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Skeletal Muscle Mitochondrial Function and Fatigability in Older Adults

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ABSTRACT

Background: Fatigability tends to increase while the capacity for mitochondrial energy production tends to decrease significantly with age. Thus, diminished mitochondrial function may contribute to higher levels of fatigability in older adults.

Methods: The relationship between fatigability and skeletal muscle mitochondrial function was examined in 30 participants aged 78.5±5.0 years (47% female, 93% white), with a BMI of 25.9±2.7kg/m^2 and usual gait-speed of 1.2±0.2m/s. Fatigability was defined using Rating of Perceived Exertion (RPE, 6-20) after a 5-minute treadmill walk at 0.72m/s. Phosphocreatine recovery in the quadriceps was measured using ^{31}P magnetic resonance spectroscopy and images of the quadriceps were captured to calculate quadriceps volume. ATPmax (mM ATP/s) and oxidative capacity of the quadriceps (ATPmax·Quadriceps Volume) were calculated. Peak aerobic capacity (VO_2peak) was measured using a modified Balke protocol.

Results: ATPmax·Quadriceps volume was associated with VO_2peak and was 162.61mM ATP•mL/s lower (p = 0.03) in those with high (RPE ≥10) vs. low (RPE ≤ 9) fatigability. Participants with high fatigability required a significantly higher proportion of VO_2peak to walk at 0.72m/s compared to those with low fatigability (58.7±19.4 vs. 44.9±13.2%, $p < 0.05$). After adjustment for age and sex, higher ATPmax was associated with lower odds of having high fatigability (*OR*: 0.34, 95% *CI*: 0.11-1.01, $p = 0.05$).

Conclusions: Lower capacity for oxidative phosphorylation in the quadriceps, perhaps by contributing to lower VO_2peak, is associated with higher fatigability in older adults.

INTRODUCTION

Fatigue is common among older adults (1) and associated with poorer physical function and disability both cross-sectionally (2) and longitudinally (3). Fatigue is primarily considered to be an energy disorder and a large proportion cannot be attributed to underlying diseases (4). It has been hypothesized that age-related decreases in mitochondrial function may contribute to higher levels of age-related fatigue (5-6). The capacity for oxidative phosphorylation in skeletal muscle is lower in older compared to younger adults; however, more recent evidence shows this difference varies across muscle groups and appears largely attributable to decreased physical activity (7-12). Peak aerobic capacity (VO_2peak) also decreases significantly with age, independent of muscle loss and physical activity level (13), and is considered a hallmark manifestation of mitochondrial disorders (14). Hence, age-related decreased capacity for mitochondrial energy production may contribute to higher levels of fatigability via lower aerobic capacity. Little is known, however, about the etiology of age-related fatigue, particularly as it relates to mitochondrial energetics (5-6).

Fatigability, similar conceptually to exercise tolerance, is fatigue anchored to an activity of a specific intensity and duration (6). Fatigability, as opposed to global fatigue, provides insight into the degree to which an individual is limited physically due to fatigue (6,15). The primary objective of this research was to determine if skeletal muscle mitochondrial oxidative capacity determined by ^{31}P magnetic resonance spectroscopy (^{31}P MRS), was related to higher levels of fatigability in older adults. We hypothesized that that those with higher compared to lower fatigability would have lower skeletal muscle oxidative capacity. We also hypothesized that physical activity would attenuate this relationship and VO_2peak would act as a mediator.

METHODS

Participants

Community-dwelling (n = 37) men and women aged 70–89 years from the Pittsburgh, PA area were enrolled into the Study of Energy and Aging Pilot. Inclusion criteria was body weight ≤ 285lbs for men and ≤ 250lbs for women; body mass index (BMI) 20–32kg/m^2; ability to walk without an assistive device and free of difficulty performing basic activities of daily living. Exclusion criteria: symptomatic cardiovascular or pulmonary disease, heart attack, angioplasty, or heart surgery within the past 3-months, or a cerebral hemorrhage within the past 6-months, stroke within the past 12-months, or chest pain during walking in the past 30-days (16). Participants were telephone screened and reassessed at the magnetic resonance imaging (MRI) center for scanner eligibility, including ability to lie in a supine position for 1-hour, no metal or other implants, joint replacements, or tattoos. Participants had to be willing and able to sign an informed consent. This study was approved by the University of Pittsburgh and California Pacific Medical Center Institutional Review Boards.

Clinical Examination and Measurements

Body height (cm) was measured using a wall-mounted stadiometer and weight (kg) with a certified and calibrated scale and used to calculate BMI, kg/m^2. Participants completed demographic, medical and disease history questionnaires. Depression was assessed using a short form of the Center for Epidemiologic Studies Depression Scale (CES-D) (17).

Physical function was measured by the Short Physical Performance Battery (SPPB), which includes a 6m walk, chair stands and balance tests (18). Usual gait-speed was derived

from the faster of two 6m trials. Time to walk 400m (s) at usual pace was also measured. Seven-day free-living physical activity was assessed using the SenseWear™ (BodyMedia Pittsburgh, PA) Pro armband. The manufacturer's proprietary algorithm was used to calculate minutes-per-day of moderate (≥ 3METs) physical activity.

VO_2Peak Test

VO_2peak was determined using a modified Balke protocol (19) and participant's usual 6m walking speed was used throughout the test. Treadmill grade was increased 2% every 2-minutes until volitional exhaustion. A resting 12-lead electrocardiogram was conducted prior to and monitored continuously during the test. VO_2 measurements were obtained with a metabolic cart (Moxus, AEI Technologies, Pittsburgh, PA). Gas analyzers and flow, using a 3L syringe, were calibrated before each test. Studies employing similar protocols in older adults report excellent reproducibility of maximal treadmill walking time (ICC = 0.87) (20), which correlates highly with VO_2peak (r = 0.92) (21).

Determination of ATPmax by ^{31}P MRS

Maximal mitochondrial ATP production (ATPmax) following an acute bout of knee extensor exercise was determined *in vivo* using ^{31}P MRS. Phosphocreatine (PCr) recovery after exercise was used to quantify rates of mitochondrial ATP production. ^{31}P MRS has been validated by animal and human studies showing that ATPmax varies in direct proportion to oxidative enzyme activity of healthy muscle (22-23) and corresponds to mitochondrial content in human muscle (24). ATPmax had good reproducibility illustrated by a high correlation (r = 0.92)

between same day repeat scans of 8 participants and Bland Altman analysis (Supplemental Figure 1).

The exercise protocol was performed in an MRI magnet (3T TIM Trio, Siemens' Medical System). Participants laid supine with the right knee (unless contraindicated) elevated at ~30^{o}. Straps were placed over the legs and a 2.5" surface RF coil tuned to ^{31}P was placed over the quadriceps. Signal was collected by a hemisphere defined by the coil radius (1.25"), which we previously confirmed using an image generated by a similarly sized coil (24). Participants kicked repeatedly as hard and as fast as they could for two bouts (30s and 36s), each followed by a 6-minute rest. The protocol was designed to deplete PCr stores by 33-66% to ensure high signal to noise defining PCr recovery without inducing acidosis (pH < 6.8), which inhibits oxidative phosphorylation. PCr recovery rate (ATPmax) was measured after exercise until PCr returned to baseline levels.

Phosphorus spectra were collected using a standard one pulse experiment to determine levels of PCr, ATP, Pi, PDE (phosphodiesters) and pH throughout exercise and recovery. PCr, Pi, PDE and ATP peak areas in the fully relaxed spectra were measured by integration using Varian VNMR 6.1C software (Varian Medical Systems, Palo Alto, CA). Areas of the PCr and Pi peaks were expressed relative to the ATP peak (Supplemental Table 2). Previous analyses of human vastus lateralis muscle biopsies revealed that ATP content accounted for the range of PCr/ATP levels determined by MRS among participants aged 65-80 (24). In contrast, PCr was stable (as was total creatine) and averaged 27 mM. Thus, as previously reported (16), we used 27 mM PCr to determine ATPmax. Changes in PCr and Pi peak areas during the tests were analyzed as previously described (25-26).

Seven participants were missing ATPmax, four due to inadequate PCr breakdown or acidosis and three because of metal deposits or claustrophobia that precluded testing.

Determination of Muscle Size and Oxidative Capacity of the Quadriceps

MRI was used to determine quadriceps volume as previously described (27). Images were collected every 3cm from hip to thigh (15–25 slices per participant). Standard stereological techniques were used to determine the largest quadriceps muscle cross-sectional area. Oxidative capacity of the quadriceps was calculated by multiplying ATPmax by quadriceps volume.

Determination of Fatigability and Measurement of VO_2 during Steady State Treadmill Test

Fatigability was determined following a 5-minute treadmill walk at 1.6mph (0.72 m/s) and 0% grade (15). This speed and duration were selected to simulate the minimal level of activity needed to function relatively independently in the community (15). Immediately following the walk participants rated their perceived exertion (RPE) using the Borg scale (6-20) (28) and were categorized as high fatigability (RPE $\geq$ 10) or low fatigability (RPE $\leq$ 9). This cut point was established in the Baltimore Longitudinal Study of Aging, which showed that this threshold is associated with substantially poorer physical functioning (15). The same test was used for each person in order to compare fatigability levels across individuals for the same standardized task (5-6). Oxygen consumption was measured during the walk and mean VO_2 was calculated. Immediately following the test blood lactate levels were assessed by finger stick using a portable lactate analyzer.

Statistical Analyses

The final analytic sample included those with valid ATPmax values (n=30). Means and standard deviations or counts and percents were calculated for the entire cohort and separately by high and low fatigability. Univariate statistics for predictors, covariates and other variables were compared between fatigability groups using t-tests, chi-squared and nonparametric tests where appropriate. Least squared age and sex adjusted means for ATPmax and ATPmax·quadriceps volume were compared between fatigability groups. Separate multivariate logistic regression models were generated to determine the odds of having high fatigability associated with increases in the primary predictors of ATPmax and ATPmax·quadriceps volume. Standard deviation increases in ATPmax and ATPmax·quadriceps volume were used to generate odds ratios for ease of interpretation, as odds ratios using original units resulted in upper limits that approached infinity. Analyses were performed using SAS v9.2.

RESULTS

Demographic Characteristics, Medical History and Physical Function

Participants were aged 78.5±5.0 years, 46.7% female, 93.3% white, BMI of 25.9±2.7kg/m^2 (Table 1) and were relatively high functioning with SPPB scores of 10.9±1.4 and usual gait-speeds of 1.2±0.2m/s. There were no differences in demographic characteristics and medical history by fatigability status (all $p > 0.05$, Table 1). However, those with high fatigability tended to have a higher prevalence of osteoarthritis compared to those with low fatigability ($p = 0.06$). Those with high fatigability were less physically active (36.8±24.5 vs. 100.6±83.7min/d moderate activity, $p < 0.05$) and had slower 400m walk times (383.5±79.3s vs. 319.5±41.4s, $p = 0.03$) compared to the low fatigability group.

Aerobic Capacity, Muscle Size and Mitochondrial Function

Aerobic capacity, muscle size and mitochondrial function, both stratified by fatigability status and overall, are shown in Table 2. Mean VO_2peak was 22.3±5.9ml/kg/min (range: 7.8-33.4), ATPmax: 0.52±0.13mM ATP/s (range: 0.30-0.83) and ATPmax•quadriceps volume: 591.25±203.93mM ATP•mL/s (range: 222.50-965.04).

Those with high fatigability had significantly lower VO_2peak (18.9±4.4 vs. 24.4±5.8ml/kg/min, $p < 0.05$) and ATPmax•quadriceps volume (493.69±203.95 vs. 656.30±181.19mM ATP•mL/s, $p < 0.05$, Figure 1) compared to those with low fatigability. ATPmax was lower in those with high fatigability compared to low fatigability (0.47±0.12 vs. 0.55±0.14mM ATP/s, $p = 0.09$, Table 2); after adjustment for age and sex, the difference became significant (0.46±0.03 vs. 0.56±0.03mM ATP/s, $p = 0.04$). Differences in ATPmax•quadriceps volume were slightly attenuated after adjusting for age and sex (519.61±48.55 vs. 639.02±39.46mM ATP•mL/s, $p = 0.07$). Mean oxygen consumption during the 5 minute, 0.72m/s treadmill bout was nearly identical between those with high and low fatigability (10.4±1.8 vs. 10.4±1.0ml/kg/min, $p > 0.99$); however, those with high fatigability required a significantly higher proportion of VO_2peak to walk at the same speed (58.7±19.4 vs. 44.9±13.2%, $p < 0.05$, Table 2). No difference in muscle volume was observed between fatigability groups ($p = 0.37$). Pearson correlations between age, MRS, VO_2peak and physical activity measures are in Supplemental Table 1. PCr, ATP, Pi, and PDE levels did not differ significantly between groups (all $p > 0.05$, Supplemental Table 2).

Relationship between Fatigability and Mitochondrial Function

One standard deviation increase in ATPmax was associated with 2.94 times lower odds of having high fatigability (*OR*: 0.34, 95% *CI*: 0.11-1.01, $p = 0.05$, Table 3) after adjustment for age and sex. One standard deviation increase in ATPmax•quadriceps volume was associated with 2.56 times lower odds of having high fatigability (*OR*: 0.39, 95% *CI*: 0.16-0.96, $p = 0.04$). Adjusting for age and sex attenuated this relationship to borderline significance (*OR*: 0.37, 95% *CI*: 0.13-1.10, $p = 0.07$, Table 4). The relationships between ATPmax and ATPmax•quadriceps volume and fatigability were attenuated after adjustment for physical activity or VO_2peak (Tables 3 and 4). Similarly quadriceps volume was not significantly associated with fatigability univariately ($p = 0.35$) or after age and sex adjustment ($p = 0.98$). Finally, adjustment for osteoarthritis had no effect on the relationship between either ATPmax or ATPmax•quadriceps volume with fatigability.

DISCUSSION

The capacity for oxidative ATP synthesis, assessed with ^{31}P MRS (ATPmax and ATPmax•muscle volume) was lower in older adults with higher levels of fatigability. To our knowledge, this is the first study to have examined the relationship between mitochondrial energetics and fatigability in older adults. The inverse association between ATPmax and fatigability is consistent with research showing patients with mitochondrial disorders possess lower levels of exercise tolerance than controls (29). This is also consistent with data from mitochondrial gene ANT1-knockout mice, a model for chronic ATP deficiency, displaying lower exercise tolerance and higher fatigability compared to wild type (30). Additionally, ATPmax and ATPmax•quadriceps volume are highly related to aerobic capacity in older adults (31), as skeletal muscle ATP production is one of the two major components comprising VO_2peak, and

both decrease with age (13,24). Reduced cardiac output, the other major component of VO_2peak, may also contribute to higher fatigability independent of ATPmax (32). Nonetheless, impaired mitochondrial energy production may be a specific factor that contributes to higher levels of fatigability via lower aerobic capacity.

Another key finding was that participants with high fatigability had to utilize a higher proportion of VO_2peak to walk at the same speed compared to those with lower fatigability. There are several mechanisms related to mitochondrial function to consider. First, it is possible that the source of energy, i.e., mitochondrial and glycolytic, to maintain walking speed was related to fatigability, however this was not reflected by blood lactate levels during steady-state walking. Second, impaired ATPmax may contribute to a reduced ability to replenish high energy phosphates (e.g. PCr) from inorganic phosphate (Pi) (33), leading to a buildup of Pi, which is associated with muscle fatigue (34). Additionally, it is possible that when mitochondrial energy production approaches maximum capacity or is depleted, a sensation of fatigue is elicited as a response (5). For example, Fiser *et al*. showed that those with slower gait-speed reached a significantly higher proportion of VO_2peak and had higher RPE (i.e. fatigability) during a sub-maximal walking test (35). Our data extend these findings by suggesting that mitochondrial function may be in the etiologic pathway of fatigability and ultimately slowed gait (16). This is also supported by our finding that time to walk 400m was significantly slower in the high fatigability group. These data also advance the "Energetic Pathway for Mobility Loss" theory postulated by Schrack *et al*. (36), which states that the maximum capacity for energy (VO_2peak) decreases with age (13), restricting the availability of energy for everyday activities eventually leading to mobility loss in older age. Our data suggest that impaired mitochondrial function may contribute to lower availability of energy and VO_2peak in older adults, which induces fatigability

and may subsequently exacerbate decreased physical activity levels and mobility. The potential role of impaired mitochondrial function in age-related mobility loss is supported by the finding of Coen *et al*. in this cohort that lower ATPmax is significantly associated with slower usual-paced 400m walking time (16). This is bolstered by two other studies showing that mitochondrial function is impaired in sedentary compared to active older adults (11) and that lower functioning older adults possess greater mitochondrial dysfunction compared to higher functioning (37). Further, fatigue is independently associated with lower SPPB scores and slower walking speeds (2) as well as a significant predictor of future functional decline (3). Thus, interventions aimed at improving mitochondrial energy production may be effective at lowering fatigability (exercise tolerance), resulting in sustainable increased physical activity levels and ultimately mobility in older adults. In order to establish directionality and causality, it is important to study the longitudinal relationships between age, physical activity, VO_2peak, fatigability, mitochondrial function and mobility.

The relationship between ATPmax and fatigability was attenuated after adjustment for physical activity. This was not surprising as ATPmax is closely associated with physical activity (8) and we did not have a large enough sample to detect an independent relationship. Mitochondrial dysfunction may be a contributor to, as well as a consequence of, age-related declines in physical activity. For example, mitochondrial dysfunction activates apoptotic pathways in skeletal muscle and activation of these pathways may contribute to sarcopenia (38), which may result in decreased physical activity levels. However, more recent work shows that aged, compared to young, skeletal muscle fibers are more susceptible to mitochondrial mediated apoptosis independent of lower oxidative capacity (9). Although decreases in physical activity likely initiate the process, lower ATPmax may exacerbate age-related decreases in physical

activity by contributing to the age-related loss of VO_2peak and exercise tolerance (i.e. higher fatigability). Further, increasing ATPmax may be a mechanism by which physical activity can lower fatigability. These relationships warrant further longitudinal study.

This study had several strengths. Mitochondrial function was measured *in vivo*, which reflects actual mitochondrial energy production in the living skeletal muscle as opposed to energy production measured in isolated mitochondria from muscle biopsy (39). Fatigability was measured following a standardized performance test, eliminating any contextual and recall biases associated with self-reported fatigability. This study also had limitations. The small sample size limited our ability to detect independent relationships. Thus, other benefits of physical activity that may lower fatigability could not be separated from higher ATPmax. Although fatigability was assessed following a performance test, a certain degree of subjectivity remained as participants rated their perceived exertion, as opposed to an observed deterioration in performance. This cohort of older adults was also relatively healthy and high functioning, thus it remains unclear whether mitochondrial function contributes to fatigability in lower functioning, more frail populations. However, it was encouraging that in a relatively homogenous cohort in regard to physical function, we saw relationships between ATPmax and fatigability.

In conclusion, we provide novel evidence showing that impaired mitochondrial function may be implicated in the etiologic pathway of age-related fatigability. Understanding the etiology of fatigability is vital to preventing and treating declines in physical function. Impaired mitochondrial function may lead to higher levels of fatigability by contributing to lower maximal aerobic or reserve capacity. Physical inactivity may initiate the process; however, improving mitochondrial function may improve fatigability, which may then increase physical activity levels due to improved exercise tolerance. The causal role of impaired mitochondrial function

and lower aerobic capacity in age-related fatigability needs to be studied longitudinally and in a larger population of older adults across a wider range of physical function.

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Table 1. Demographic Characteristic by High and Low Fatigability

	High Fatigability (RPE ≥ 10, *n* = 12)	**Low Fatigability (RPE ≤ 9, *n* = 18)**	**Entire Cohort (*n* = 30)**
Age, years	79.0 (4.6)	78.2 (5.3)	78.5 (5.0)
Gender, % female	58.3 (7)	38.9 (7)	46.7 (14)
Race, % white	100 (12)	88.9 (16)	93.3 (28)
Body Weight, kg	72.3 (15.6)	71.6 (11.2)	71.9 (12.9)
Body Mass Index, kg•$(m^2)^{-1}$	26.2 (3.0)	25.7 (2.6)	25.9 (2.7)
Moderate Physical Activity, min•day^{-1}	36.8 (24.5)	100.6 (83.7)*	71.2 (66.5)
Smoker, Current/Former	16.7 (2)	44.4 (8)	33.3 (10)
Alcohol Intake, 6+ drinks•$week^{-1}$	8.3 (1)	0.0 (0)	3.3 (1)
Diabetes, yes	0 (0.0)	4.8 (1)	3.3 (1)

History of Myocardial Infarction, yes	16.7 (2)	5.46 (1)	10.0 (3)
History of COPD, yes	8.3 (1)	0.0 (0)	3.3 (1)
History of Osteoarthritis, yes	50.0 (6)	16.7 (3)	30.0 (9)
History of Cancer, yes	41.7 (5)	61.1 (11)	53.3 (16)
CES-D Score	7.7 (3.3)	6.8 (2.8)	7.2 (3.0)
Usual Gait Speed, m•s^{-1}	1.1 (0.2)	1.3 (0.2)	1.2 (0.2)
Time to walk 400m, s	383.5 (79.3)	319.5 (41.4)*	343.8 (65.5)
SPPB Score, 0-12	10.8 (1.5)	10.9 (1.3)	10.9 (1.4)

Notes: Values are mean ± (*SD*) or % (*n*), RPE = rating of perceived exertion, COPD = Chronic Obstructive Pulmonary Disorder, CES-D = Center for Epidemiologic Studies Depression Scale SPPB = short physical performance battery, * = significant difference between fatigability groups, $p < 0.05$

Table 2. Perceived Exertion, Aerobic Capacity, Lactate Levels, Mitochondrial Function and Muscle Size by High and Low Fatigability

	High Fatigability (RPE ≥ 10, *n* = 12)	**Low Fatigability (RPE ≤ 9, *n* = 18)**	**Entire Cohort (*n* = 30)**
VO_2peak, mL•kg^{-1}•min^{-1}	18.9 (4.4)	24.4 (5.8)*	22.3 (5.9)
VO_2peak, mL•min^{-1}	1326.5 (324.7)	1730.0 (432.5)*	1576.9 (436.9)
VO_2 during steady state walk, mL•kg^{-1}•min^{-1}	10.4 (1.0)	10.4 (1.8)	10.4 (1.5)
VO_2 during steady state walk, mL•min^{-1}	744.3 (150.4)	738.8 (156.9)	741.0 (151.8)
% of peak VO_2 reached during steady state walk	58.7 (19.4)	44.9 (13.2)*	50.2 (16.9)

Blood lactate end of steady state walk	1.81 (1.09)	1.63 (1.02)	1.71 (1.03)
ATPmax, mM ATP•s^{-1}	0.47 (0.12)	0.55 (0.14)	0.52 (0.13)
Quadriceps volume, mL	1081.2 (427.1)	1204.1 (307.9)	1154.9 (358.5)

Notes: Values are mean ± (*SD*), RPE = rating of perceived exertion, ATP = adenosine triphosphate, * = significant difference between fatigability groups, $p < 0.05$

Table 3. Logistic Regression Models for the Association between High Fatigability and ATPmax or ATPmax·Quadriceps Volume

	ATPmax		**ATPmax·Quadriceps Volume**	
Model	**Odds Ratio* (95% CI)**	**Wald X^2 *p*-value****	**Odds Ratio* (95% CI)**	**Wald X^2 *p*-value****
Model 1,unadjusted	0.45 (0.20 – 1.17)	0.11	0.39 (0.16 – 0.96)	0.04
Model 2[†]	0.34 (0.11 – 1.01)	0.05	0.37 (0.13 – 1.10)	0.07
Model 3[‡]	0.57	0.35	0.46	0.22

	(0.18 – 1.84)		(0.13 – 1.59)	
Model 4[§]	0.41 (0.09 – 1.83)	0.24	0.62 (0.14 – 2.71)	0.53

Notes: * = per standard deviation increase, [†] = adjusted for age and sex, [‡] = adjusted for age, sex and VO_2peak, [§] = adjusted for age, sex and physical activity, ***p*-value corresponds to the relationship between ATPmax or ATPmax·Quadriceps Volume and Fatigability respectively.

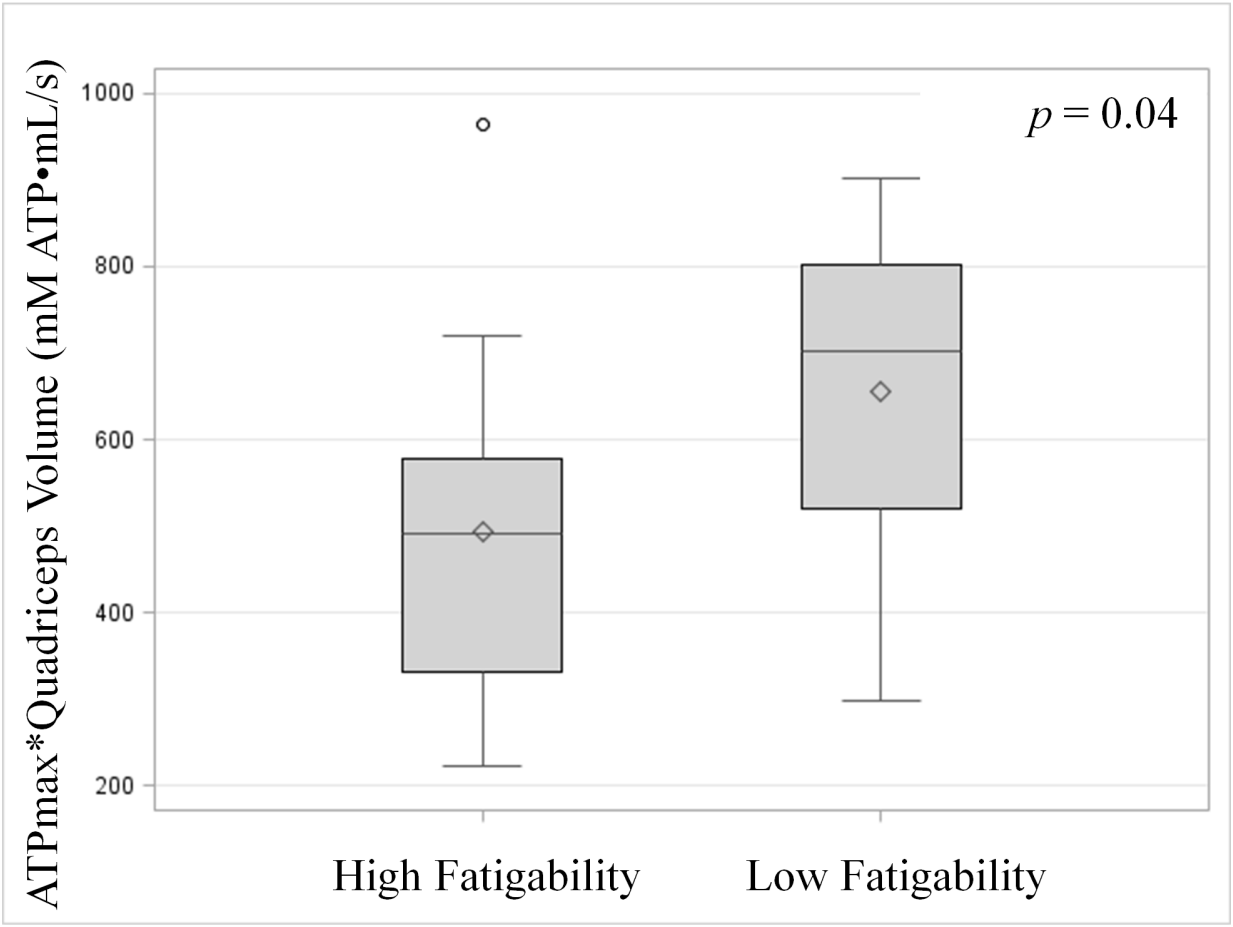

Figure 1. Oxidative Capacity of the Quadriceps by Low and High Fatigability

Captions for Illustrations

Figure 1.

Maximal mitochondrial ATP production defined as phosphocreatine recovery in the quadriceps, following an acute bout of exercise, measured by ^{31}P magnetic resonance spectroscopy, multiplied by quadriceps volume. Fatigability was defined using Rating of Perceived Exertion (RPE, 6-20) after a 5-minute treadmill walk at 0.72m/s. High fatigability = RPE $\geq$10 and low fatigability = RPE $\leq$ 9.