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Skeletal Muscle Mitochondrial Energetics are Associated with Maximal Aerobic Capacity and Walking Speed in Older Adults

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ABSTRACT

Background: Lower ambulatory performance with aging may be related to a reduced oxidative capacity within skeletal muscle. We examined the associations between skeletal muscle mitochondrial capacity and efficiency with walking performance of the elderly. **Methods:** Thirty-seven older adults (mean age 78yrs; 21 male and 16 female) completed an aerobic capacity (VO_2 peak) test and measurement of preferred walking speed over 400 meters. Maximal coupled (State 3; St3) mitochondrial respiration was determined by high-resolution respirometry in saponin-permeabilized myofibers obtained from percutanous biopsies of vastus lateralis (n=22). Maximal phosphorylation capacity (ATP_{max}) of vastus lateralis was determined in vivo by ^{31}P magnetic resonance spectroscopy (n=30). Quadriceps contractile volume was determined by magnetic resonance imaging. Mitochondrial efficiency (max ATP production/ max O_2 consumption) was characterized using ATP_{max} per St3 respiration (ATP_{max}/St3). **Results:** *In vitro* St3 respiration was significantly correlated with *in vivo* ATP_{max} (r^2=0.47, P=0.004). Total oxidative capacity of the quadriceps (St3*quadriceps contractile volume) was a determinant of VO_2 peak (r^2=0.33, P=0.006). ATP_{max} (r^2=0.158, P=0.03) and VO_2 peak (r^2=0.475, P<0.0001) were correlated with preferred walking speed. Inclusion of both ATP_{max}/St3 and VO_2 peak in a multiple linear regression improved the prediction of preferred walking speed (r^2=0.647, P<0.0001), suggesting that mitochondrial efficiency is an important determinant for preferred walking speed. **Conclusions:** Lower mitochondrial capacity and efficiency were both associated with slower walking speed within a group of older participants with a wide range of function. In addition to aerobic capacity, lower mitochondrial capacity and efficiency likely play roles in slowing gait speed with age.

INTRODUCTION

Aging is associated with reduced mitochondrial capacity (1, 2) and the loss of muscle mass and strength (3, 4), which could potentially predispose individuals to frailty and a slower preferential walking speed (5, 6). Preferred walking speed is lower in older adults (7, 8), and slower gait speed is a strong, independent predictor of disability, healthcare utilization, nursing home admission and mortality (9-11). A number of studies have found a close relationship between VO_2peak and walking speed in the elderly (12, 13), which suggests that the decline in aerobic capacity contributes to, and may be predictive of, slower walking speed with age. A reduced efficiency of locomotion is also apparent in the elderly, which leads to an increased metabolic cost of walking (14).

Aging is also associated with declines in both the capacity and efficiency of energy supply in muscle. Several studies using a variety of techniques have reported reduced capacity to generate ATP with age (1, 15, 16). The age-related changes in skeletal muscle mitochondrial function apparent in these studies are consistent with their likely role in the parallel loss of aerobic capacity (1, 16). In addition, reduced mitochondrial efficiency (energy conversion of O_2 uptake into ATP generation) has been reported in a variety of tissues in vitro and in vivo (17-19). The importance of mitochondrial efficiency is that it could impact the ability to generate ATP during ambulation (16) as well as movement efficiency (20-22). One study found that walking speed in patients with peripheral arterial disease was related to their capacity for ATP generation assessed by phosphorus magnetic resonance spectroscopy (^{31}P MRS) (23). While the loss of muscle mitochondrial function has been widely hypothesized to contribute to the decline in VO_2 max and slowing of locomotion with age, there currently is insufficient published evidence to show that reductions in available energy results in a decline in customary walking speed with

aging and disease (11). Furthermore, the potential role of mitochondrial efficiencey in energy availablity for walking has not been examined.

The goal of this study was to test the hypothesis that reduced mitochondrial capacity and efficiency are associated with slower walking speed in older adults. We combined *in vitro* (respirometry) and *in vivo* (^{31}P MRS) measurements of mitochondrial function and related them to whole body aerobic capacity (VO_2 peak) and preferred walking speed in a group of older men and women. High resolution respirometry of permeabilized fibers isolated from muscle biopsy specimens yielded mitochondrial oxidative capacity (State 3 or St3 respiration). These measures of mitochondrial capacity at cellular level were extended to the muscle tissue level by assessing quadriceps muscle volume with magnetic resonance imaging. ^{31}P MRS was used to determine the maximum mitochondrial ATP production (ATP_{max}) in vivo, which was combined with St3 respiration to yield an index of mitochondrial efficiency (ATP_{max}/St3). Our study tested the paradigm that muscle mitochondrial properties impact walking speed and that the mitochondria capacity and efficiency may be associated with the decline in mobility with age.

METHODS

Recruitment: Participants were community dwelling, ambulatory men and women aged 70-89 years from the Pittsburgh, PA area. A telephone interview was initially conducted to determine eligibility. The inclusion criteria were: age 70-89 years; body weight ≤ 285 lbs for men, ≤ 250 lbs for women; body mass index (BMI) 20-32 kg/m^2; ability to walk without the assistance of a device or another person; free of basic activities of daily living (ADL) disability, defined as no difficulty getting in and out of bed or chairs, and no difficulty walking across a small room; no history of hip fracture; no heart attack, angioplasty, or heart surgery within the past 3 months, no cerebral hemorrhage within the past 6 months, stroke within the past 12 months, or chest pain during walking in the past 30 days; no symptomatic cardiovascular or pulmonary disease; no regular pain, aching, or stiffness in the legs, hips, knees, feet, or ankles when walking; no bilateral difficulty bending or straightening fully the knees; not regularly taking Coumadin, Plavix, Aggrenox, Ticlid, or Agrylin/Xagrid. All participants provided written informed consent. The study was approved by the University of Pittsburgh Institutional Review Board.

Testing Schedule: The clinic examination involved three visits. During the first visit potential participants were asked to read and sign an informed consent document. Measurements included height, weight, blood pressure and resting pulse. A physical examination was also conducted along with a review of clinical information including self-reported physical function, medical history, and medication inventory. A physical activity scale for the elderly (PASE) questionairre was completed and a final summary score was calculated (24). A short physical performance battery (SPPB) was conducted and an overall score was calculated (25). A 400-

meter walk test was conducted to determine self-selected walking speed. Participants were also given a 5-minute practice session on the treadmill to become acquainted with treadmill walking prior to the VO_2 peak test conducted in a subsequent visit. The second visit involved ^{31}P magnetic resonance spectroscopy (MRS) and imaging and a graded exercise test to determine VO_2 peak. The third visit involved muscle tissue collection.

400 meter walk test: The 400 meter walk test assessed the participant's ability to complete a 400 meter walking course in 15 minutes or less without sitting down or stopping, without help or the use of any assistive device. Participants were instructed to complete the distance at their usual pace and without overexerting themselves. Participants were remined to walk at their usual pace every lap. Seated blood pressure and pulse were reviewed for safety before the walk. Prefered walking speed was calculated as total meters walked/total time in seconds.

VO_2 peak test: Maximal whole body oxygen consumption (VO_2 peak) was determined by a graded treadmill exercise test (26). A resting 12-lead electrocardiogram (ECG) was conducted prior to the VO_2 peak test to screen for cardiac arrhythmias. To ensure participant safety, continuous ECG monitoring was also performed during the VO_2 peak test. During the test, the participant's self-selected usual walking speed was used and the treadmill grade was increased by 2% every 2 minutes until attainment of peak VO_2. The test was terminated as per the criteria outlined in the American College of Sports Medicine (ACSM) guidelines (26).

Muscle Biopsy procedure and preparation of permeabilized muscle fiber bundle: Percutaneous biopsies were obtained at the University of Pittsburgh's Clinical Translational

Research Center (CTRC) on a morning after an overnight fast. Participants were instructed not to perform physical exercise 48 h prior to the muscle biopsy procedure. Muscle biopsy samples were obtained from the middle region of the musculus vastus lateralis as described previously (27). Following the procedure, the biopsy specimen was immediately blotted dry of blood and interstitial fluid and dissected free of any connective tissue and intermuscular fat. A portion of the biopsy specimen (~10 mg) was immediately placed in ice-cold BIOPS solution (10 mM Ca-EGTA buffer, 0.1 M free calcium, 20 mM imidazole, 20 mM taurine, 50 mM potassium 2-(n-morpholino)-enthanosulfonic acid, 0.5 mM dithio- threitol, 6.56 mM $MgCl_2$, 5.77 mM ATP and 15 mM phosphocreatine, pH 7.1). The individual muscle fibers in the sample were then gently teased apart in a petri dish containing ice cold BIOPS solution using fine-nosed forceps and a dissecting microscope (Leica Microsystems, Heerbrugg, Switzerland). The fiber bundles were then permeabilized with saponin (2 ml of 50 ug/ml saponin in BIOPS solution) for 20 min at 4^{o}C on an orbital shaker, and then washed twice for 10 min at 4^{o}C with Mir05 respiration medium (0.5 mM EGTA, 3 mM $MgCl_2.6H_2O$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, and 1 g/L BSA, pH 7.1) on an orbital shaker (28). The permeabilized muscle fiber bundles were then placed into the respiration chambers of an Oxygraph 2K (Oroboros Inc, Innsbruck, Aus).

Mitochondrial respiration protocol: Measurement of oxygen consumption in permeablized fibers was conducted over a ~1hr 40 min period, at 37^{o}C and in the oxygen concentration range 220-150 nmol O_2/ml (see supplemental method for full protocol). Following the assay, the fiber bundles were recovered and dried. A dry weight was then determined with an analytical balance (Mettler Toledo, XS105). Steady state O_2 flux for each respiratory state was determined and

normalized to fiber bundle weight using Datlab 4 software (Oroboros Inc., Innsbruck, Aus).

Determination of ATP_{max} *by* ^{31}P *MRS:* Maximal mitochondrial ATP production *in vivo* (ATP_{max}) following an acute bout of knee extensor exercise was determined using phosphorus magnetic resonance spectroscopy (^{31}P MRS). Recovery of phosphocreatine (PCr) levels after exercise is used to characterize rates of mitochondrial ATP resynthesis (production). The validity of this method is confirmed by animal and human studies showing that ATP_{max} varies in direct proportion to the oxidative enzyme activity of healthy muscle (29, 30) and corresponds with mitochondrial content in human muscle (16). Repeat measurements of muscle ATP_{max} have been shown to agree to within about 7% (31).

Exercise Protocol: The exercise protocol was designed to deplete [PCr] of the quadriceps muscles with minimal acidification to achieve a high [ADP] and thus maximize oxidative phosphorylation [30]. Participants lay supine in the scanner's bore with the knee supported in about 30^{o} of flexion. Sandbags and padding were placed on both sides of the ankle and knee for support and straps placed across the distal leg, thigh and hips restricted limb movement. Participants performed strong, fast contractions of the quadriceps muscle at the highest rate possible for 24 - 36 s, followed by 6 min of rest. Most participants repeated this protocol twice, with 2 different exercise times, to assure that in at least one bout [PCr] was reduced by 33 – 66% of basal and that muscle pH did not fall <6.80 during recovery. Participants were trained to perform the exercise before entering the magnet.

^{31}P MRS: We collected phosphorus spectra using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System, Erlanger, Germany) (see supplemental method). A standard

one pulse experiment was used to determine the levels of PCr, ATP, P_i, and pH throughout exercise and recovery.

PCr, Pi, 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 P_i peaks were expressed relative to the ATP peak and quantified using a resting PCr value of 27 mM as determined from biopsies of human vastus lateralis muscle (16). Changes in PCr and Pi peak areas during the experiments were analyzed as previously described (32, 33).

Determination of Muscle size: We used MR imaging to determine quadriceps cross-sectional area and volume according to a previously described method (see supplemental method) (35). Using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System, Erlanger, Germany), we collected images every 3 cm from the hip to the thigh (15-25 slices per subject). The patient lay supine for imaging. Standard stereologic techniques were used to determine the largest muscle CSA for the quadriceps (35). Subcutaneous and intramuscular fat and other non-contractile tissues were excluded from the calculation of muscle contractile CSA.

Statistical Analysis: All data are presented as mean ± standard deviation unless otherwise stated. Pearson correlation coefficients were used to examine relationships between variables. A multiple linear regression model was used to predict preferred walking speed from VO_2 peak and mitochondrial efficiency (ATP_{max}/State 3 respiration).

RESULTS

Participant Characteristics: A total of 179 potential participants were screened by telephone interview. Of those interviewed, 99 individuals were ineligible and 43 were not interested inparticipating. A total of 37 older adults (21 men, 16 women); who were normal weight to slightly overweight were studied (Table 1). The group had on average a relatively low and widely ranging level of aerobic fitness defined by VO_2 peak (Table 1). This group of older subjects also had a fairly wide range of preferred walking speed, and the SPPB scores were indicative of low to moderate lower extremity function (Table 1).

Muscle Magnetic Resonance Measurements: Due to exclusions from MRI, e.g., history of metal work or claustrophobia, a subsample of individuals (n = 30; 16 men, 14 women) had ATP_{max} determined by ^{31}P MRS. The average ATP resynthesis rate (ATP_{max}) was 0.52 mM ATP/sec and covered a >2.5-fold range (0.32-0.83) in agreement with reports on older adults (16). The quadriceps contractile muscle size determined from MRI was also consistent with values previously reported for older participants (36). The coefficient of variation for repeat determinations on 8 participants was 7.2% for ATP_{max}, and 3% for quadriceps volume.

Respirometry Measurements: A subsample of individuals (n = 22; 12 men, 10 women) had muscle biopsies that were studied by high-resolution respirometry. The maximal coupled respiratory capacity (State 3 respiration) of vastus lateralis permeabilized fiber bundles ranged >5-fold (Table 1). The non-phosphorylating rate of respiration (State 4 respiration) displayed a similar wide range as State 3. A respiratory control ratio (state 3/state 4) of 11.8 indicated good preparation of permeabilized muscle fiber bundles (37). These data are in agreement with previous respirometry measurements on permeabilized fibers from elderly subjects (75 yrs old) with State 3 and State 4 respiration averaging 199 and 24 pmol s^{-1} mg DW^{-1}, respectively, based

on Figure 1 from ref. (38) and wet/dry weight conversion from ref. (39). The coefficient of variation of the respirometry assay for this study was determined to be 16.9 % (State 3 respiration, determined from 6 participants). A representitive oxygraph is presented in the supplemental section (Figure S1)

Associations among in vitro and in vivo muscle and whole-body oxidative capacity: Maximal coupled, State 3 respiration (r^2=0.47, P=0.004). and maximal uncoupled respiration (r2=0.47, P=0.002) (table S1) measured in intact muscle fibers from the biopsy was significantly correlated with whole muscle ATP_{max}, indicating a close relationship between the measurements of oxidative capacity of permeabilized fibers and phosphorylation capacity of intact muscle.

The relationship of muscle respiratory and oxidative capacity to whole body aerobic capacity was evaluated by combining respirometry data and MRI measurements of quadriceps contractile volume. A measure of muscle oxidative capacity was derived from the product of state 3 respiration and the volume of quadriceps muscle ($St3 \bullet V_Q$). Figure 3 shows that variation in VO_2 peak is significantly correlated with the quadriceps' oxidative capacity (r^2=0.33, P<0.0061). These associations were consistent for both men and women. This finding is also consistent with a prior study in elderly subjects (36).

The Impact of Energetics on Walking Speed: The range of VO_2 peak among participants accounted for 48% of the variation in preferred walking speed over 400m (Figure 4, Panel A: r^2=0.48, P<0.0001), in agreement with previous studies of energetics in older adults (12, 13). It was also found that ATP_{max} accounted for 15.8% of the variation in preferred walking speed (Figure 4, Panel B: r^2 = 0.158, P = 0.03). The impact of mitochondrial efficiency (ATP_{max}/St3) on the relationship between aerobic capacity (VO_2peak) and walking performance was tested using a multiple linear regression. Table 2 shows that independent of VO_2peak, mitochondrial

efficiency was borderline associated with preferred walking speed (ATP_{max}/St3; P = 0.057). Together, however, VO_2peak and ATP_{max}/St3, predicted ~65% of the variation in preferred walking speed (Figure 5, $r^2 = 0.647$, P<0.0001) as compared to 47.5% of the variation by VO_2 peak alone. Adding gender to the model did not significantly affect these associations. The correlations between all respirometry states, MRS, VO_2 peak and walking speed are presented in the supplemental section (Table S1).

DISCUSSION

This study provides novel evidence obtained at the cellular, tissue and whole-body level that skeletal muscle mitochondrial capacity and efficiency are associated with preferred walking speed in older men and women. First, we found that muscle oxidative and phosphorylation capacities, whole body aerobic capacity, and walking speed all varied many fold among older adults in accordance with their fairly broad range in function. Second, muscle mitochondrial capacity and efficiency along with whole body aerobic capacity were directly associated with walking speed. Aerobic capacity (VO_2 peak) varied in proportion to muscle respiratory capacity as measured by State 3 respiration (oxidative capacity) of permeabilized fibers combined with quadriceps volume (Fig. 3). Aerobic capacity and mitochondrial capacity were also strongly correlated with walking speed (Fig. 4). Our third key finding was that muscle mitochondrial efficiency (ATP_{max}/St3) provided independent explanatory power to predict walkng speed, additional to that provided by VO_2 peak alone. These data indicate that muscle mitochondrial capacity and efficiency are associated with ambulatory performance of older adults.

Mitochondrial capacity of muscle fibers: We first compared mitochondrial respiratory capacity determined from permeabilized muscle fibers against phosphorylation capacity of whole muscle. We found that State 3 respiration was directly proportional to ATP_{max} over the many-fold range of properties found among these participants. Thus, a higher oxidative capacity of the muscle fiber is reflected in a higher oxidative phosphorylation capacity in whole muscle. This finding is in agreement with studies correlating mitochondrial content and enzymatic activity of muscle biopsies with ATP_{max} (16, 29). Thus, for the first time we compare two separate measures of mitochondrial energetics, determined *in vitro* by respirometry and *in vivo* by ^{31}P-MRS, in

older adults and find a correspondence between mitochondrial respiratory capacity and whole muscle phosphorylation capacity.

Impact of metabolic capacity and efficiency on mobility: Our key question was whether or not – and the extent to which - metabolic capacity and efficiency are related to walking performance. We tested the hypothesis that walking speed is not only affected by whole body aerobic capacity (VO_2 peak) but also by muscle mitochondrial efficiency (ATP_{max}/St3). The contribution of both aerobic capacity and mitochondrial efficiency on walking speed is apparent in a multiple linear regression model shown in Table 2. In this model, the positive coefficient for VO_2 peak implies that a higher aerobic capacity is associated with a faster preferred walking speed. Similarly, the positive coefficient for ATP_{max}/St3 implies that greater mitochondrial efficiency has a beneficial effect on walking speed. Together, aerobic capacity and mitochondria efficiency accounted for 64.7% of the variation in walking speed, whereas VO_2 peak alone accounted for only 47.5% of the variation. The contribution of mitochondrial efficiency, independent of VO_2 peak, can be further highlighted by examining data from individual subjects. For example, for two subjects with similar VO_2peaks, one subject had a higher walking speed (1.5 m/sec) and high mitochondrial efficiency (7.9×10^3 (mM ATP/sec)/(pmol O_2 /sec*mg DW)), while the second had a lower walking speed (0.74 m/sec) and low mitochondrial efficiency (2.3×10^3 (mM ATP/sec)/(pmol O_2 /sec*mg DW)). Alterations in mitochondrial efficiency of ATP production may be caused by reduced inner mitochondrial membrane leak, or by reduced electron leak from the electron transport chain. Further studies are warranted to determine the causal factors mediating mitochondrial efficiency in the elderly. This is the first study, to our knowledge, to demonstrate that greater muscle mitochondrial efficiency may play a direct role in gait speed in the elderly.

Muscle impact on metabolic capacity: Here, we combine mitochondrial respiratory capacity (St 3) with quadriceps contractile volume (V_Q) to extend the oxidative capacity of the muscle fibers to that of the whole quadriceps. We found that the oxidative capacity of the quadriceps accounted for 33% of the variation in VO_2 peak (Fig. 3), a finding that is in agreement with a prior study of individuals 20-80 yrs old (36). This agreement suggests that mitochondria play an important role in determining cardiorespiratory fitness in older individuals. In contrast, a study of young and master endurance-trained athletes concluded that cardiac output and O_2 delivery to the muscle likely sets the limits to the aerobic capacity (40). These highly active older adults may well have reached the limits to oxygen delivery, as found in younger athletes (average age: 26.1 years old) (41). However, direct measurements demonstrating an O_2 delivery limit in athletic individuals (41) fail to find a similar limitation in more sedentary people (42). The lack of O_2 delivery limitation in less active older participants is evident in the scaling of maximum O_2 uptake in proportion to the muscle's capacity for O_2 consumption in the older adults that is apparent in Figure 3 and reported previously (36). These data indicate that in non-athletic older participants with a wide variation in physical function, muscle mitochondrial capacity is an important factor in addition to the cardiovascular system in determining VO_2 peak across age. Thus these data suggest that interventions to enhance muscle mitochondria could have important effects to improve exercise tolerance and function in relatively sedentary older adults.

There are some potential limitations and caveats to this study. Firstly, the strong relationship between VO_2 peak and quadriceps oxidative capacity (Fig. 3) is dependent on one or two data points. A larger study with more participants would provide a more definitive view of this relationship. Secondly, although the range of functional performance of the older adult

participants was fairly broad, we studied 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 gait speed. Nevertheless, we believe that these findings may be clinically relevant, since walking speed has recently been identified as an important determinant of health and mortality in older men and women (9, 10). Thirdly, despite the lack of significant gender effect on these associations, our study was not adequately powered to examine gender-specific associations. Thus larger studies are warranted to determine whether or not mitochondrial energetics are more or less strongly associated with function in men and women specifically.

In conclusion, muscle mitochondrial capacity and efficiency are related to walking speed in older adults, and that the loss of mitochondrial capacity and efficiency with age may be important contributors to the reduction in mobility and increase in disability. Future prospective longitudinal studies should determine whether mitochondrial energetics predicts the decline in walking speed and function as well as incident mobility limitations.

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TABLE 1: **Descriptive, metabolic and physiological data for study participants.**

n	37 (M=21, F=16)
Age (yrs)	78.3 ± 4.9 (60-89)
Weight (Kg)	72.0 ± 12 (53-97)
BMI (Kg/m^2)	25.7 ± 2.6 (21.4-31.2)
Quadriceps contractile volume (ml)	1159 ± 324 (589-1886), n=30
ATPmax (mM ATP/s)	0.52 ± 0.1 (0.32-0.83), n=30
State 3 respiration (pmol/(s*mg DW)	174 ± 68 (52-303), n=22
State 4 respiration (pmol/(s*mg DW)	16.4 ± 7.1 (4.0-30.8), n=22
Respiratory control ratio	11.8 ± 5.1 (6.1-26), n=22
Mitochondrial efficiency; ATPmax/state 3 resp. ((mM ATP/sec)/(pmol O_2 /sec*mg DW))	3.5 ± 1.7 $x10^3$ (1.8 $x10^3$-7.9 $x10^3$), n=18
Quad. oxidative capacity; state 3 resp. x muscle vol. ((pmol O_2 /sec*mg DW)* ml muscle)	209 ± 89.4$x10^3$ (78$x10^3$- 488$x10^3$), n=22
VO_2 Peak (ml/min)	1551.5 ± 408 (750-2724)
VO_2 Peak (ml/KgBW/min)	22.0 ± 5.5 (7.8-33.4)
Preferred walking speed over 400m (m/sec)	1.2 ± 0.2 (0.74 -1.58)
SPPB Score	10.9 ± 1.3 (7-12)
PASE Score	133 ± 55 (15-274)

Values are average ± standard deviation (Min-Max). DW, dry weight of tissue; BW, body weight; SPPB, short performance physical battery; PASE, physical activity scale for the elderly.

TABLE 2: Multiple linear regression results for the model of preferred walking speed as a function of aerobic capacity per body mass (VO_2peak; ml/kgBW/min) and mitochondrial efficiency (ATP_{max}/St3).

Analysis of Variance

Variable	Coefficient	Standard Error	F value	P value
Intercept	0.4561	0.143	10.11	0.0062
ATP_{max}/St3	37.786	18.3	4.26	0.0567
VO_2peak	0.0272	0.005	24.18	0.0002

Summary of Stepwise Selection

Step	Variable Entered	Partial R-Squared	Model R-Squared
1	VO_2peak	0.4753	0.4753
2	ATP_{max}/St3	0.1714	0.6467

FIGURE LEGENDS

FIGURE 1: Concept map illustrating age related changes in muscle physiology and how they contribute to reduced walking speed in the elderly. This study examined the relationships between muscle mitochondrial capacity/efficiency, aerobic capacity and walking speed in the elderly. VO_2 peak = maximal oxygen consumption during maximal dynamic exercise. This is an index of whole body aerobic capacity.

FIGURE 2: Pearson correlation of maximum respiratory capacity with maximum oxidative phosphorylation in muscle. State 3 respiration in permeabilized fiber bundles was determined by high resolution respirometry. Maximum oxidative phosphorylation (ATPmax) elicited by exercise was determined by ^{31}P MRS. DW: Dry Weight.

FIGURE 3: Pearson correlation of whole body aerobic capacity with muscle oxidative capacity. Aerobic capacity (VO_2 peak) was determined by a graded exercise test. Muscle oxidative capacity was defined as the product of state 3 respiration and quadriceps contractile volume (State 3 respiration * Quad Contractile Vol.). DW: Dry Weight.

FIGURE 4: Pearson correlation of preferred walking speed with whole body aerobic capacity and muscle mitochondrial capacity. Panel A; preferred walking speed versus VO_2 peak. Panel B; preffered waslking speed versus ATPmax. Aerobic capacity normalized to body weight (ml/kgBW/min) was determined by a graded exercise test. Preferred walking speed was determined over a 400m walk test. ATPmax was determiend by ^{31}P magnetic resonance spectroscopy (MRS).

FIGURE 5: Pearson correlation of predicted preferred walking speed versus measured preferred walking speed. Walking speed was predicted from aerobic capacity (VO_2 peak) and muscle mitochondrial efficiency (ATP_{max}/St3) by multiple linear regression (Table 2). Measured preferred walking speed was determined over a 400m walk test.

FIGURE 1:

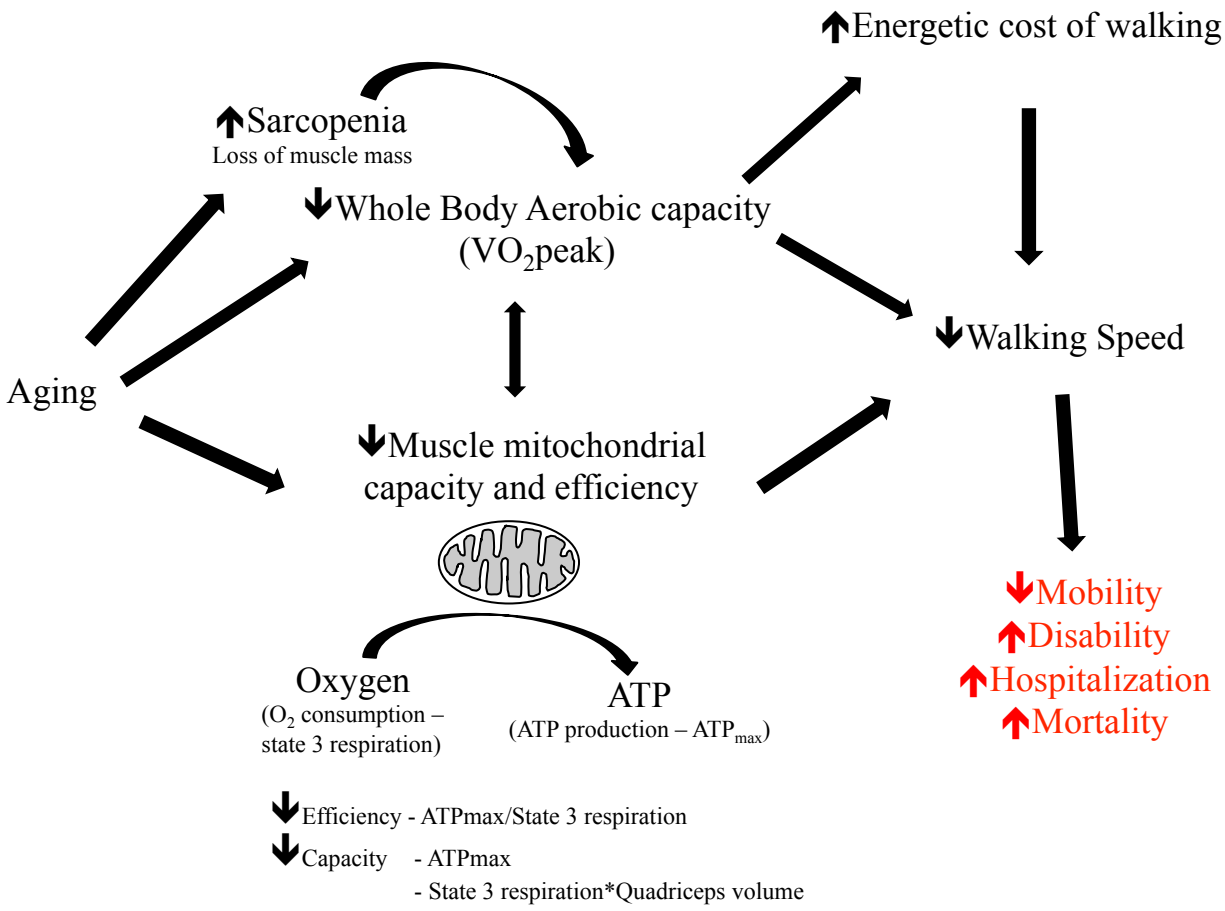

↑Energetic cost of walking
↑Sarcopenia
Loss of muscle mass
↓Whole Body Aerobic capacity
(VO_2peak)
↓Walking Speed
Aging
↓Muscle mitochondrial capacity and efficiency
↓Mobility
↑Disability
↑Hospitalization
↑Mortality
Oxygen
(O_2 consumption – state 3 respiration)
ATP
(ATP production – ATP_{max})
↓Efficiency - ATPmax/State 3 respiration
↓Capacity - ATPmax
- State 3 respiration*Quadriceps volume

FIGURE 2

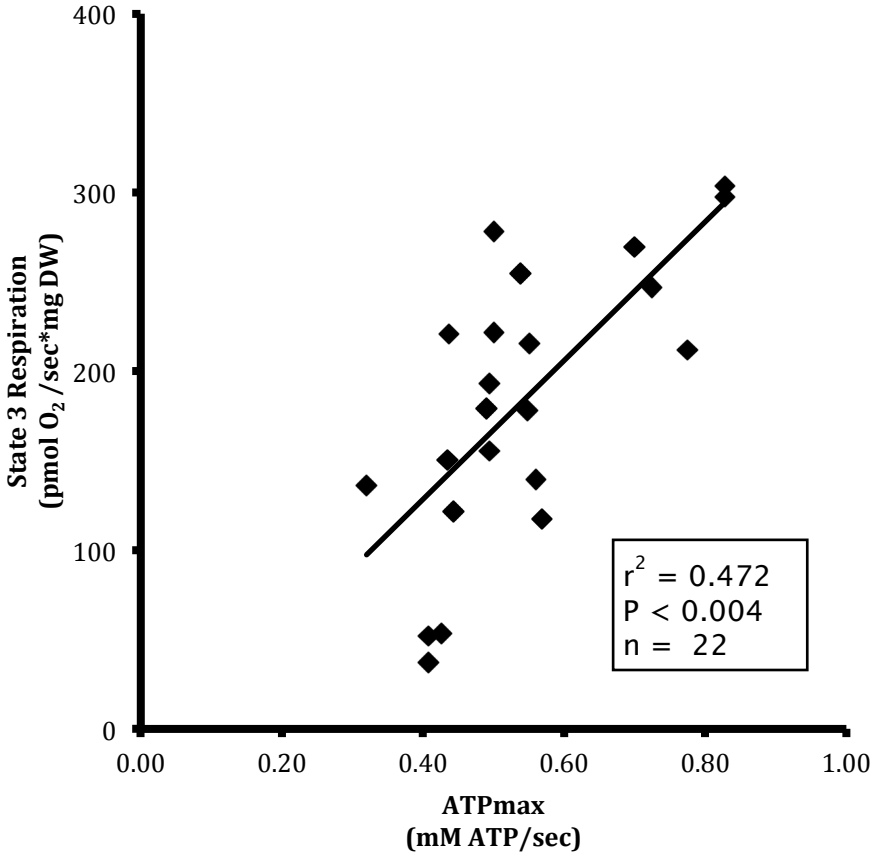

400
300
200
100
0
State 3 Respiration
(pmol O_2 /sec*mg DW)
0.00
0.20
0.40
0.60
0.80
1.00
ATPmax
(mM ATP/sec)
r^2 = 0.472
P < 0.004
n = 22

FIGURE 3

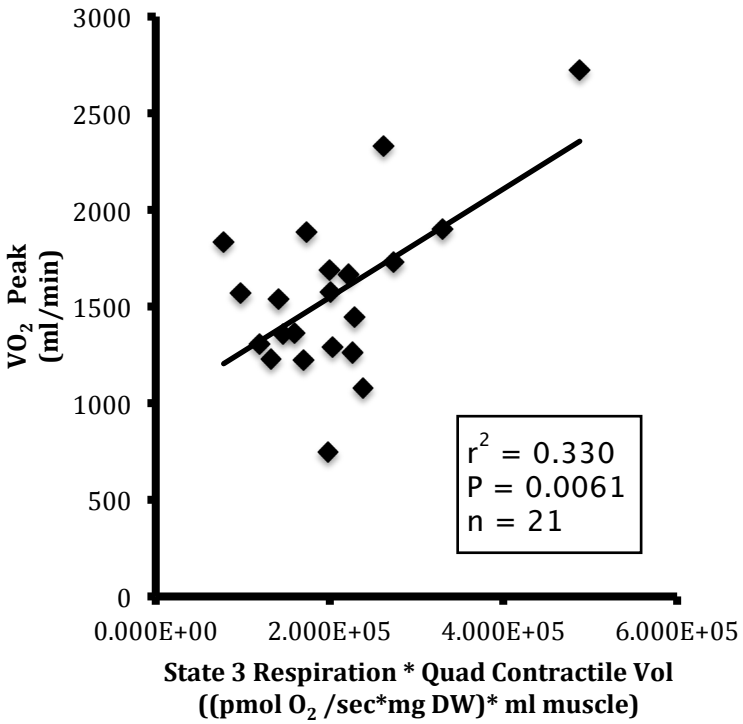
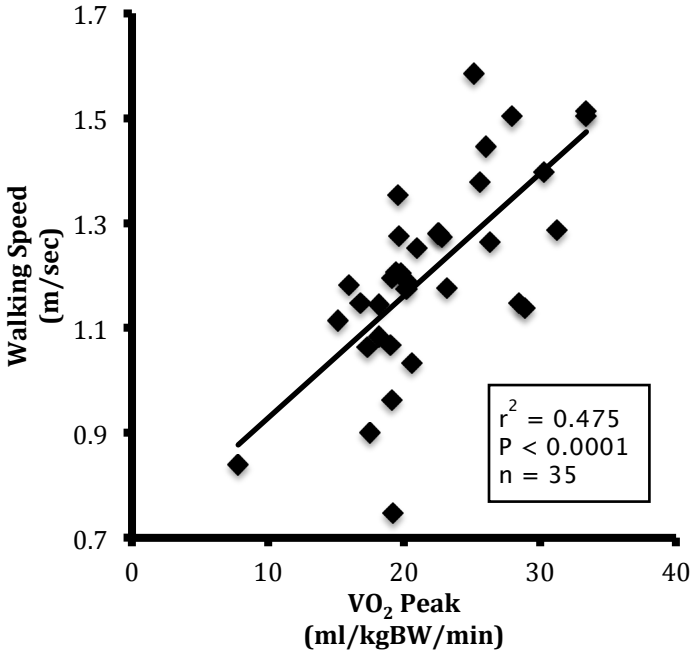

3000
2500
2000
1500
1000
500
0
VO_2 Peak (ml/min)
r^2 = 0.330
P = 0.0061
n = 21
0.000E+00
2.000E+05
4.000E+05
6.000E+05
State 3 Respiration * Quad Contractile Vol
((pmol O_2 /sec*mg DW)* ml muscle)

FIGURE 4:

A


1.7
1.5
1.3
1.1
0.9
0.7
Walking Speed (m/sec)
0
10
20
30
40
VO2 Peak (ml/kgBW/min)
r2 = 0.475
P < 0.0001
n = 35

B

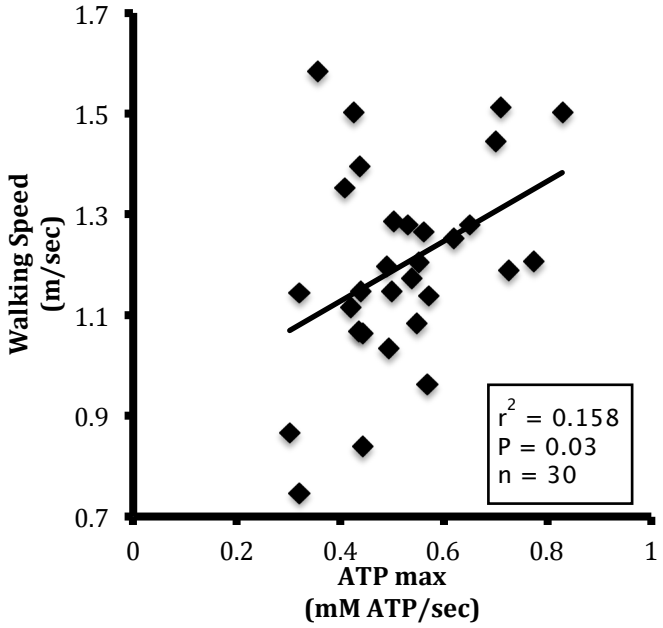
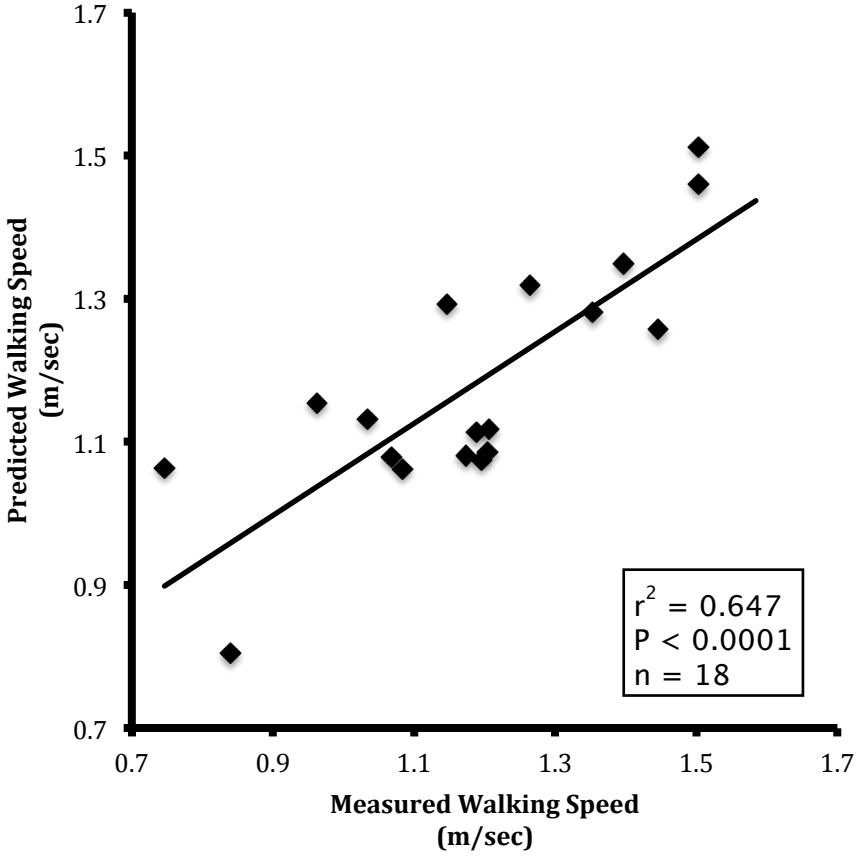

1.7
1.5
1.3
1.1
0.9
0.7
Walking Speed (m/sec)
0
0.2
0.4
0.6
0.8
1
ATP max (mM ATP/sec)
r2 = 0.158
P = 0.03
n = 30

FIGURE 5:


1.7
1.5
1.3
1.1
0.9
0.7
Predicted Walking Speed (m/sec)
0.7
0.9
1.1
1.3
1.5
1.7
Measured Walking Speed (m/sec)
r2 = 0.647
P < 0.0001
n = 18

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SUPPLEMENTAL:

Methods:

Mitochondrial respiration protocol: Measurement of oxygen consumption in permeablized fibers was conducted over a ~1hr 40 min period, at 37^{o}C and in the oxygen concentration range 220-150 nmol O_2/ml. State 4 respiration (non-phosphorylating inner membrane proton leak) was measured following the addition of saturating concentrations of malate (2 mM), pyruvate (5 mM) and glutamate (10 mM). Maximal respiration supported by electron flux through complex I was measured with the addition of ADP (5 mM). The integrity of the outer mitochondrial membrane was assessed by the addition of cytochrome C (10 mM). State 3 respiration (maximal coupled respiration) with convergent electron flux through complex I and complex II was achieved by adding saturating concentrations of succinate (10 mM). Maximal uncoupled respiration was achieved with the addition of FCCP (1 μM). The complex I inhibitor rotenone (2 μM) was then added to measure the rate of respiration through complex II alone. Finally, antimycin A (5 μM) was added to inhibit complex III and thus total ETC respiration. Following the assay, the fiber bundles were recovered and dried. A dry weight was then determined with an analytical balance (Mettler Toledo, XS105). Steady state O_2 flux for each respiratory state was determined and normalized to fiber bundle weight using Datlab 4 software (Oroboros Inc., Innsbruck, Aus).

^{31}P MRS: We collected phosphorus spectra using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System, Erlanger, Germany). A 2.5" surface RF coil tuned to ^{31}P was placed over the vastus lateralis muscle. After shimming on water proton to optimize B_0 field homogeneity and determining the optimal pulse power, we collected a fully-relaxed, high resolution ^{31}P spectrum of the resting muscle (16 free-induction decays [FID] with a 16 s interpulse delay, spectral width of ± 5000 Hz, and 2048 data points). Following this, a standard

one pulse experiment under partially saturated conditions (1.5 s interpulse delay) was used to determine the levels of PCr, ATP, P_i, and pH throughout exercise and recovery. Four FIDs were averaged per spectrum, resulting in a time resolution of 6 s.

The FIDs were line-broadened with the half-height width of the resting PCr peak and Fourier-transformed into spectra. PCr, Pi, 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 P_i peaks were expressed relative to the ATP peak and quantified using a resting PCr value of 27 mM as determined from biopsies of human vastus lateralis muscle (16). Changes in PCr and Pi peak areas during the experiments were analyzed as previously described (32, 33). A monoexponential fit of [PCr] recovery following exercise yielded the recovery time constant (τ) for use in calculating ATP_{max}: $ATP_{max} = [PCr]_{rest}/ \tau_{PCr}$ (34). Finally, we determined pH from the chemical shift of the P_i peak relative to the PCr peak [33].

Determination of Muscle size: We used MR imaging to determine quadriceps cross-sectional area and volume according to a previously described method (35). Using a 3T TIM Trio magnetic resonance scanner (Siemen's Medical System, Erlanger, Germany), we collected axial plane T_1-weighted, 2D spin echo images every 3 cm from the hip to the thigh (15-25 slices per subject). Our collection parameters were: TR/TE = 600/10, 5 mm slice thickness, 25 mm interslice interval, 320 x 320, and 2 NEX. The patient lay supine for imaging. Standard stereologic techniques were used to determine the largest muscle CSA for the quadriceps (35). Quadriceps volume was calculated as: $\Sigma(CSA_{slice}$ x slice thickness). Subcutaneous and intramuscular fat and other non-contractile tissues were excluded from the calculation of muscle contractile CSA.

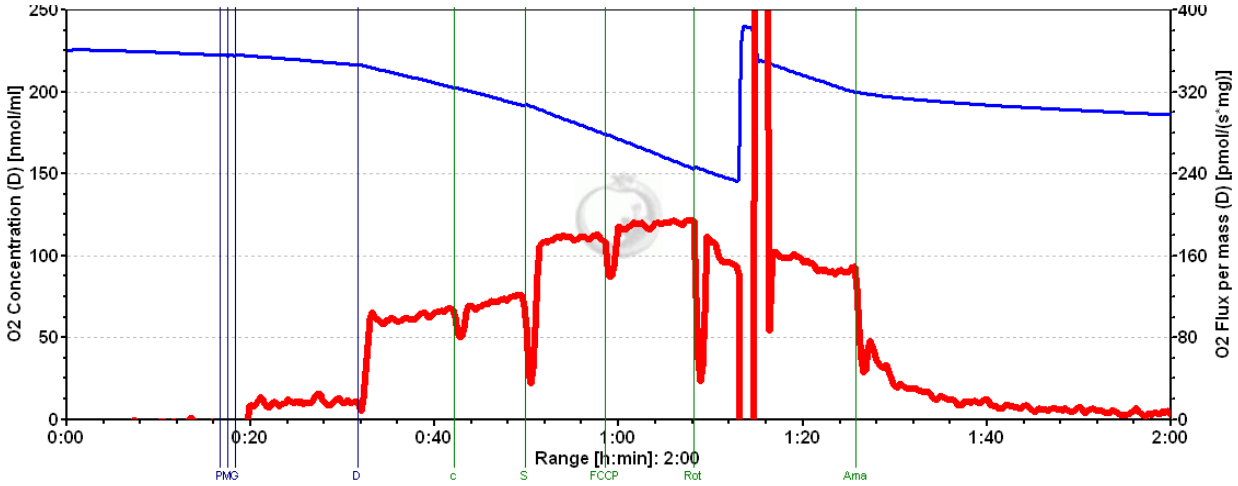

FIGURE S1. Representitative oxygraph generated from the substrate/inhibitor/uncoupler titration protocol employed in this study. The red line represents O_2 flux and the blue line represents O_2 concentration within the respiration chamber. Additions during the protocol included; P: Pyruvate, M:Malate, G: Glutamate, D: ADP, c: Cytochrome C, S: Succinate, FCCP: carbonyl cyanide p-trifluoromethoxy-phenylhydrazone, Rot: Rotenone, Ama: Antimycin A. The protocol was typically run over 1hr 40min.

		Pearson Correlation Coefficient													
		VO2 Peak	VO2 Peak	400 M walk speed	ATPmax	ATPmax/State3	Quadriceps Vol.	State 3*Quad Vol.	(State 4) PMG	PMGD	PMGDC	(State 3) PMGSD	F	ROT	AMA
VO2 Peak (ml/min)	Pearson Correlation	1	.763**	.462**	0.12	-0.04	.446*	.578**	0.364	0.19	0.197	0.317	0.346	.427*	-0.065
	Sig. (2-tailed)		0	0.005	0.536	0.873	0.011	0.006	0.095	0.396	0.379	0.151	0.114	0.047	0.774
VO2 Peak (ml/min/kgBW)	Pearson Correlation	.763**	1	.693**	.369*	-0.052	0.042	0.354	.487*	0.416	0.399	0.408	.487*	0.401	-0.082
	Sig. (2-tailed)	0		0	0.049	0.838	0.817	0.115	0.022	0.054	0.066	0.06	0.022	0.064	0.717
400 M walk speed (m/sec)	Pearson Correlation	.462**	.693**	1	.398*	0.278	-0.077	-0.005	0.263	0.291	0.275	0.249	0.313	0.104	-0.241
	Sig. (2-tailed)	0.005	0		0.03	0.264	0.664	0.984	0.225	0.178	0.204	0.252	0.145	0.637	0.269
ATPmax (mM ATP/s)	Pearson Correlation	0.12	.369*	.398*	1	-0.22	-0.205	0.34	0.255	.756**	.763**	.687**	.689**	.484*	0.066
	Sig. (2-tailed)	0.536	0.049	0.03		0.38	0.277	0.168	0.307	0	0	0.004	0.002	0.042	0.793
ATPmax/State3 respiration	Pearson Correlation	-0.04	-0.052	0.278	-0.22	1	.496*	-.616**	-.665**	-.540*	-.558*	-.784**	-.702**	-.781**	-.571*
(mM ATP/s)/(pmol/(s*mg DW)	Sig. (2-tailed)	0.873	0.838	0.264	0.38		0.036	0.006	0.003	0.021	0.016	0	0.001	0	0.013
Quadriceps Vol. (ml)	Pearson Correlation	.446*	0.042	-0.077	-0.205	.496*	1	0.192	-0.318	-.461*	-.452*	-.450*	-0.378	-0.227	-0.126
	Sig. (2-tailed)	0.011	0.817	0.664	0.277	0.036		0.392	0.149	0.031	0.035	0.036	0.083	0.31	0.575
State 3*Quad Vol.	Pearson Correlation	.578**	0.354	-0.005	0.34	-.616**	0.192	1	.572**	.571**	.589**	.759**	.755**	.825**	0.393
(pmol/(s*mg DW)*ml	Sig. (2-tailed)	0.006	0.115	0.984	0.168	0.006	0.392		0.005	0.006	0.004	0	0	0	0.071
(State 4) PMG	Pearson Correlation	0.364	.487*	0.263	0.255	-.665**	-0.318	.572**	1	.656**	.642**	.688**	.720**	.691**	.501*
(pmol/(s*mg DW)	Sig. (2-tailed)	0.095	0.022	0.225	0.307	0.003	0.149	0.005		0.001	0.001	0	0	0	0.015
PMGD (pmol/(s*mg DW)	Pearson Correlation	0.19	0.416	0.291	.756**	-.540*	-.461*	.571**	.656**	1	.994**	.809**	.848**	.679**	0.33
	Sig. (2-tailed)	0.396	0.054	0.178	0	0.021	0.031	0.006	0.001		0	0	0	0	0.125
PMGDC (pmol/(s*mg DW)	Pearson Correlation	0.197	0.399	0.275	.763**	-.558*	-.452*	.589**	.642**	.994**	1	.823**	.863**	.709**	0.337
	Sig. (2-tailed)	0.379	0.066	0.204	0	0.016	0.035	0.004	0.001	0		0	0	0	0.116
(State 3) PMGSD	Pearson Correlation	0.317	0.408	0.249	.687**	-.784**	-.450*	.759**	.688**	.809**	.823**	1	.963**	.863**	0.346
(pmol/(s*mg DW)	Sig. (2-tailed)	0.151	0.06	0.252	0.004	0	0.036	0	0	0	0		0	0	0.106
F (pmol/(s*mg DW)	Pearson Correlation	0.346	.487*	0.313	.689**	-.702**	-0.378	.755**	.720**	.848**	.863**	.963**	1	.899**	0.339
	Sig. (2-tailed)	0.114	0.022	0.145	0.002	0.001	0.083	0	0	0	0	0		0	0.113
ROT (pmol/(s*mg DW)	Pearson Correlation	.427*	0.401	0.104	.484*	-.781**	-0.227	.825**	.691**	.679**	.709**	.863**	.899**	1	.484*
	Sig. (2-tailed)	0.047	0.064	0.637	0.042	0	0.31	0	0	0	0	0	0		0.019
AMA (pmol/(s*mg DW)	Pearson Correlation	-0.065	-0.082	-0.241	0.066	-.571*	-0.126	0.393	.501*	0.33	0.337	0.346	0.339	.484*	1
	Sig. (2-tailed)	0.774	0.717	0.269	0.793	0.013	0.575	0.071	0.015	0.125	0.116	0.106	0.113	0.019	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

P=Pyruvate, M=Malate, G=Glutamate, D=ADP, C=Cytochrome C, S=Succinate, F=FCCP, ROT=Rotenone, AMA=Antimycin A, DW=Dry Weight, BW=Body Weight

TABLE S1: Pearson Correlation Coefficient Matrix including respirometry, MRS, VO_2peak and 400m walking speed data.