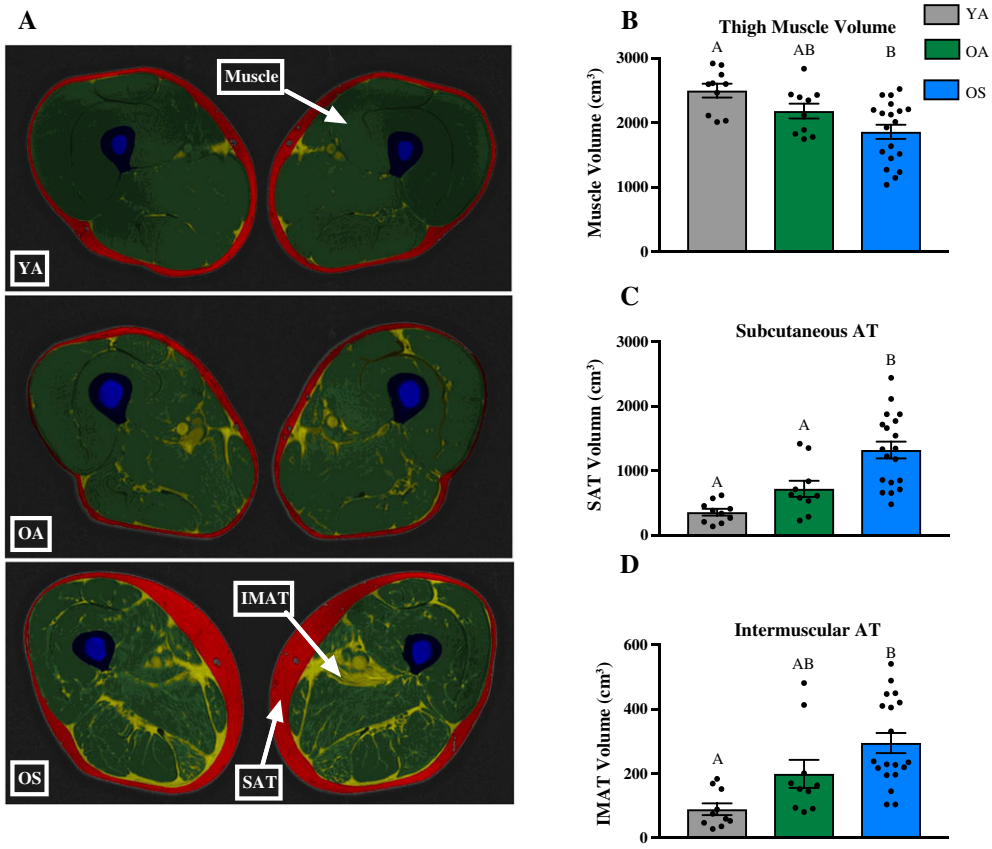
cardiovascular fitness and greater BMI compared to the active groups indicating deconditioning and increased adiposity typically associated with a sedentary lifestyle. Weight and sex ratios were similar between groups. The results of the DXA and MRI scans and representative mid-thigh MR images are presented in Table 1 and Figure 1, respectively. Whole body fat mass, and mid-thigh SAT and IMAT volumes were greater in the OS group, indicating greater adiposity. Whole body lean mass was lower in the OS group, although the difference was not statistically significant (P = 0.065, Table 1). Left mid-thigh lean mass (Table 1) and thigh muscle volume were significantly lower in the OS group when compared to the YA group (Figure 1). Skeletal muscle index was similar between groups and above sex-specific population based cut-offs indicating the absence of low muscle mass and thus sarcopenia.[2] Hand grip strength was lower in both older groups, compared to the YA group.

Muscle contractile performance and physical function

Left leg peak torque, total work, average power, and 1-RM were greater in the YA group compared with both older groups (Table 2 and Figure 2A). To determine if differences in leg skeletal muscle mass between groups may explain differences in muscle contractile performance, leg 1-RM was normalized to left leg lean mass (from regional DXA), to produce an index of muscle quality (Figure 2B). The YA group had significantly greater normalized 1-RM compared to the OS group. However, OA had similar normalized 1-RM to YA indicating that physical activity rescued some aspects of age-associated decline of muscle quality.

There was no difference in the SPPB score between OA and OS (11.9 vs. 11.5, $P > 0.05$). However, when the individual test results were considered, group differences were

Figure 1 Mid-thigh muscle and adipose tissue volume. Panel A, representative mid-thigh MR images from young active (YA), old active (OA), and old sedentary (OS) participants. Skeletal muscle is coloured dark green. Subcutaneous adipose tissue (SAT) is coloured red. Intermuscular adipose tissue (IMAT) is coloured light green. Panel B, mid-thigh muscle volume was higher in the YA group when compared with OS. Physical activity partially preserved mid-thigh muscle volume in the OA group. Panel C, SAT volume was higher in the OS group when compared with YA and OA groups. Panel D, IMAT volume was higher in the OS group when compared with YA. Physical activity partially prevented IMAT accumulation in the OA group. The letters ^{A} and ^{B} denote significant differences between groups ($P < 0.05$, one-way analysis of variance followed by post-hoc Tukey test). Data presented are mean ± standard error of the mean.



DOI: 10.1002/jcsm.12272

Table 2 Muscle contractile performance by isokinetic dynamometry

	YA (n = 10)	OA (n = 10)	OS (n = 19)	ANOVA P-value
BIODEX (Left leg, 120°/s)				
Peak torque (Nm)	114 ± 11.2 [a]	99 ± 8.4 [b]	87 ± 5.6 [b]	<0.0001
Total work (W)	929 ± 79 [a]	594.4 ± 50 [b]	532 ± 38 [b]	<0.0001
Average power (J)	195 ± 16 [a]	128 ± 10 [b]	111 ± 8.6 [b]	<0.0001
1 repetition maximum (Kg)	51 ± 4.6 [a]	36 ± 3.4 [b]	26 ± 2.4 [b]	<0.0001

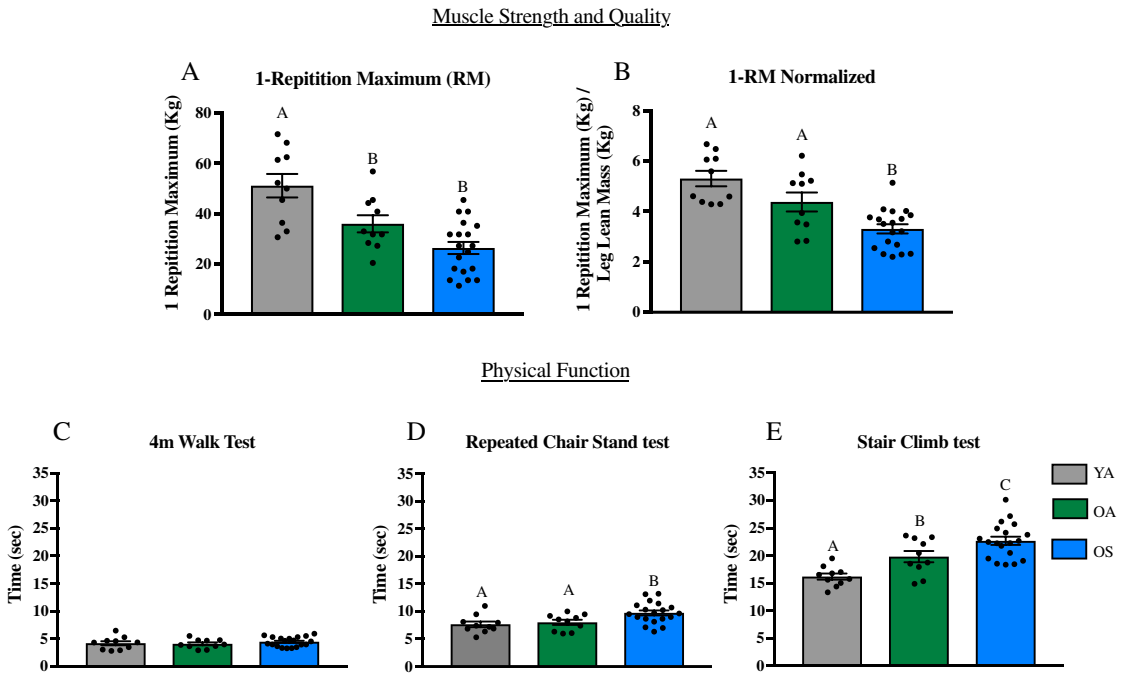
Mean ± standard deviation for all subjects. ANOVA, analysis of variance; Nm, newton meters; W, watt; J, joule; OA, old Active; OS, old sedentary; YA, young Active.
[a], [b]indicate significant differences between groups ($P < 0.05$).

apparent. The results are presented in Figure 2. Interestingly, the duration of the test seemed to differentiate physical function between the three groups. There was no difference between groups for the time taken to walk 4 m (Figure 2C). Repeated chair stand took longer to complete for the OS group, when compared to the active groups (Figure 2D). Finally, time to complete the stair climb test was significantly different between all three groups, with the OS group having the poorest performance (Figure 2E).

Skeletal muscle mitochondrial energetics are affected by physical activity and not chronological age

We employed ^{31}P MRS to examine *in vivo* mitochondrial energetics. This approach assesses maximal capacity of the mitochondria to generate ATP (ATP_{max}) following muscle contraction and integrates all aspects of mitochondrial content and function including oxygen consumption, Ca^{2+}

Figure 2 Muscle contractile performance and physical function. Panel A, left leg 1-repitition maximum (1-RM) was lower in the old active (OA) and old sedentary (OS) groups, compared with young active (YA). Panel B, muscle quality (1-RM/left leg lean mass) was similar between YA and OA groups and lower in the OS group. Physical function was assessed via 4-m walk test (from short physical performance battery), repeated chair stand (from short physical performance battery), and stair climb test. Panel C, there was no difference between groups in time to complete 4-m walk test. Panel D, time to complete the repeated chair stand test was higher in the OS group when compared with the YA and OA groups. Panel E, time to complete the stair climb test was higher in the OS group when compared with YA and OA groups and higher in the OA when compared with YA. The letters [A], [B], and [C] denote significant differences between groups ($P < 0.05$, one-way analysis of variance followed by post-hoc Tukey test). Data presented are mean ± standard error of the mean.



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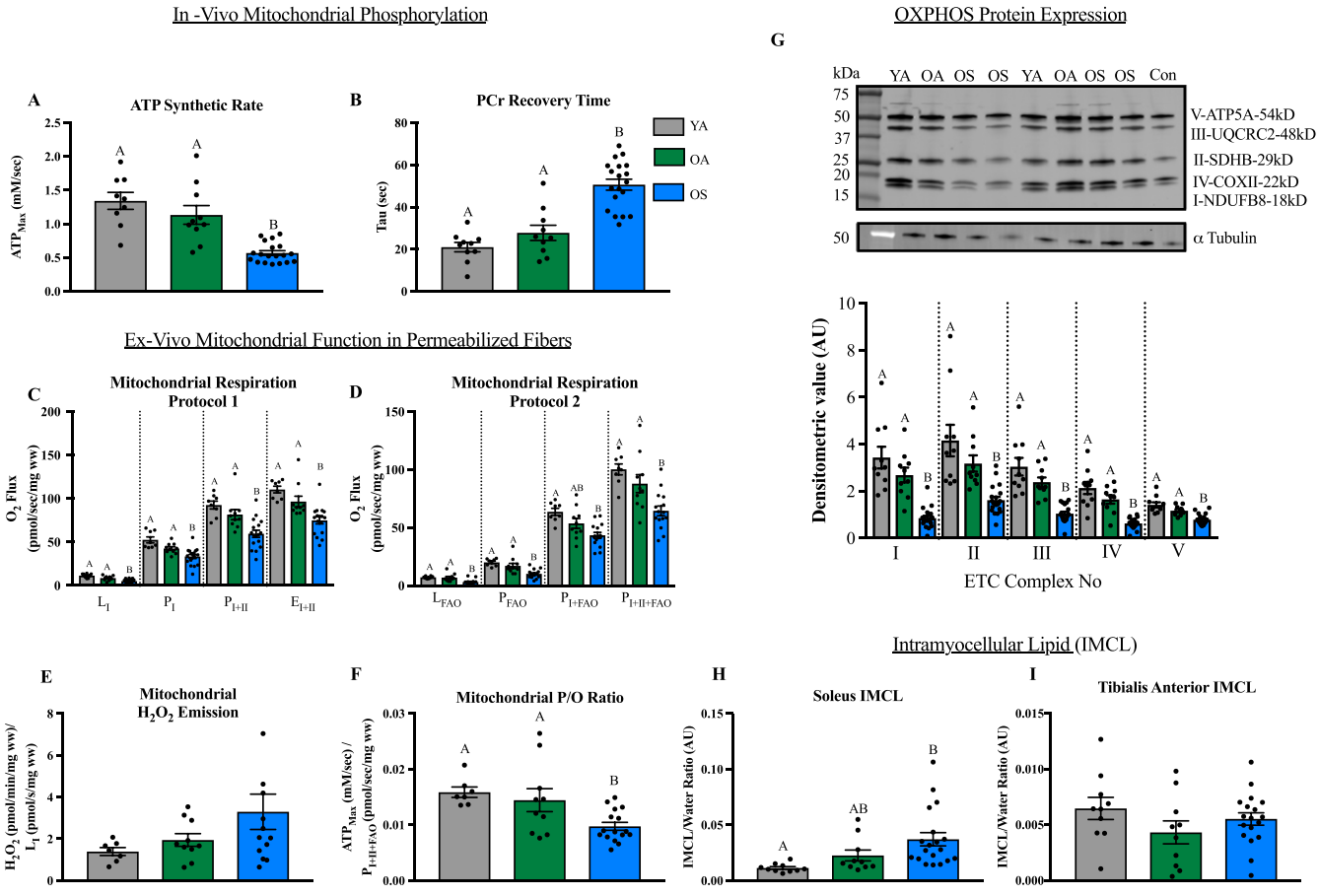
handling, and redox, all working together to produce ATP.[52] We observed that both YA and OA groups had similar maximal ATP synthetic rate and PCr recovery time following muscle contraction and that the OS group had poorer mitochondrial energetics (Figure 3A and 3B). These results indicate that maximal ATP synthesis of muscle is impacted by physical activity (or inactivity) to a greater degree than chronological age *per se*.

To complement ^{31}P MRS measurements, we employed an *ex vivo* approach to assess respiratory capacity of the electron transfer system (ETS) in muscle biopsy specimens. Measuring respiration in permeabilized muscle fibres is a widely employed approach to specifically assess mitochondrial ETS function. In the first assay protocol, which utilized carbohydrate-derived substrates, we found that OS had lower LEAK (L_I), CI and CI + II supported OXPHOS respiration (P_I and P_{I+II}, respectively), and maximal ETS capacity (E_{I+II}) compared with the OA and YA groups that were similar (Figure 3C). The second assay protocol used fatty acid (palmitoyl-carnitine) and carbohydrate-derived substrates. Again, we observed that the OS group had lower LEAK

Figure 3 Skeletal muscle mitochondrial energetics and intramyocellular lipid. Panels A and B. Maximal mitochondrial phosphorylation capacity synthetic rate (ATP_{max}) and phosphocreatine (PCr) recovery time were determined by ^{31}P magnetic resonance spectrocopy. Panel A, ATP_{max} was lower in old sedentary (OS) compared with both young active (YA) and old active (OA) groups. Panel B, PCr recovery time was significantly longer for OS compared with OA and YA groups. Panels C and D. Mitochondrial respiratory capacity measured in permeabilized myofibers by high-resolution respirometry. Panel C, OS had lower LEAK respiration (L_I, 5 mM glutamate, and 2 mM malate), adenosine diphosphate (4 mM) stimulated maximal complex I (P_I) and complex I and II (P_{I+II}, 10 mM succinate) oxidative phosphorylation (OXPHOS) respiration, and maximal electron transfer system capacity (E_{I+II}, FCCP, 2 μM steps), when compared to YA and OA groups. Panel D, OS had lower fatty acid supported LEAK respiration (L_{FAO}, 2 mM malate, and 25uM Palmitoylcarnitine), adenosine diphosphate (4 mM) stimulated maximal OXPHOS respiration supported by FAO (P_{FAO},), and complex I and II supported FAO ($P_{I+II+FAO}$, 2 mM malate, 5 mM glutamate, and 10 mM succinate), when compared with OA and YA groups. Panel E, mitochondrial H_2O_2 emission in permeabilized myofibers was not different between groups. Panel F, mitochondrial efficiency (ATP_{max}/P_{I+II} respiration; P/O ratio) was lower in the OS group when compared with YA and OA groups. Panels G, OXPHOS protein expression was lower in the OS group compared to YA and OA. Panels H and I, skeletal muscle intramyocellular lipid (IMCL) content. Panel H, *soleus* IMCL accumulation was higher in the OS when compared with YA. Physical activity partially prevented *soleus* IMCL accumulation in the OA group. Panel I, *tibialis anterior* IMCL content was similar between groups. The letters ^{A} and ^{B} denote significant differences between groups ($P < 0.05$, one-way analysis of variance and Tukey's honest significance difference post-hoc test). Data presented are mean ± standard error of the mean.



DOI: 10.1002/jcsm.12272

(L_{FAO}), and CI + II + FAO supported OXPHOS ($P_{I+ II+FAO}$) respiration (Figure 3D). Finally, the cytochrome c response for all groups was <10%, indicating maintenance of mitochondrial membrane integrity. These finding support the observation of lower ATP_{max} in the OS group and that is likely due to reduced ETS respiratory capacity. These finding clearly indicate that the aetiology of lower mitochondria energetics in ageing muscle is primarily due to low physical activity.

Mitochondria are also a source of redox signalling, an aspect of mitochondrial function that is reported to be dysfunctional with high fat feeding, obesity, and ageing. We assessed mitochondrial H_2O_2 emission in permeabilized fibre bundles via real time fluorometric monitoring of the Amplex Red reaction. We found that reactive oxygen species (ROS) emission tended to be higher in the OS group but did not reach statistical significance (Figure 3E). We also examined whether an index of mitochondrial efficiency (P/O; ATP_{max} – maximal ATP synthesis / P_{I+II} – maximal mitochondrial oxygen consumption) was different between groups. We previously reported that this index of mitochondrial efficiency was related to 400-m walk time in older adults.[10] Here, we show that older sedentary individuals have lower mitochondrial P/O ratio compared with both active groups (Figure 3F). Mitochondrial OXPHOS protein expression, one protein for each of the five electron transport chain subunits, was measured as an index of mitochondrial content (Figure 3G). We observed that both YA and OA groups had similar OXPHOS protein expression, and the OS group had lower levels of each of the five OXPHOS proteins. These data indicate that lower mitochondrial respiratory capacity and ATP_{max} were likely due to lower mitochondrial content.

Soleus intramyocellular lipid is elevated in older sedentary individuals

Mitochondrial dysfunction in ageing often occurs in parallel with accumulation of IMCL, which in turn may contribute to dysfunctional muscle metabolism. We used proton MR spectroscopy to assess IMCL in *soleus* and *tibialis anterior* muscle groups (Figure 3H and 3I). Interestingly, IMCL was elevated in the *soleus* of the OS group compared to YA, whereas *tibialis anterior* IMCL content was not different between groups. This result raises the interesting question that age and physical activity may have muscle group specific effects on IMCL, and potentially mitochondrial function, which in turn may be linked to the distinct muscle fibre compositions of these muscle groups.

Mitochondrial and exercise efficiency are related and both are reduced with physical inactivity and not chronological age

A reduction in work (or exercise) efficiency has been reported to contribute to increased cost of movement (walking and cycling) in older adults. We measured exercise efficiency during a submaximal exercise bout to examine the impact of physical activity and ageing. The group average heart rate reserve that was achieved during the exercise bout was 72% (YA), 71.5% (OA), and 68% (OS), and this equated to 61% (YA), 63% (OA), and 64% (OS) of VO_2 peak (mL/Kg/min) indicating all three groups exercised at the same relative workload. We found that both GE and NE efficiencies were similar between the active groups and lower in the OS group (Figure 4A and 4B). Interestingly, when we examined data from all subjects (n = 39), we found that mitochondrial P/O ratio was also positively related to both GE (R^2 = 0.22, P = 0.01) and NE (R^2 = 0.13, P = 0.049), indicating a link between mitochondrial efficiency in muscle and whole body exercise efficiency. In addition, PCr recovery time following contraction was negatively related to GE (R^2 = 0.22, P = 0.01) and NE (R^2 = 0.13, P = 0.049), suggesting that mitochondrial ATP synthetic capacity is related to exercise efficiency.

Figure 4 Submaximal exercise efficiency. The old sedentary (OS) group had lower gross (Panel A) and net (Panel B) exercise efficiency compared to the active groups. The letters [A] and [B] denote significant differences between groups ($P < 0.05$, one-way analysis of variance and Tukey's honest significance difference post-hoc test). Data presented are mean ± standard error of the mean.

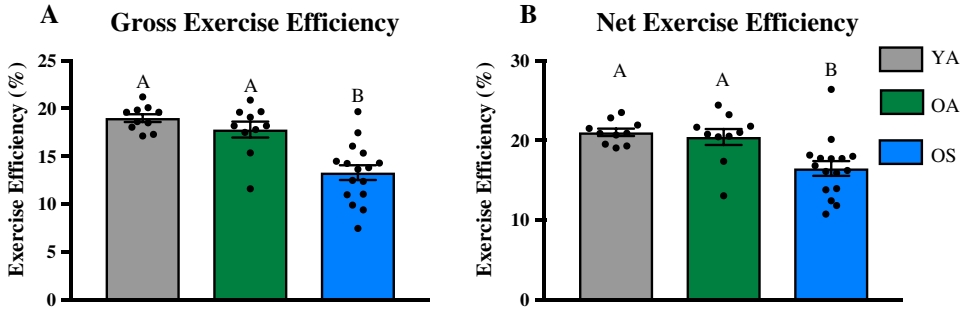


Table 3 Bivariate analysis to explore the relationships between energetics, muscle quality, and physical function

Measurement	Variables	1-RM (Kg)/(kg)		Gait speed (m/s)		Stair climb test (s)		Repeated chair stand (s)	
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Mitochondrial energetics	†ATP_{max} (mM/s)	0.457	**0.016***	0.303	0.133	−0.046	0.818	−0.188	0.358
	τ (s)	−0.334	**0.088**	−0.236	0.246	0.131	0.516	0.210	0.304
	Maximal coupled respiration (pmol/s/mg ww)	−0.016	0.939	−0.182	0.395	−0.192	0.358	−0.054	0.803
	†H_2O_2 (pmol/min/mg dw)	0.389	**0.054***	−0.076	0.724	−0.329	0.108	−0.271	0.200
	P/O ratio [(mM/s)/(pmol/s/mg ww)]	0.545	**0.007***	0.263	0.226	0.276	0.202	0.114	0.606
Muscle lipid content	†IMAT volume (cm^3)	−0.487	**0.010***	−0.372	**0.061**	0.253	0.203	0.348	**0.081**
	†*Soleus* IMCL (AU)	−0.530	**0.004***	−0.594	**0.001***	0.345	**0.078**	0.388	**0.050***
Cardiovascular fitness	†VO_2 peak (L/min)	0.165	0.410	0.235	0.248	−0.338	**0.085**	−0.362	**0.069**
Physical activity	Steps per day (steps/24 h)	0.294	0.154	0.398	**0.054***	−0.351	**0.085**	−0.301	0.153
Exercise efficiency	Gross efficiency (%)	0.411	**0.046***	0.244	0.251	−0.239	0.260	−0.322	0.126
	Net efficiency (%)	0.340	0.104	0.188	0.379	−0.211	0.323	−0.244	0.251

Data are from the old active and old sedentary groups only (total $n = 29$). Correlations are adjusted for age and sex. ATP_{max}, maximal mitochondrial phosphorylation capacity; IMAT, intermuscular adipose tissue; IMCL, intramyocellular lipid; RM, repetition maximum.
*indicates statistically significant partial correlation ($P < 0.05$) and bold *P*-values indicate $P < 0.10$.
†indicate variables that were log transformed.

Mitochondrial energetics and IMCL relate to muscle quality and physical function in older adults

To further explore the relationship between mitochondrial energetics, muscle quality, and physical function, we conducted partial correlations while controlling for the effects of age and sex in the OA and OS groups only (Table 3, $n = 29$). We found that muscle quality (1-RM/leg lean mass) was independently explained by mitochondrial energetics (ATP_{max}, τ, respiration, and P/O ratio) and muscle lipid content (IMAT, *soleus* IMCL) variables. Interestingly, *soleus* IMCL content was also associated with gait speed, stair climb test, and repeated chair stand time.

We next constructed multiple regression models to more deeply explore how combinations of variables explain variation in muscle quality and physical function (Table 4 and Figure 5). Although including age and sex as covariates, we found that ATP_{max} and *soleus* IMCL explained 60.6% of the variation in muscle quality. *Soleus* IMCL was the single strongest predictor of gait speed explaining 30.3% of variation. Finally, 54.6% of the variation in time to complete stair climb test was explained by a combination of ATP_{max}, leg lean mass, and VO_2peak.

Discussion

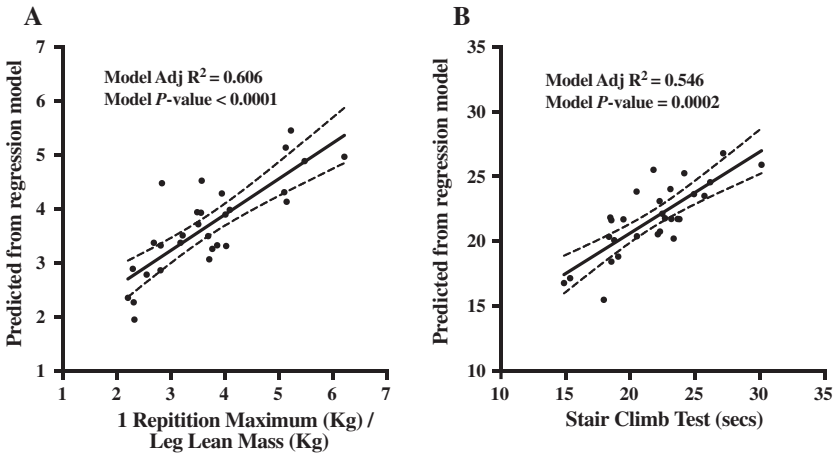
The paradigm of age-associated mitochondrial dysfunction in human skeletal muscle continues to generate considerable debate. In addition, the clinical relevance of mitochondrial dysfunction in ageing has not been clearly defined. A deeper understanding is critical if mitochondria are to be a feasible therapeutic target for age-related conditions including sarcopenia and loss of physical function. We rationalized that studying well-phenotyped older cohorts across a wide range of physical activity, principally endurance exercise, and comparing with a YA group would unveil a

Table 4 Multiple regression to explore sources of variation in muscle quality, and physical function

Parameters	1-RM (kg/kg)		Gait speed (m/s)		Stair climb test (s)	
Adj R^2 (Model *P*)	0.606 (<0.0001)		0.303 (0.0083)		0.546 (0.0002)	
	β	*P*	β	*P*	β	*P*
†Age (years)	−0.336	0.017*	0.09	0.596	−0.007	0.963
Sex	−0.465	0.004*	0.258	0.153	−0.037	0.864
†ATP_{max} (mM/s)	0.319	0.03*	—	—	0.656	0.003*
†*Soleus* IMCL (AU)	−0.349	0.008*	−0.581	0.001*	—	—
Leg lean mass (Kg)	—	—	—	—	1.276	<0.001*
†VO_2peak (L/min)	—	—	—	—	−1.746	<0.001*

Data for multiple regression models are from the old active and old sedentary groups only (total $n = 29$). ATP_{max}, maximal mitochondrial phosphorylation capacity; IMCL, intramyocellular lipid; RM, repetition maximum.
*indicate statistically significant contribution to the multiple regression model ($P < 0.05$).
†indicate variables that were log transformed.

Figure 5 Scatter plots depicting actual measured versus predicted normalized 1 repetition maximum (A) and time taken to complete stair climb test (B). Data are from multiple regression models in Table 4 and are from the old active and old sedentary groups only (total *n* = 29). For (A): age, sex, maximal adenosine triphosphate synthetic rate, and *soleus* IMCL significantly contributed to the model. For (B), maximal adenosine triphosphate synthetic rate, leg lean mass, and VO_2peak significantly contributed to the model. Best-fit trend line and 95% confidence intervals are included.



range of mitochondrial function in skeletal muscle. This, in turn, allowed us to more clearly examine the impact of age *per se* on mitochondrial energetics and the relationships between mitochondrial energetics with clinically relevant assessments of physical function. Indeed, the OS group steps/day average was below the 5,000 step threshold for sedentary behaviour for healthy adults.[53] Here, we show that physical activity largely mitigates the negative effects of a sedentary lifestyle on mitochondrial energetics, exercise efficiency, and certain aspects of muscle and physical function in older adults. Furthermore, novel evidence obtained at the myocellular and whole-body level complements and extends recent findings by St-Jean-Pelletier *et al*. and indicate that mitochondrial energetics is a determinant of exercise efficiency, muscle quality, and physical function.[33]

The influence of chronological age and physical activity on physical function and muscle quality

Age-associated loss in the capacity for mobility is an increasingly important focus in the field of gerontology. Although the consensus is that the aetiology of mobility loss is multifactorial, much work remains to identify specific key physiological factors that underlie mobility loss with ageing. Among the physical function tests used, we found that the longer duration of the stair climb test effectively separated the three groups, whereas the short duration 4-m walk test did not. These results are in line with the observations of Choi *et al*. and suggest that maintaining physical activity into old age, and as a result cardiovascular fitness and muscle bioenergetics, preserves physical function in longer more difficult tests of physical function.[11] Interestingly, age-associated loss of muscle strength (1-RM) and muscle contractile performance assessed by isokinetic dynamometry was evident, despite a physically active lifestyle in the OA group. However, in our study, physical activity did seem to partially preserve muscle quality (strength per muscle mass). The lack of preservation of strength with endurance type physical activity may be due to a relatively lower engagement of type II fibres during endurance exercise (running, swimming, and cycling) compared with type I fibres that, in turn, may not be sufficient to protect against age-associated preferential atrophy of type II fibres.[54] In this respect, our data support the notion that resistance exercise may be needed to effectively offset loss of muscle strength with ageing.

Physical activity, not chronological age, influences mitochondrial energetics of muscle

A key finding was that mitochondrial energetics of skeletal muscle was greatly influenced by physical activity status and not chronological age. There are numerous reports of decrements in mitochondrial oxidative capacity with ageing.[15–20] However, it is important to note that many of these investigations do not characterize important aspects of participant phenotype that likely influence the relationship between age and mitochondrial function, including objectively measured daily physical activity level and cardiorespiratory fitness.[17,18] Many have also used isolated mitochondria,[15,18,20] an experimental approach that may confound assays of mitochondrial function in ageing.[55]

In contrast to the aforementioned studies, others who control for participant phenotype have failed to find age-related changes in mitochondrial respiration in permeabilized myofibers.[21–23,25,56] Our study is the first to comprehensively interrogate mitochondrial energetics (*in vivo* and *ex vivo*) in YA and older groups who are well-phenotyped in terms of objectively measured physical activity, cardiovascular fitness,

and body composition. Our results are in line with two recent reports showing that physically active young and old groups have similar protein markers of mitochondrial content and respiration, data that underscore the importance of considering physical activity when studying mitochondrial function in ageing.[21,33] Our findings also extend this work and suggest that lower ATP synthesis capacity in the OS group is likely due to reduced ETS respiratory capacity. We also assessed mitochondrial H_2O_2 emission in permeabilized fibre bundles and found that ROS emission tended to be higher in the OS group but did not reach statistical significance. However, this is interesting to consider along with the observation that mitochondrial P/O ratio is reduced in older sedentary adults. Both observations are in line with the "uncoupling to survive" theory, which posits that mitochondria become more uncoupled with age, and in this case, sedentary ageing, to prevent the deleterious effect of elevated ROS emission.[57] Mitochondrial OXPHOS protein expression was similar between YA and OA groups and was lower in the OS group, indicating that lower mitochondrial respiratory capacity, and ATP_{max} are likely due to lower mitochondrial content, although the role of altered post translational modifications (lysine acetylation) of protein, cardiolipin profile, and cristae structure cannot be ruled out.

Taken together, the data clearly indicate that reduced physical activity, cardiovascular fitness, and increased adiposity largely explain reductions in mitochondrial energetics in skeletal muscle from older adults.[33] Interestingly, VO_2peak was lower in OA compared to YA, despite similar muscle mitochondrial energetics. This suggests a detrimental ageing effect on other physiological determinants of VO_2peak, potentially cardiac output or muscle perfusion[58] that is not completely abrogated by aerobic physical activity.

Impact of chronological age and physical activity on intramyocellular lipid

Elevated IMCL has been reported in *vastus lateralis* of older adults and can occur along with mitochondrial dysfunction and insulin resistance.[32,33] We extend these findings to show that IMCL is elevated in *soleus* but not *tibialis anterior* muscle of OS compared with YA participants. It is not apparent why IMCL accumulation might occur specifically in *soleus* vs. *tibialis anterior*. However, *soleus* has a higher proportion of oxidative type I fibres[59] compared with the *tibialis anterior*,[60] and it has been shown that ectopic lipid accumulates to a greater extent in type I fibres of *vastus lateralis* compared with type II fibres in the context of obesity,[61,62] sedentary ageing,[33] and ageing with metabolic syndrome.[63] Studies in diaphragm muscle indicate that mitochondrial respiratory chain dysfunction can precede lipid droplet accumulation in individual muscle fibres during inactivity induced by mechanical ventilation.[64] In addition, type I fibre IMCL accumulation associates with poor contractile function.[62] Evidence in rodents confirm that *soleus* is particularly susceptible to the negative effects of physical inactivity (immobilization and hind limb unloading), on mitochondrial dysfunction, insulin resistance, and loss of contractile function. Interestingly, several studies have shown preserved *in vivo* mitochondrial function in the *tibialis anterior* muscle of older adults compared to young, when physical activity is controlled.[65–67] In contrast, age-related declines in oxidative capacity of plantar flexor muscles (including *soleus*) have been described using *in vitro* and *in vivo* approaches.[68–70] The mechanisms underlying muscle specific effects are not clear but may include age-related changes in patterns of muscle use,[71,72] as well as fibre type specific susceptibly to age-related changes in mitochondrial function.[27] These factors could in turn could stave off age-associated IMCL accumulation in the *tibialis anterior*. Taken together, these reports suggest that *soleus* may be more susceptible to IMCL accumulation with sedentary behaviour in ageing and raises the interesting question that age and physical activity may have muscle group specific effects on IMCL content. Whereas others have reported an association between IMCL and contractile function and have speculated that IMCL may increase internal resistance within the myofibril,[62] further studies are needed to understand the nature of the association between IMCL, muscle, and physical function.

Impact of chronological age and physical activity on exercise efficiency and mitochondrial P/O ratio

Ageing is also associated reduced exercise efficiency, defined as an increase in the energy expenditure of exercise, which likely contribute to impairments in physical activity.[35–37] Exercise efficiency can be improved with endurance training in both young[73] and older adults.[14,37] Alterations in contractile coupling is the conventional explanation for differences in exercise efficiency,[74] although a role for mitochondria in exercise efficiency has been previously suggested.[75] Others have shown that dietary nitrite improves mitochondrial and exercise efficiency during exercise[76,77] and that markers of mitochondrial content and function,[14] and the proportion of type I fibres[73] associate with exercise efficiency, all evidence suggesting that mitochondria play a role in mediation of exercise efficiency. In addition, our group previously reported that P/O ratio, calculated as ATP_{max}/State 3 respiration (P_{I+II}), was related to 400-m walk time in older adults.[10] Here, we extend those findings to show that mitochondrial P/O ratio is reduced in older sedentary adults when compared to young and old physically active groups, evidence indicating that reduced physical activity contributes to reduced mitochondrial efficiency in skeletal muscle from older adults. In addition, P/O ratio was related to both NE and GE during submaximal exercise, data that for the first time suggest that skeletal muscle mitochondrial efficiency is linked with whole body exercise efficiency in older adults.

Alterations in mitochondrial efficiency of ATP production may be caused by altered inner mitochondrial membrane proton leak (uncoupling), possibly in response to greater ROS emission, or by reduced electron leak from the electron transport chain. Others have shown that mitochondrial uncoupling occurs in ageing[78,79]; however, further investigations into molecular mechanisms of mitochondrial uncoupling are needed to further understand the relationship with reduced exercise efficiency and likely the capacity for mobility in older adults.

Mitochondrial energetics associate with muscle quality and physical function

Loss of muscle strength and quality underlie functional decline and predict mobility limitations in older adults.[80,81] A recent report from Zane *et al*., showed that, in 326 participants from the Baltimore Longitudinal Study of Aging, mitochondrial phosphorylation capacity corresponds with muscle strength and quality in older adults.[12] Here, with a smaller cohort of older adults (n = 29) but with a wide range of physical activity level, we further this observation to unveil that multiple aspects of mitochondrial energetics (ATP_{max}, P/O ratio, and H_2O_2 emission) and muscle lipid content (IMAT and IMCL) strongly associate with muscle quality, supporting the hypothesis that they are likely important biological factors in loss of muscle quality with ageing. In addition, multiple regression modelling showed that the combination of age, sex, ATP_{max}, and IMCL together explained 60.6% of the variation of muscle quality in older adults.

These data are supported by preclinical evidence showing that mitochondrial reactive oxygen species can affect muscle mass by depressing protein synthesis[82–84] and impairing postprandial AKT phosphorylation, a major driver of the mammalian target of rapamycin pathway and protein synthesis.[49] Genetic models of elevated mitochondrial ROS show a premature ageing phenotype including loss of muscle mass and contractile properties.[85] Interestingly, *soleus* IMCL was significantly associated with many of the muscle and physical function measurements. In line with this observation, others have shown that IMCLs are linked with slower myofiber contraction, force, and power development in obese older adults,[62] and specific lipid species may mediate fatigue and weakness.[86] We also found that the combination of ATP_{max}, VO_2peak, and leg lean mass explained 54% of the variation in time taken to complete the repeated chair stand. These data support an emerging paradigm that cardiovascular and muscle bioenergetics play an important role in the aetiology of mobility loss with ageing,[10–12] thus, should be considered as a feasible therapeutic target to delay age-associated loss of muscle function and sarcopenia.

Our study has a few potential limitations that should be noted. We did not characterize all potential covariates that may influence the relationship between age and mitochondrial capacity and efficiency, including muscle fibre type. Also, although our study groups were balanced for sex ratio, we did not have enough statistical power to adequately investigate sex differences in ageing, physical activity, and mitochondrial energetics, likely an important biological factor. Thus, larger studies are needed to generate the next level of evidence that mitochondrial energetics are linked with physical function in men and women specifically. In addition, this study included few very low functioning people. Inclusion of more very low functioning older adults may have further strengthened the observed relationships between muscle mitochondrial capacity/efficiency and physical function.

In summary, this study complements and extends recent findings by St-Jean-Pelletier *et al*.,[33] and provides a number of novel and clinically relevant observations in well-phenotyped young and older adults, including (i) the variation in physical activity (predominantly endurance exercise), largely explains 'age-associated' deficits in mitochondrial energetics and efficiency in older adults, (ii) mitochondrial ATP_{max} and efficiency in skeletal muscle are linked with exercise efficiency, and (iii) skeletal muscle ATP_{max} and IMCL are strongly associated with muscle contractile function and physical function in older adults.

Authors contributions

GD, RAS, XZ, EAC, HHC, and PMC contributed to study execution, researched the data, and reviewed and approved the manuscript. FY and GD assisted with statistical analysis. GD contributed to data interpretation and writing the manuscript. All co-authors reviewed and approved the manuscript. PMC contributed to the study concept and design, statistical analysis, interpretation of the data, and wrote the manuscript. PMC is the guarantor of the data.

Conflict of interest

No potential conflicts of interest relevant to this article were reported. All authors declare that the submitted work has not been published before (neither in English nor in any other language) and that the work is not under consideration for publication elsewhere. A subset of this data was presented as a short talk at the Myology Institute, Muscle Biology Conference held on 8–10 March 2017 at the University of Florida, Gainesville, FL, USA, and the Cell Symposia on Exercise Metabolism held on 21–23 May in Gothenburg, Sweden.

Acknowledgements

The authors gratefully appreciate the contributions of our study participants and acknowledge the excellent technical assistance of the imaging, recruitment, clinic, calorimetry,

laboratory, and nutrition core staff at TRI-MD, Florida Hospital. Medical oversight, for this study, was provided by Richard E. Pratley, MD and Christian Meyer, MD. This study was supported by funding from the National Institutes of Health | National Institute on Aging (K01 AG044437) awarded to PMC. The authors certify that they comply with the ethical guidelines for authorship and publishing of the *Journal of Cachexia, Sarcopenia and Muscle*.[87]

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