Giovanna Distefano[1],*, Robert A. Standley[1],*, Xiaolei Zhang[1], Elvis A. Carnero[1], Fanchao Yi, Heather H. Cornnell[1], Paul M. Coen[1,2]

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

Author affiliations:

[1]Translational Research Institute for Metabolism and Diabetes, Florida Hospital, 301 East Princeton Street, Orlando, FL 32804.

[2] Sanford Burnham Prebys Medical Discovery Institute at Lake Nona, 6400 Sanger Rd, Orlando, FL 32827.

*co-first authors

Corresponding author:

Paul M. Coen, Ph.D.

Paul.Coen@flhosp.org

Phone: (407)303-1306

Fax: (407)303-7199

ACKNOWLEDGEMENTS

ABSTRACT (word count:340/400)

Background: The concept of mitochondrial dysfunction in aging muscle is highly controversial. In addition, emerging evidence suggests that reduced muscle oxidative capacity and efficiency underlie the etiology 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=2F/8M, age=31.2 ± 5.4 yrs.). Older Active (OA, n=2F/8M, age=67.5 ± 2.7 yrs.) and Older Sedentary (OS, n=8F/11M, age=70.7 ± 4.7 yrs.). Participants completed a graded exercise test to determine fitness (VO$_2$peak), a submaximal exercise test to determine exercise efficiency, and daily physical activity (PA) was recorded using a tri-axial armband accelerometer. Mitochondrial energetics were determined by 1) ^{31}P magnetic resonance spectroscopy and 2) respirometry of fiber bundles from vastus lateralis biopsies. Quadriceps function was assessed by isokinetic dynamometry and physical function by the short physical performance battery (SPPB) and stair climb test.

Results: Daily PA energy expenditure was significantly lower in OS, compared to YA and OA groups. Despite fitness being higher in YA compared to OA and OS, mitochondrial respiration, ATP$_{Max}$, 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 was significantly greater for OS. Interestingly, ATP$_{Max}$ 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 aging associated conditions including sarcopenia, dynapenia and loss of physical function.

Keywords: Aging, cardiovascular fitness, skeletal muscle, mitochondria, physical function

INTRODUCTION

The progressive loss of muscle mass, strength and physical function that occurs with aging 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 United States, sarcopenia is poised 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 etiology 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 ICD-10-CM code designation [5].

Physical function is a key aspect of contemporary clinical definitions of sarcopenia [2], and requires the integration of multiple physiological systems, including cardiopulmonary, vestibular, sensory and muscular. A decline in skeletal muscle mitochondrial capacity also characteristic of aging and has recently been studied as an underlying factor for low physical function [6], slower walking speed [7-9], fatigability [10], and loss of exercise efficiency with aging [11]. However, it should be noted that there is still considerable debate regarding the true nature of aging-associated mitochondrial dysfunction. While many studies have supported an age-related decrease in mitochondrial capacity [12-17], several others have failed to find these associations [18-22]. 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 [15], cardiovascular fitness, and adiposity [17], all of which likely confound the relationship between mitochondrial capacity and age [16, 23, 19, 24, 25]. In addition, the various analytical approaches employed to assess mitochondrial function [26], including the use of isolated mitochondria [12, 15, 17] which has been shown to exaggerate the observed deficit in mitochondrial function in aging [27], may contribute to the lack of clarity. Impaired mitochondrial function is also linked with elevated intramyocellular lipids (IMCL) in aging [28] and which have been recently linked to the pre-frail phenotype [29]. 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 aging 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 [30]. This in turn could impede activities of daily living in older adults [31, 32], potentially contributing to future risk for mobility disability. While the underlying cause of low exercise efficiency with aging is unknown, mitochondrial energetics may be involved [11, 33]. 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 PA 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 (±4.5kg in preceding 6 months), had a BMI between 20-35 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 (≤150mmHg systolic, ≤ 90mmHg diastolic). A note from the participant's PCP/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 (21-40 yrs.) Active (YA), Older Active (OA) and Older Sedentary (OS, 65-90 yrs.). Participants were considered active if they engaged in aerobic exercise (running, cycling, swimming) at least 3 days a week without extensive layoff 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, resting 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, 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, 50% carbohydrate) and 15 minutes later the muscle biopsy procedure was conducted. Participants then completed the acute exercise bout on a cycle ergometer, all as described below.

Daily physical activity. The monitor used for this study was the SenseWear® Pro Armband (BodyMedia Inc., Pittsburgh, PA). This activity monitor uses 3-axis accelerometer, galvanic skin response and skin temperature to estimate energy expenditure at a one-minute resolution [34]. The SenseWear® Armband has been demonstrated to be a valid, accurate and reliable method when compared to indirect calorimetry [34], and doubly labeled water [35]. 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 [7, 36]. 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 [37].

Assessment of body composition. Dual energy X-ray absorptiometry (DXA) scans were performed using a GE Lunar iDXA whole-body scanner and analyzed with enCORE software. Coefficients of variation (CV) vary by tissue type and range from 0.5% to 1.1%. Magnetic resonance imaging (MRI) was performed on a 3T Philips Acheiva magnet and was used to assess of intermuscular (IMAT) and subcutaneous fat (SAT). Volumetric measurement of fat was performed under standardized conditions with subjects in a supine position. T1 weighted imaging sequences were performed across the thighs. The resultant images were analyzed 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 3T Philips Acheiva magnet. Assessment of Intramyocellular lipids (IMCL) was by proton (^{1}H) spectroscopy using multiple single voxel acquisitions. Ratios of IMCL to water were determined using the AMARES (Advances Method for Accurate, Robust and Efficient Spectral fitting) algorithm within the 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 ATP_{Max} was performed as previously described [38, 39].

The Phosphocreatine (PCr) recovery time constant (tau) and the PCr level in oxygenated muscle at rest

$(PCr)_{rest}$ were used to calculate maximum mitochondrial capacity (ATP_{Max}). After the fully relaxed

spectrum (TR 15s, TE 0.17s, NSA 48) was obtained, the ATP_{Max} experiment was performed by obtaining

^{31}P spectra, with one summed spectrum acquired every 6 seconds 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 seconds. After kicking stopped, the volunteer remained still for an additional ~8

minutes to allow the PCr peak to return to baseline. The entire ATP_{Max} acquisition took 10 minutes. The

experimental spectra (6sec each) consisted of 4 (NSA) partially saturated free induction decays (TR =

1.5s, TE = 0.13s). The areas/heights of the peaks in each ^{31}P MRS spectrum during the experiment were

determined using the AMARES (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 Pi chemical shifts and to calculate tau [40]. The CV for the ATP_{Max} measured in the

same participant in one 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 1 repetition maximum (1-RM), where the starting weight was 90% and 60% of body weight for

males and females, 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 [41]. 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 hours prior to the biopsy procedure. Prior to the biopsy resting metabolic rate was determined while resting in the supine position over 30 mins 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 anesthesia (2% buffered lidocaine) as described previously [42]. A portion of the biopsy specimen was placed in ice-cold preservation buffer (BIOPS) for analysis of mitochondrial respiration [7].

Submaximal exercise test. Participants performed a 6-minute warm-up consisting of light cycling on an electronically braked cycle ergometer. After the warm-up, subjects cycled for 40 minutes at approximately 70% of heart rate reserve (HRR) which was calculated as HRmax during the VO_2peak test minus supine resting HR. Heart rate, perceived exertion and blood pressure data were collected every 5 mins 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 minutes of starting the test, and to calculate exercise efficiency parameters.

High-resolution respirometry and ROS emission. Permeabilized myofiber bundles (~1-3mg each) were prepared immediately after the biopsy procedure, as previously described [43]. Briefly, individual fibers were gently teased apart in a petri dish containing ice-cold BIOPS media. The fiber bundles were then permeabilized with saponin (2ml of 50µg/ml) for 20 minutes and washed twice (10 minutes each) in Buffer Z (105mM K-MES, 30mM KCl, 10mM KH_2PO4, $5mMMgCl_2$-$6H_2O$, 5mg/ml BSA, 1mM EGTA, pH7.4 with KOH). Mitochondrial respiration was evaluated by high-resolution respirometry (Oxygraph-2k, 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 (25µM).

In protocol 1, complex I supported LEAK (L_I) respiration was determined through the addition of malate (2mM) and glutamate (5mM). ADP (4mM) was added to elicit complex I supported oxidative phosphorylation (OXPHOS) (P_I) respiration. Succinate (10mM) was then added to elicit complex I and II supported OXPHOS (P_{I+II}) respiration. Cytochrome c (10μM) was added to assess the integrity of the outer mitochondrial membrane. Finally, a titer of FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone) (2μM steps) was performed to determine the maximal electron transfer system (E_{I+II}) capacity or maximal uncoupled respiration. In protocol 2, FAO supported LEAK (FAO_L) respiration was determined through the addition of palmitoylcarnitine (25μM) and malate (2mM). ADP (4mM) was added to elicit FAO supported OXPHOS (FAO_P). Cytochrome c (10μM) was added to access mitochondrial integrity. Glutamate (5mM) was then added to elicit complex I and FAO_P ($CI_\&FAO_P$) respiration. Succinate was added (10mM) to stimulate complex I and II and FAO_P ($CI+II_\&FAO_P$). Steady state O_2 flux for each respiratory state was determined and normalized to myofiber bundle wet weight using Datlab 5 software (Oroboros Instruments, Innsbruck, Austria).

Measurement of H_2O_2 emission by mitochondria was measured in from permeabilized muscle fiber 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 [44]. 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 [45]. Energy expenditure during steady-state exercise (20 to 40 minutes) was calculated using the Brouwer equation as previously described [46]. 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$_{(kcal/min)}$-REE$_{(kcal/min)}$)*100.

Statistics. Group differences were determined using a one-way ANOVA with a Tukey's post hoc test for individual group comparisons. The distribution of sex and race across groups were determined by Chi-

squared test. To measure associations between variables while adjusting for age and gender, the partial

correlation analyses were conducted. The 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: Young Active (YA, n=10), Old Active (OA, n=10), or Old Sedentary (OS, n=19). The characteristics of the study groups are shown in table 1. By study design, the OS group had lower objectively measured daily energy expenditure (EE), activity EE and steps per day, compared to the active groups. The OS group had lower cardiovascular fitness and greater BMI compared to the active groups indicating deconditioning and increased adiposity typically associated with a sedentary lifestyle. Weight and sex ratio 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. While whole body lean mass was similar between groups (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 (SMI) was similar between groups and above sex-specific population based cutoffs 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-repitition maximum were greater in the YA group compared to 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 PA 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 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 4m (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 mitochondrial to generate ATP (ATPmax) following muscle contraction and integrates all aspects of mitochondrial content and function including oxygen consumption, Ca^{2+} handling and redox, all working together to produce ATP [47]. 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 2A&B). 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 fibers 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 to the OA and YA groups which 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 (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 etiology of lower mitochondria energetics in aging muscle primarily due to low physical activity.

Mitochondria are also a source of redox signaling, an aspect of mitochondrial function that is reported to be dysfunctional with high fat feeding, obesity and aging. We assessed mitochondrial H_2O_2 emission in permeabilized fiber bundles via real time fluorometric monitoring of the Amplex Red reaction. We found that 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 / PI+II – maximal mitochondrial oxygen consumption) was different between groups. We previously reported that this index of mitochondrial efficiency was related to 400m walk time in older adults [7]. Here we show that older sedentary individuals have lower mitochondrial P/O ratio compared to both active groups (figure 3F).

Soleus intramyocellular lipid is elevated in older sedentary individuals. Mitochondrial dysfunction in aging often occurs in parallel with accumulation of intramyocellular lipid, which in turn may contribute to dysfunctional muscle metabolism. We used proton MR spectroscopy to assess intramyocellular lipid in soleus and tibialis anterior muscle groups (figure 3G&H). Interestingly, IMCL was elevated in the soleus of the OS group compared to YA, while 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 fiber 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, cycling) in older adults. We measured exercise efficiency during a submaximal exercise bout to examine the impact of physical activity and aging. The group average HRR that was achieved during the exercise bout was 72% (YA), 71.5% (OA) and 68% (OS) percent 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 gross (GE) and net (NE) efficiencies were similar between the active groups and lower in the OS group (figure 4A&B). 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.

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 (1RM/leg lean mass) was independently explained by mitochondrial energetics (ATP$_{Max}$, Tau, respiration, P/O ratio) and muscle lipid content (IMAT, soleus IMCL) variables. Interestingly, soleus IMCL content was also associated with gait speed, step test time 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). While 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 step test was explained by a combination of ATP$_{Max}$, leg lean mass and VO$_2$peak.

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 aging 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 to a young active group, would unveil a 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 5000 step threshold for sedentary behavior for healthy adults [48]. Here, we show that PA 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 indicate that mitochondrial energetics is a determinant of exercise efficiency, muscle quality and physical function.

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. While the consensus is that the etiology of mobility loss is multifactorial, much work remains to identify specific key physiological factors that underlie mobility loss with aging. 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 4m walk test did not. These results are in line with the observations of Choi et al., and suggest that maintaining PA into old age, and as a result cardiovascular fitness and muscle bioenergetics, preserves physical function in longer more difficult tests of physical function [8]. 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 PA did seem to partially preserve muscle quality (strength per muscle

mass). The lack of preservation of strength with endurance type PA may be due to a relatively lower engagement of type II fibers during endurance exercise (running, swimming, cycling) compared to type I fibers, that in turn may not be sufficient to protect against age-associated preferential atrophy of type II fibers [49]. In this respect, our data support the notion that resistance exercise may be needed to effectively offset loss of muscle strength with aging.

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 aging [12-17]. 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 [15, 14]. Many have also used isolated mitochondria [12, 15, 17], an experimental approach that may confound assays of mitochondrial function in aging [50].

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 [19, 20, 22, 51, 18]. Our study is the first to comprehensively interrogate mitochondrial energetics (in vivo and ex vivo) in active young 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 aging [18, 52]. 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 fiber 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 aging, to prevent the deleterious effect of elevated ROS emission [53].

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 [52]. Interestingly, VO$_2$peak was lower in OA compared to YA, despite similar muscle mitochondrial energetics. This suggests a detrimental aging effect on other physiological determinants of VO$_2$peak, potentially cardiac output or muscle perfusion [54], that is not completely abrogated by aerobic physical activity.

Impact of chronological age and physical activity on intramyocellular lipid.

Elevated intramyocellular lipid (IMLC) has been reported in vastus lateralis of older adults and can occur along with mitochondrial dysfunction and insulin resistance [55, 52]. We extend these findings to show that IMCL is elevated in soleus but not tibialis anterior muscle of OS compared to YA participants. It is not apparent why IMCL accumulation might occur specifically in soleus verses TA. However, soleus has a higher proportion of oxidative type I fibers [56] compared to the TA [57] and it has been shown that ectopic lipid accumulates to a greater extent in type I fibers of vastus lateralis compared to type II fibers in the context of obesity [58, 59], sedentary aging [52] and aging with metabolic syndrome [60]. In addition, type I fiber IMCL accumulation associates with poor contractile function [59]. Evidence in rodents confirm that soleus is particularly susceptible to the negative effects of physical inactivity (immobilization, hind limb unloading), on mitochondrial dysfunction, insulin resistance and loss of contractile function. Interestingly, some reports indicate that that the oxidative capacity of the TA is increased with aging, possibly to compensate changes in gait biomechanics [61, 62], which in turn could stave off age-associated IMCL accumulation. Taken together these reports suggest that soleus may be more susceptible to IMCL accumulation with sedentary behavior in aging and raises the interesting question that age and physical activity may have muscle group specific effects on IMCL content and subsequently on physical function.

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

Aging is also associated reduced exercise efficiency, defined as an increase in the energy expenditure of exercise, which likely contribute to impairments in physical activity [32, 31, 30]. Exercise efficiency can be improved with endurance training in both young [63] and older adults [32, 11]. Alterations in contractile coupling is the conventional explanation for differences in exercise efficiency [64], although a role for mitochondria in exercise efficiency has been previously suggested [65]. Others have shown that dietary nitrite improves mitochondrial and exercise efficiency during exercise [66, 67] and that markers of mitochondrial content and function [11], and the proportion of type I fibers [63] 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 (PI+II), was related to 400m walk time in older adults [7]. 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 which for the first time suggests 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 aging [68, 69], 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 [70, 71]. 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 [9]. Here, with a smaller cohort of older adults (n=29) but with a wide range of PA level, we further this observation to unveil that multiple aspects of mitochondrial energetics (ATPmax, P/O ratio, 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 aging. In addition, multiple regression modelling showed that the combination of age, sex, ATPmax 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 [72-74] and impairing postprandial AKT phosphorylation, a major driver of the mTOR pathway and protein synthesis [44]. Genetic models of elevated mitochondrial ROS show a premature aging phenotype including loss of muscle mass and contractile properties [75]. Interestingly, soleus IMCL was significantly associated with many of the muscle and physical function measurements. In line with this observation, other have shown that intramyocellular lipids are linked with slower myofiber contraction, force and power development in obese older adults [59] and specific lipid species may mediate fatigue and weakness [76]. We also found that the combination of ATPmax, VO2peak 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 etiology of mobility loss with aging [7-9], 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 fiber type. Also, while our study groups were balanced for sex ratio, we did not have enough statistical power to adequately investigate sex differences in aging, 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 provides a number of novel and clinically relevant observations in well phenotyped young and older adults, including; 1) the variation in physical activity (predominantly endurance exercise), largely explains "age-associated" deficits in mitochondrial energetics and efficiency in older adults, 2) mitochondrial ATPmax and efficiency in skeletal muscle are linked with exercise efficiency, 3) skeletal muscle ATPmax nad 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.

DISCLOSURE STATEMENT

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 March 8-10, 2017 at the University of Florida, Gainesville, FL, USA, and the Cell Symposia on Exercise Metabolism held on May 21-23 in Gothenburg, Sweden.

REFERENCES

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. The Journal of nutrition. 1997;127(5 Suppl):990S-1S.

2. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age and ageing. 2010;39(4):412-23. doi:10.1093/ageing/afq034.

3. Clark BC, Manini TM. Functional consequences of sarcopenia and dynapenia in the elderly. Current opinion in clinical nutrition and metabolic care. 2010;13(3):271-6. doi:10.1097/MCO.0b013e328337819e.

4. Morley JE. Pharmacologic Options for the Treatment of Sarcopenia. Calcified tissue international. 2015. doi:10.1007/s00223-015-0022-5.

5. Anker SD, Morley JE, von Haehling S. Welcome to the ICD-10 code for sarcopenia. Journal of cachexia, sarcopenia and muscle. 2016. doi:10.1002/jcsm.12147.

6. Joseph AM, Adhihetty PJ, Buford TW, Wohlgemuth SE, Lees HA, Nguyen LM et al. The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. Aging cell. 2012;11(5):801-9. doi:10.1111/j.1474-9726.2012.00844.x.

7. Coen PM, Jubrias SA, Distefano G, Amati F, Mackey DC, Glynn NW et al. Skeletal muscle mitochondrial energetics are associated with maximal aerobic capacity and walking speed in older adults. The journals of gerontology Series A, Biological sciences and medical sciences. 2013;68(4):447-55. doi:10.1093/gerona/gls196.

8. Choi S, Reiter DA, Shardell M, Simonsick EM, Studenski S, Spencer RG et al. 31P Magnetic Resonance Spectroscopy Assessment of Muscle Bioenergetics as a Predictor of Gait Speed in the Baltimore Longitudinal Study of Aging. The journals of gerontology Series A, Biological sciences and medical sciences. 2016;71(12):1638-45. doi:10.1093/gerona/glw059.

9. Zane AC, Reiter DA, Shardell M, Cameron D, Simonsick EM, Fishbein KW et al. Muscle strength mediates the relationship between mitochondrial energetics and walking performance. Aging cell. 2017. doi:10.1111/acel.12568.

10. Santanasto AJ, Glynn NW, Jubrias SA, Conley KE, Boudreau RM, Amati F et al. Skeletal Muscle Mitochondrial Function and Fatigability in Older Adults. The journals of gerontology Series A, Biological sciences and medical sciences. 2014. doi:10.1093/gerona/glu134.

11. Broskey NT, Boss A, Fares EJ, Greggio C, Gremion G, Schluter L et al. Exercise efficiency relates with mitochondrial content and function in older adults. Physiol Rep. 2015;3(6). doi:10.14814/phy2.12418.

12. Trounce I, Byrne E, Marzuki S. Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. Lancet. 1989;1(8639):637-9.

13. Tonkonogi M, Fernstrom M, Walsh B, Ji LL, Rooyackers O, Hammarqvist F et al. Reduced oxidative power but unchanged antioxidative capacity in skeletal muscle from aged humans. Pflugers Archiv : European journal of physiology. 2003;446(2):261-9. doi:10.1007/s00424-003-1044-9.

14. Porter C, Hurren NM, Cotter MV, Bhattarai N, Reidy PT, Dillon EL et al. Mitochondrial respiratory capacity and coupling control decline with age in human skeletal muscle. American

journal of physiology Endocrinology and metabolism. 2015:ajpendo 00125 2015. doi:10.1152/ajpendo.00125.2015.

15. Boffoli D, Scacco SC, Vergari R, Solarino G, Santacroce G, Papa S. Decline with age of the respiratory chain activity in human skeletal muscle. Biochimica et biophysica acta. 1994;1226(1):73-82.

16. Lanza IR, Short DK, Short KR, Raghavakaimal S, Basu R, Joyner MJ et al. Endurance exercise as a countermeasure for aging. Diabetes. 2008;57(11):2933-42. doi:10.2337/db08-0349.

17. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S et al. Decline in skeletal muscle mitochondrial function with aging in humans. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(15):5618-23. doi:10.1073/pnas.0501559102.

18. Gouspillou G, Sgarioto N, Kapchinsky S, Purves-Smith F, Norris B, Pion CH et al. Increased sensitivity to mitochondrial permeability transition and myonuclear translocation of endonuclease G in atrophied muscle of physically active older humans. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2014;28(4):1621-33. doi:10.1096/fj.13-242750.

19. Hutter E, Skovbro M, Lener B, Prats C, Rabol R, Dela F et al. Oxidative stress and mitochondrial impairment can be separated from lipofuscin accumulation in aged human skeletal muscle. Aging cell. 2007;6(2):245-56. doi:10.1111/j.1474-9726.2007.00282.x.

20. Larsen S, Hey-Mogensen M, Rabol R, Stride N, Helge JW, Dela F. The influence of age and aerobic fitness: effects on mitochondrial respiration in skeletal muscle. Acta Physiol (Oxf). 2012;205(3):423-32. doi:10.1111/j.1748-1716.2012.02408.x.

21. Rasmussen UF, Krustrup P, Kjaer M, Rasmussen HN. Human skeletal muscle mitochondrial metabolism in youth and senescence: no signs of functional changes in ATP formation and mitochondrial oxidative capacity. Pflugers Archiv : European journal of physiology. 2003;446(2):270-8. doi:10.1007/s00424-003-1022-2.

22. Gram M, Vigelso A, Yokota T, Hansen CN, Helge JW, Hey-Mogensen M et al. Two weeks of one-leg immobilization decreases skeletal muscle respiratory capacity equally in young and elderly men. Experimental gerontology. 2014;58:269-78. doi:10.1016/j.exger.2014.08.013.

23. Safdar A, Hamadeh MJ, Kaczor JJ, Raha S, Debeer J, Tarnopolsky MA. Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. PLoS One. 2010;5(5):e10778. doi:10.1371/journal.pone.0010778.

24. Proctor DN, Sinning WE, Walro JM, Sieck GC, Lemon PW. Oxidative capacity of human muscle fiber types: effects of age and training status. Journal of applied physiology. 1995;78(6):2033-8.

25. Russ DW, Kent-Braun JA. Is skeletal muscle oxidative capacity decreased in old age? Sports medicine. 2004;34(4):221-9.

26. Perry CG, Kane DA, Lanza IR, Neufer PD. Methods for assessing mitochondrial function in diabetes. Diabetes. 2013;62(4):1041-53. doi:10.2337/db12-1219.

27. Picard M, Ritchie D, Wright KJ, Romestaing C, Thomas MM, Rowan SL et al. Mitochondrial functional impairment with aging is exaggerated in isolated mitochondria compared to permeabilized myofibers. Aging cell. 2010;9(6):1032-46. doi:10.1111/j.1474-9726.2010.00628.x.

28. Cree MG, Newcomer BR, Katsanos CS, Sheffield-Moore M, Chinkes D, Aarsland A et al. Intramuscular and liver triglycerides are increased in the elderly. The Journal of clinical endocrinology and metabolism. 2004;89(8):3864-71. doi:10.1210/jc.2003-031986.

29. Haran PH, Rivas DA, Fielding RA. Role and potential mechanisms of anabolic resistance in sarcopenia. Journal of cachexia, sarcopenia and muscle. 2012;3(3):157-62. doi:10.1007/s13539-012-0068-4.

30. Mian OS, Thom JM, Ardigo LP, Narici MV, Minetti AE. Metabolic cost, mechanical work, and efficiency during walking in young and older men. Acta physiologica. 2006;186(2):127-39. doi:10.1111/j.1748-1716.2006.01522.x.

31. Martin PE, Rothstein DE, Larish DD. Effects of age and physical activity status on the speed-aerobic demand relationship of walking. Journal of applied physiology. 1992;73(1):200-6.

32. Woo JS, Derleth C, Stratton JR, Levy WC. The influence of age, gender, and training on exercise efficiency. Journal of the American College of Cardiology. 2006;47(5):1049-57. doi:10.1016/j.jacc.2005.09.066.

33. Conley KE, Jubrias SA, Cress ME, Esselman P. Exercise efficiency is reduced by mitochondrial uncoupling in the elderly. Experimental physiology. 2013;98(3):768-77. doi:10.1113/expphysiol.2012.067314.

34. Berntsen S, Hageberg R, Aandstad A, Mowinckel P, Anderssen SA, Carlsen KH et al. Validity of physical activity monitors in adults participating in free-living activities. Br J Sports Med. 2010;44(9):657-64. doi:10.1136/bjsm.2008.048868.

35. Mackey DC, Manini TM, Schoeller DA, Koster A, Glynn NW, Goodpaster BH et al. Validation of an armband to measure daily energy expenditure in older adults. The journals of gerontology Series A, Biological sciences and medical sciences. 2011;66(10):1108-13. doi:10.1093/gerona/glr101.

36. Dube JJ, Coen PM, DiStefano G, Chacon AC, Helbling NL, Desimone ME et al. Effects of acute lipid overload on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance. American journal of physiology Endocrinology and metabolism. 2014;307(12):E1117-24. doi:10.1152/ajpendo.00257.2014.

37. Thompson WR GN, Pescatello LS. ACSM's Guidlines for Exercise Testing and Prescription. 8 ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins; 2010.

38. Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, Conley KE. Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. J Physiol. 2003;553(Pt 2):589-99. doi:10.1113/jphysiol.2003.045872.

39. Blei ML, Conley KE, Kushmerick MJ. Separate measures of ATP utilization and recovery in human skeletal muscle. J Physiol. 1993;465:203-22.

40. Blei ML, Conley KE, Odderson IB, Esselman PC, Kushmerick MJ. Individual variation in contractile cost and recovery in a human skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America. 1993;90(15):7396-400.

41. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J Gerontol. 1994;49(2):M85-94.

42. Pruchnic R, Katsiaras A, He J, Kelley DE, Winters C, Goodpaster BH. Exercise training increases intramyocellular lipid and oxidative capacity in older adults. American journal of

physiology Endocrinology and metabolism. 2004;287(5):E857-62. doi:10.1152/ajpendo.00459.2003.

43. Coen PM, Menshikova EV, Distefano G, Zheng D, Tanner CJ, Standley RA et al. Exercise and Weight Loss Improve Muscle Mitochondrial Respiration, Lipid Partitioning and Insulin Sensitivity Following Gastric Bypass Surgery. Diabetes. 2015. doi:10.2337/db15-0809.

44. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin CT et al. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. The Journal of clinical investigation. 2009;119(3):573-81. doi:10.1172/JCI37048.

45. Amati F, Dube JJ, Shay C, Goodpaster BH. Separate and combined effects of exercise training and weight loss on exercise efficiency and substrate oxidation. Journal of applied physiology. 2008;105(3):825-31. doi:10.1152/japplphysiol.90384.2008.

46. Brouwer E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. Acta Physiol Pharmacol Neerl. 1957;6:795-802.

47. Conley KE, Jubrias SA, Esselman PE. Oxidative capacity and aging in human muscle [published erratum appears in J Physiol 2001 Jun 15;533 Pt 3:921]. The Journal of physiology. 2000;526.1:203-10.

48. Tudor-Locke C, Hatano Y, Pangrazi RP, Kang M. Revisiting "how many steps are enough?". Med Sci Sports Exerc. 2008;40(7 Suppl):S537-43. doi:10.1249/MSS.0b013e31817c7133.

49. Purves-Smith FM, Sgarioto N, Hepple RT. Fiber typing in aging muscle. Exercise and sport sciences reviews. 2014;42(2):45-52. doi:10.1249/JES.0000000000000012.

50. Picard M, Taivassalo T, Ritchie D, Wright KJ, Thomas MM, Romestaing C et al. Mitochondrial structure and function are disrupted by standard isolation methods. PLoS One. 2011;6(3):e18317. doi:10.1371/journal.pone.0018317.

51. Distefano G, Standley RA, Dube JJ, Carnero EA, Ritov VB, Stefanovic-Racic M et al. Chronological Age Does not Influence Ex-vivo Mitochondrial Respiration and Quality Control in Skeletal Muscle. The journals of gerontology Series A, Biological sciences and medical sciences. 2016. doi:10.1093/gerona/glw102.

52. St-Jean-Pelletier F, Pion CH, Leduc-Gaudet JP, Sgarioto N, Zovile I, Barbat-Artigas S et al. The impact of ageing, physical activity, and pre-frailty on skeletal muscle phenotype, mitochondrial content, and intramyocellular lipids in men. Journal of cachexia, sarcopenia and muscle. 2016. doi:10.1002/jcsm.12139.

53. Brand MD. Uncoupling to survive? The role of mitochondrial inefficiency in ageing. Experimental gerontology. 2000;35(6-7):811-20.

54. Beere PA, Russell SD, Morey MC, Kitzman DW, Higginbotham MB. Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. Circulation. 1999;100(10):1085-94.

55. Gueugneau M, Coudy-Gandilhon C, Theron L, Meunier B, Barboiron C, Combaret L et al. Skeletal muscle lipid content and oxidative activity in relation to muscle fiber type in aging and metabolic syndrome. The journals of gerontology Series A, Biological sciences and medical sciences. 2015;70(5):566-76. doi:10.1093/gerona/glu086.

56. Gollnick PD, Sjodin B, Karlsson J, Jansson E, Saltin B. Human soleus muscle: a comparison of fiber composition and enzyme activities with other leg muscles. Pflugers Archiv : European journal of physiology. 1974;348(3):247-55.

57. Henriksson-Larsen KB, Lexell J, Sjostrom M. Distribution of different fibre types in human skeletal muscles. I. Method for the preparation and analysis of cross-sections of whole tibialis anterior. The Histochemical journal. 1983;15(2):167-78.

58. Coen PM, Hames KC, Leachman EM, DeLany JP, Ritov VB, Menshikova EV et al. Reduced skeletal muscle oxidative capacity and elevated ceramide but not diacylglycerol content in severe obesity. Obesity (Silver Spring). 2013;21(11):2362-71. doi:10.1002/oby.20381.

59. Choi SJ, Files DC, Zhang T, Wang ZM, Messi ML, Gregory H et al. Intramyocellular Lipid and Impaired Myofiber Contraction in Normal Weight and Obese Older Adults. The journals of gerontology Series A, Biological sciences and medical sciences. 2015. doi:10.1093/gerona/glv169.

60. Gueugneau M, Coudy-Gandilhon C, Theron L, Meunier B, Barboiron C, Combaret L et al. Skeletal Muscle Lipid Content and Oxidative Activity in Relation to Muscle Fiber Type in Aging and Metabolic Syndrome. The journals of gerontology Series A, Biological sciences and medical sciences. 2014. doi:10.1093/gerona/glu086.

61. Tevald MA, Foulis SA, Kent JA. Effect of age on in vivo oxidative capacity in two locomotory muscles of the leg. Age. 2014;36(5):9713. doi:10.1007/s11357-014-9713-5.

62. Larsen RG, Callahan DM, Foulis SA, Kent-Braun JA. Age-related changes in oxidative capacity differ between locomotory muscles and are associated with physical activity behavior. Appl Physiol Nutr Metab. 2012;37(1):88-99. doi:10.1139/h11-135.

63. Mogensen M, Bagger M, Pedersen PK, Fernstrom M, Sahlin K. Cycling efficiency in humans is related to low UCP3 content and to type I fibres but not to mitochondrial efficiency. J Physiol. 2006;571(Pt 3):669-81. doi:10.1113/jphysiol.2005.101691.

64. Coyle EF, Sidossis LS, Horowitz JF, Beltz JD. Cycling efficiency is related to the percentage of type I muscle fibers. Med Sci Sports Exerc. 1992;24(7):782-8.

65. Whipp BJ, Wasserman K. Efficiency of muscular work. J Appl Physiol. 1969;26(5):644-8.

66. Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO et al. Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metab. 2011;13(2):149-59. doi:10.1016/j.cmet.2011.01.004.

67. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. Acta physiologica. 2007;191(1):59-66. doi:10.1111/j.1748-1716.2007.01713.x.

68. Amara CE, Shankland EG, Jubrias SA, Marcinek DJ, Kushmerick MJ, Conley KE. Mild mitochondrial uncoupling impacts cellular aging in human muscles in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(3):1057-62. doi:10.1073/pnas.0610131104.

69. Conley KE, Jubrias SA, Esselman PC. Oxidative capacity and ageing in human muscle. J Physiol. 2000;526 Pt 1:203-10.

70. Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. The journals of gerontology Series A, Biological sciences and medical sciences. 2006;61(10):1059-64.

71. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. The journals of gerontology Series A, Biological sciences and medical sciences. 2005;60(3):324-33.

72. Shenton D, Smirnova JB, Selley JN, Carroll K, Hubbard SJ, Pavitt GD et al. Global translational responses to oxidative stress impact upon multiple levels of protein synthesis. J Biol Chem. 2006;281(39):29011-21. doi:M601545200 [pii]
10.1074/jbc.M601545200.

73. Pham FH, Sugden PH, Clerk A. Regulation of protein kinase B and 4E-BP1 by oxidative stress in cardiac myocytes. Circ Res. 2000;86(12):1252-8.

74. Zhang L, Kimball SR, Jefferson LS, Shenberger JS. Hydrogen peroxide impairs insulin-stimulated assembly of mTORC1. Free Radic Biol Med. 2009;46(11):1500-9. doi:S0891-5849(09)00127-0 [pii]
10.1016/j.freeradbiomed.2009.03.001.

75. Muller FL, Song W, Liu Y, Chaudhuri A, Pieke-Dahl S, Strong R et al. Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. Free Radic Biol Med. 2006;40(11):1993-2004. doi:10.1016/j.freeradbiomed.2006.01.036.

76. Nikolova-Karakashian MN, Reid MB. Sphingolipid metabolism, oxidant signaling, and contractile function of skeletal muscle. Antioxidants & redox signaling. 2011;15(9):2501-17. doi:10.1089/ars.2011.3940.

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 colored dark green. Subcutaneous adipose tissue (SAT) is colored red. Intermuscular adipose tissue (IMAT) is colored light green. Panel B, Mid-thigh muscle volume was higher in the YA group when compared to OS. Physical activity partially preserved mid-thigh muscle volume in the OA group. Panel C, Subcutaneous adipose tissue (SAT) volume was higher in the OS group when compared to YA and OA groups. Panel D, Intermuscular adipose tissue (IMAT) volume was higher in the OS group when compared to 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 ANOVA followed by post-hoc Tukey test). Data presented are Mean ± SEM.

Figure 2. Muscle contractile performance and physical function. Panel A, Left leg 1-repitition maximum (1-RM) was lower in the OA and OS groups, compared to 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* 4m walk test (from SPPB), repeated chair stand (from SPPB), and stair climb test. Panel C, There was no difference between groups in time to complete 4m walk test. Panel D, Time to complete the repeated chair stand test was higher in the OS group when compared to the YA and OA groups. Panel E, Time to complete the stair climb test was higher in the OS group when compared to YA and OA groups, and higher in the OA when compared to YA. The letters [A, B and C] denote significant differences between groups ($P < 0.05$, One-way ANOVA followed by post-hoc Tukey test). Data presented are Mean ± SEM.

Figure 3. Skeletal muscle mitochondrial energetics and intramyocellular lipid. Maximal ATP synthetic rate (ATP_{Max}) and phosphocreatine (PCr) recovery time were determined by ^{31}P MRS. Panel A, ATP_{Max} was lower in OS compared to both YA and OA groups. Panel B, PCr recovery time was significantly longer for OS compared to OA and YA groups. Mitochondrial respiratory capacity measured in permeabilized myofibers by high-resolution respirometry. Panel C, OS had lower LEAK respiration (L_I, 5mM glutamate & 2mM Malate), ADP (4mM) stimulated maximal complex I (P_I) and complex I and II (P_{I+II}, 10mM succinate) OXPHOS respiration, and maximal electron transfer system capacity (E_{I+II}, FCCP titer 0-2μM), when compared to YA and OA groups. Panel D, OS had lower fatty acid supported LEAK respiration (L_{FAO}, 2mM Malate & 25uM Palmitoylcarnitine), ADP (4mM) stimulated maximal OXPHOS respiration supported by FAO (P_{FAO},), and complex I and II supported FAO ($P_{I+II+FAO}$, 2mM Malate, 5mM Glutamate & 10mM succinate), when compared to 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 to YA and OA groups. Panels G and H, Skeletal muscle intramyocellular lipid (IMCL) content. Panel G, Soleus IMCL accumulation was higher in the OS when compared to YA. Physical activity partially prevented soleus IMCL accumulation in the OA group. Panel F, Tibialis anterior IMCL content was similar between groups. The letters [A&B] denote significant differences between groups ($P < 0.05$, One-way ANOVA and Tukey's honest significance difference post-hoc test). Data presented are Mean ± SEM.

Figure 4. Exercise efficiency and its association with mitochondrial energetics and intermuscular adipose tissue. The OS group had lower Gross (Panel A) and Net (Panel B) exercise efficiency compared to the active groups**.** The letters [A&B] denote significant differences between groups (P < 0.05, One-way ANOVA and Tukey's honest significance difference post-hoc test). Data presented are Mean ± SEM.

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	2F/8M	2F/8M	8F/11M	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/24hr)	3108± 270 [A]	2565±555 [B]	2117±404 [C]	<0.0001
Daily AEE (kcal/24hr)	1833±369 [A]	1248±545 [B]	488±335 [C]	<0.0001
Steps per day (steps/24hr)	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 [AB]	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±SD. [A, B and C] indicate significant differences between groups (p<0.05). * Sex distribution across groups were determined by Chi Square test. Abbreviations: 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.

Table 2. Muscle Contractile performance by isokinetic dynamometry

	YA (n=10)	OA (n=10)	OS (n=19)	*ANOVA* *P*-Value
BIODEX (Left Leg, 120°/sec)				
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

Data: Mean+SD for all subjects. [A, B] indicate significant differences between group ($p < 0.05$).
Abbreviations: Nm, newton meters; W, Watt; J, joule; sec, second.

Table 3. Partial correlations

Measurement	Variables	1 RM ((Kg)/(kg))		Gait speed (m/sec)		Step Test (sec)		Repeated Chair Stand (sec)	
		r	p	r	p	r	p	r	p
Mitochondrial energetics	ATP$_{Max}$ (mM/sec)	0.457	**0.016***	0.303	0.133	-0.046	0.818	-0.188	0.358
	Tau (sec)	-0.334	**0.088**	-0.236	0.246	0.131	0.516	0.210	0.304
	Maximal coupled respiration (pmol/sec/mg ww)	-0.016	0.939	-0.182	0.395	-0.192	0.358	-0.054	0.803
	H$_2$O$_2$ (pmol/min/mg dw)	0.389	**0.054***	-0.076	0.724	-0.329	0.108	-0.271	0.200
	P/O ratio ((mM/sec)/(pmol/sec/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 and Physical activity	VO$_2$ peak (L/min)	0.165	0.410	0.235	0.248	-0.338	**0.085**	-0.362	**0.069**
	Steps per day (steps/24h)	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 for partial correlations are from the OA and OS groups only (total n = 29). Correlations are adjusted for Age and Sex. [*] indicate statistically significant partial correlation (p<0.05) and bold p-values indicate p<0.10. Abbreviations: ATP$_{Max}$, maximal mitochondrial phosphorylation capacity; IMAT, intermuscular adipose tissue; IMCL, intramyocellular lipid; RM, repetition maximum.
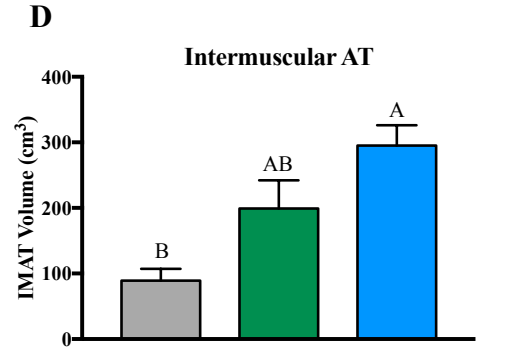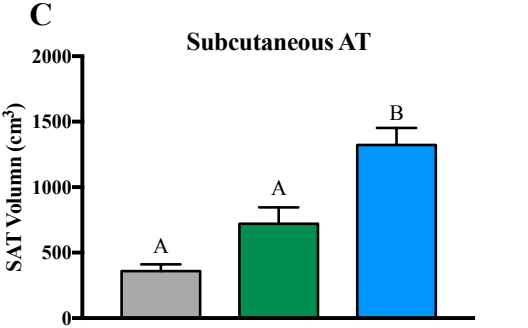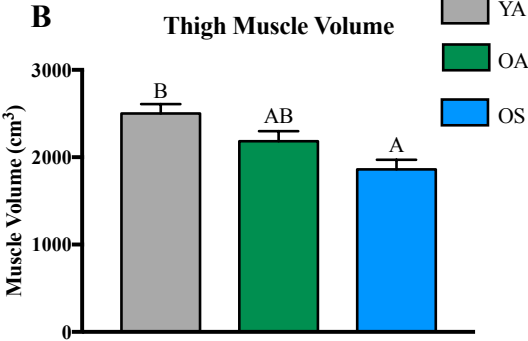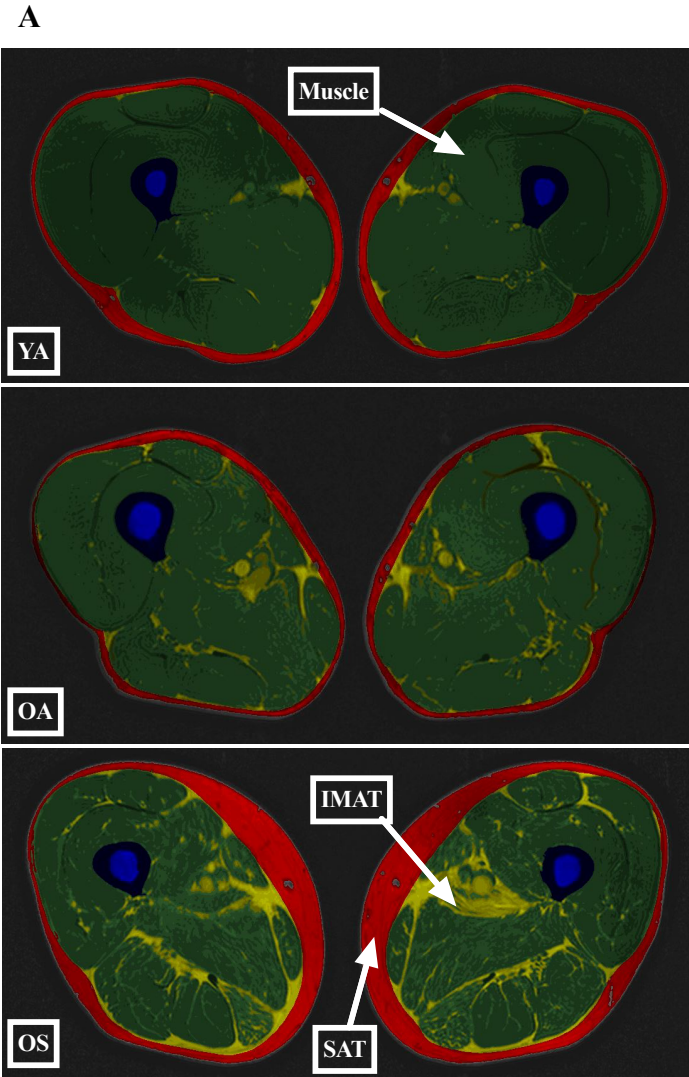
Table 4. Regression models

Parameter	1 RM (kg/kg)		Gait speed (m/sec)		Step test (sec)	
Adj R^2 (*Model p*)	0.606 (<0.0001)		0.303 (0.0083)		0.546 (0.0002)	
	β	p	β	p	β	p
Age (yrs)	-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/sec)	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 OA and OS groups only (total n = 29). [*] indicate statistically significant contribution to the multiple regression model ($p<0.05$). Abbreviations: ATP_{Max}, maximal mitochondrial phosphorylation capacity; IMCL, intramyocellular lipid; RM, repetition maximum.

FIGURE 1

FIGURE 2

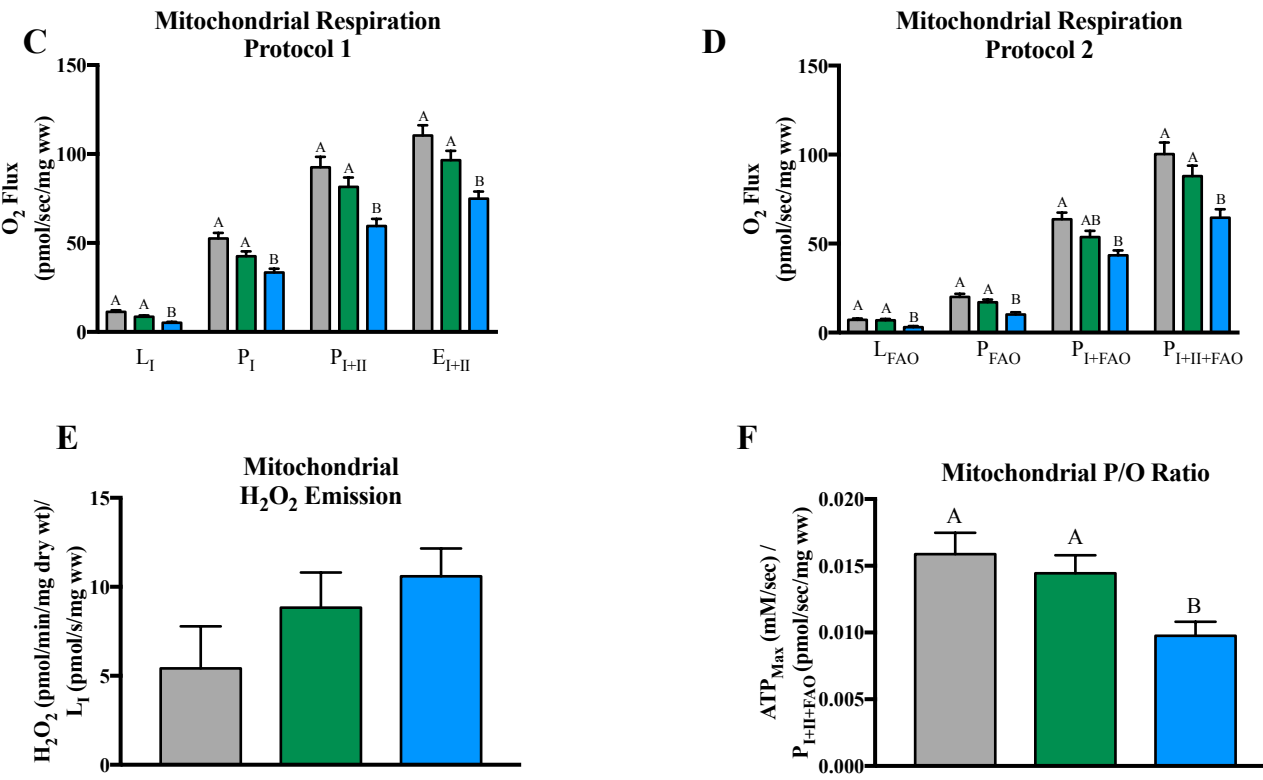## Muscle Strength and Quality

A 1-Repitition Maximum (RM)

B 1-RM Normalized

Physical Function

C 4M Walk Test

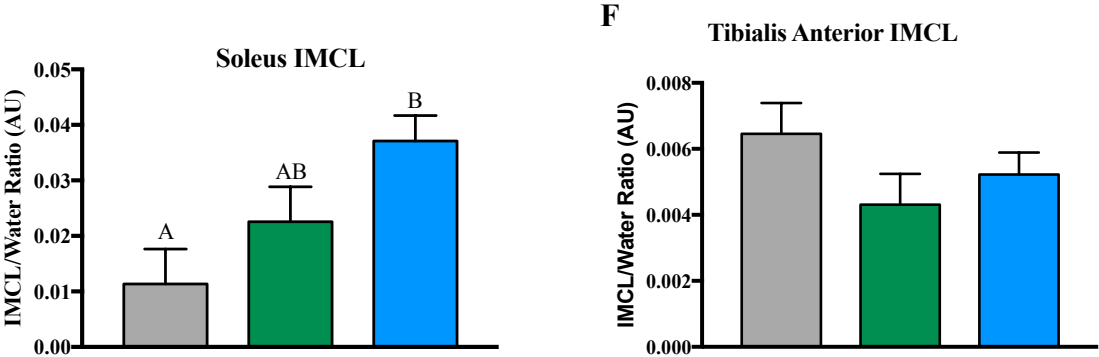**D** Repeated Chair Stand test

E Stair Climb test
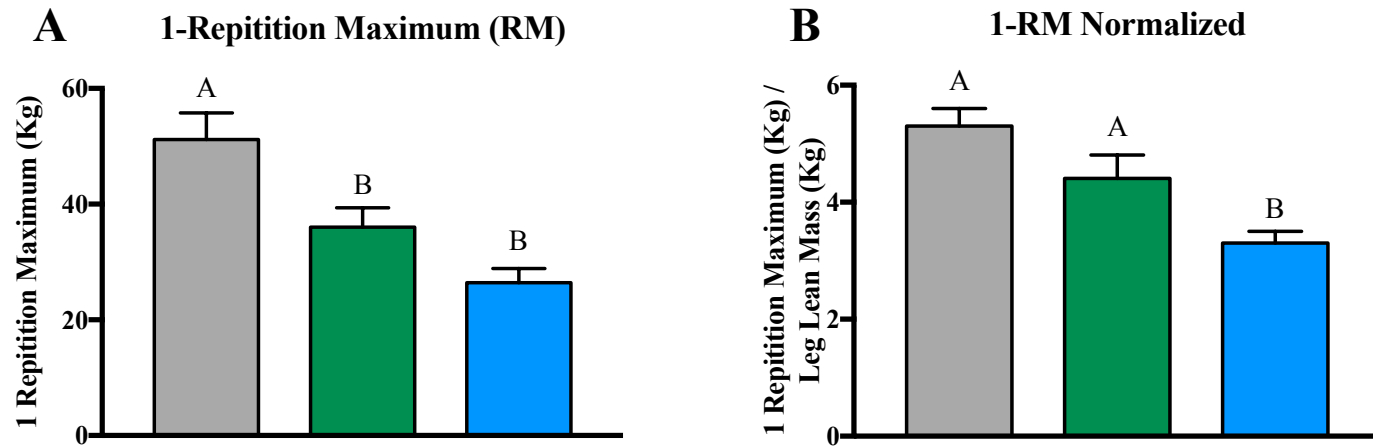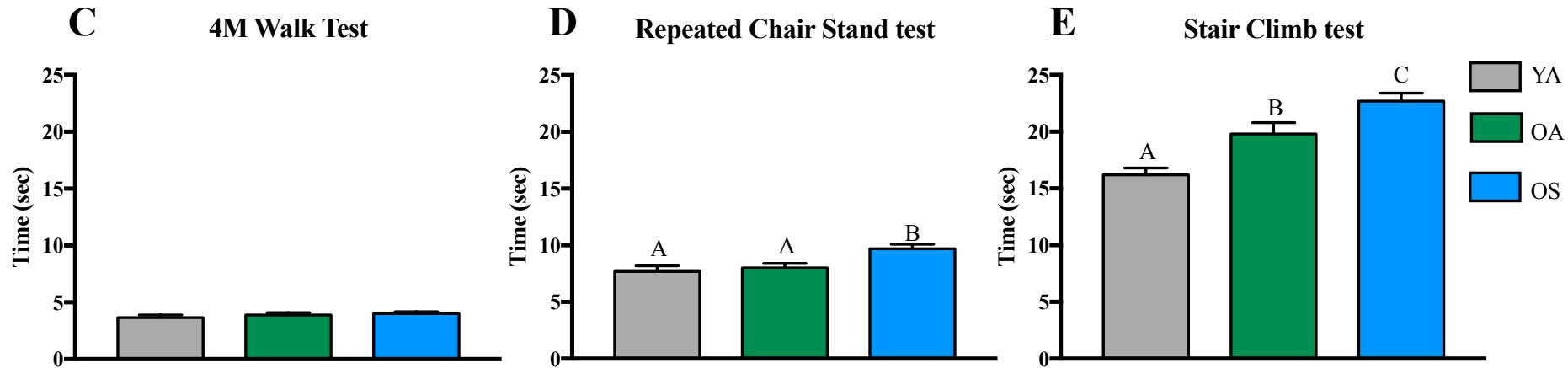
FIGURE 3

In -Vivo Mitochondrial Phosphorylation
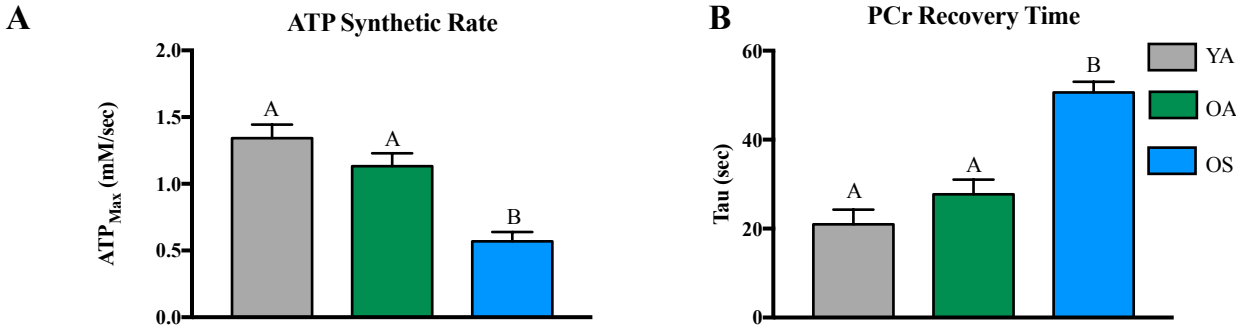
Ex-Vivo Mitochondrial Function in Permeabilized Fibers

Intramyocellular Lipid (IMCL)

FIGURE 4

A **Gross Exercise Efficiency**

B **Net Exercise Efficiency**