Evidence of Preserved Oxidative Capacity and Oxygen Delivery in the Plantar Flexor Muscles With Age

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Abstract

Studies examining the effect of aging on skeletal muscle oxidative capacity have yielded equivocal results; however, these investigations may have been confounded by differences in oxygen (O2) delivery, physical activity, and small numbers of participants. Therefore, we evaluated skeletal muscle oxidative capacity and O2 delivery in a relatively large group (N = 40) of young (22 ± 2 years) and old (73 ± 7 years) participants matched for physical activity. After submaximal dynamic plantar flexion exercise, phosphocreatine (PCr) resynthesis (31P magnetic resonance spectroscopy), muscle reoxygenation (near-infrared spectroscopy), and popliteal artery blood flow (Doppler ultrasound) were measured. The phosphocreatine recovery time constant (Tau) (young: 33 ± 16; old: 30 ± 11 seconds), maximal rate of adenosine triphosphate (ATP) synthesis (young: 25 ± 9; old: 27 ± 8 mM/min), and muscle reoxygenation rates determined by the deoxyhemoglobin/myoglobin recovery Tau (young: 48 ± 5; old: 47 ± 9 seconds) were similar between groups. Similarly, although tending to be higher in the old, there were no significant age-related differences in postexercise popliteal blood flow (area under the curve: young: 1,665 ± 227 vs old: 2,404 ± 357 mL, p = .06) and convective O2 delivery (young: 293 ± 146 vs old: 404 ± 191 mL, p = .07). In conclusion, when physical activity and O2 delivery are similar, oxidative capacity in the plantar flexors is not affected by aging. These findings reveal that diminished skeletal muscle oxidative capacity is not an obligatory accompaniment to the aging process.

Key Words: 31P MRS—Blood flow—Muscle oxygenation—Aging

The preservation of skeletal muscle oxidative capacity is essential to maintain mobility, and therefore, health across the human lifespan. Previous studies have reported both a reduction (1–3) and preservation (4–6) of lower-limb skeletal muscle oxidative capacity with advancing age. In fact, accumulating evidence suggests that decreases in skeletal muscle oxidative capacity are not merely a consequence of advancing
age, but are more likely attributable to either a general or muscle-specific decline in physical activity. Specifically, $^{31}$P magnetic resonance spectroscopy ($^{31}$P MRS) data suggest that the age-associated decrement in oxidative capacity of distal muscle groups in the lower limb is attenuated when young and old participants are matched for physical activity (4,5). Building upon this concept, but at the muscle-specific level, it has recently been postulated that age-associated alterations in oxidative capacity may be highly dependent on the functional demands of the muscle of interest (7). For instance, with age, differences in lower-extremity gait kinematics reveal decreases in plantar flexor peak power that are compensated for by increased peak power in the hip extensors (8,9). In combination, these findings highlight the importance of physical activity as a determinant of skeletal muscle metabolic changes with age.

Postexercise phosphocreatine (PCr) resynthesis, measured by $^{31}$P MRS and analyzed as either the recovery Tau or an estimated maximal rate of oxidative ATP production ($V_{max}$) (10), is a measure of muscle oxidative mitochondrial density and the function of the respiratory chain complexes (11). Although often overlooked, this index is highly dependent on muscle $O_2$ availability (12,13). Thus, it is important to note that blood flow (BF) and subsequently $O_2$ delivery to the lower limbs is generally considered to decline with age (6,14–17). However, a few studies that have assessed both oxidative capacity and $O_2$ availability have generated conflicting evidence in terms of the impact of aging on the plantar flexor muscles (6,18). Indeed, Wray and colleagues (19) recently reported improved oxidative capacity mediated by enhanced tissue perfusion after acute antioxidant administration in the calf muscles of elderly participants. With a differing approach, Layec and colleagues (20) documented an unchanged PCr recovery Tau in older individuals despite an increased arterial $O_2$ content achieved by breathing a hyperoxic gas mixture. Although both of these conflicting studies were from our group, each was not without caveats. Specifically, the discrepancies in these findings may have been related to the potential for hyperoxic vasoconstriction to counteract the beneficial effect of increased arterial $O_2$ content, antioxidants to exert a nitric oxide–mediated effect on mitochondrial efficiency (21), and their low sample size.

With the recognition that physical activity, $O_2$ availability, and limited numbers of participants in prior magnetic resonance spectroscopy studies may have confounded the interpretation of PCr recovery kinetics data, it seems necessary to comprehensively re-examine the consequences of advancing age on mitochondrial function in vivo. Therefore, we used $^{31}$P MRS, Doppler ultrasound, and near-infrared spectroscopy (NIRS) to evaluate skeletal muscle oxidative capacity and $O_2$ delivery in a relatively large group of young and old participants ($n = 40$) matched for physical activity. With the tenet that physical activity, and not age, determines metabolic changes in skeletal muscle across the lifespan, we hypothesized that any evidence of reduced skeletal muscle oxidative capacity in the old could be accounted for by attenuated end-exercise popliteal BF and convective $O_2$ delivery. Confirmation of this hypothesis would provide support for the concept that skeletal muscle oxidative capacity is preserved with age.

**Methods**

**Participants**

A total of 40 participants, equally represented by young ($n = 20$), old ($n = 20$), and gender (10 males and 10 females in each group) participated in this study. All participants were nonsmokers, normotensive (<140/90 mmHg), not taking any medication recognized to alter BF or metabolism, and no evidence of overt cardiovascular disease. Participants were recruited based on being moderately physically active (assessed by both interview and accelerometer), aged between 18 and 25 years for the young, and older than 65 years of age for the old. To minimize the influence of female hormones, young female participants were tested during the follicular phase of the menstrual cycle, although old women were postmenopausal and not taking any form of estrogen-replacement therapy. All participants reported to the laboratory for testing in a fasted state (>8 hours postprandial) and refrained from caffeine or strenuous exercise before the studies (>24 hours). The protocol was approved by the Human Research Protection Program at the University of Utah and the Salt Lake City VAMC, and written informed consent was obtained from all participants before participation.

**Exercise Protocol**

On the first laboratory visit, all participants were familiarized with supine plantar flexion exercise performed in a whole body MRI system (Siemens Trio 3T, Erlangen, Germany). Maximum plantar flexion work rate ($WR_{max}$) was determined by performing a graded test to maximum effort, as described previously (22). In brief, individual $WR_{max}$ was determined by performing an incremental dynamic plantar flexion exercise until exhaustion (0.5–1 W increments per min, frequency of 1 Hz) in the supine position. During experimental trials, participants performed constant-load submaximal plantar flexion at ~40% of $WR_{max}$ (frequency of 1 Hz) for 5 minutes followed by 5 minutes of recovery lying supine in the superconducting magnet to facilitate $^{31}$P data collection. During a separate visit, participants performed the same submaximal plantar flexion exercise outside the magnet to facilitate NIRS and BF (Doppler ultrasound) data collection. Trials were counterbalanced to control for an order effect.

**$^{31}$P MRS**

Magnetic resonance spectroscopy was performed using a clinical 3T MRI system (Tim-Trio, Siemens Medical Solutions, Erlangen, Germany) operating at 49.9 MHz for $^{31}$P resonance. $^{31}$P MRS data were acquired with a $^{31}$P-$^1$H dual surface coil with linear polarization (Rapid biomedical GmbH, Rimpar, Germany) positioned around the calf at its maximum diameter. The $^1$H single-loop coil diameter was 125 mm surrounding a 110-mm $^1$H coil loop. After a three-plane scout proton image, advanced localized volume shimming was performed. Before each experiment, three fully relaxed spectra were acquired at rest with three averages per spectrum and a repetition time of 30 seconds. Then, magnetic resonance spectroscopy data acquisition was performed throughout the rest–exercise–recovery protocol using a free-induction-decay pulse sequence with a 2.56-millisecond adiabatic-half-passage excitation RF pulse and the following parameters (repetition time = 2 seconds, receiver bandwidth = 5 kHz, 1024 data points, and three averages per spectrum). Saturation factors were quantified by the comparison between fully relaxed (TR = 30 seconds) and partially relaxed spectra (TR = 2 seconds).

As described previously (23), relative concentrations of [PCr], inorganic phosphate [Pi], and [ATP] were obtained by a time-domain fitting routine using the AMARES algorithm (24) incorporated into the CSIARO software (25). The free cytosolic adenosine diphosphate [ADP] was calculated from [PCr] and pH using the creatine kinase equilibrium constant ($K_{eq} = 1.66 \times 10^9 \text{M}^{-1}$) and assuming that PCr represents 85% of the total creatine content (26). The resting concentrations were calculated from the average peak areas of the three relaxed spectra (TR = 30 seconds; N = 3) recorded at rest.
and assuming a resting 8.2 mM ATP concentration. When Pi splitting was evident, the pH corresponding to each Pi pool was calculated separately as pH1 and pH2 on the basis of the chemical shift of each peak relative to PCr. The overall muscle pH was then calculated as pH = pH1 (areaPi/total Pi area) + pH2 (areaPi/total Pi area) (27).

**Popliteal BF and O2 Delivery**

Measurements of popliteal artery blood velocity and vessel diameter were performed in the popliteal fossa of the leg, proximal to the branching of the medial inferior genicular artery, with a Logic 7 Doppler ultrasound system (General Electric Medical Systems, Milwaukee, WI). With a linear transducer operating at an imaging frequency of 14 MHz, vessel diameter was determined at a perpendicular angle along the central axis of the scanned area. Blood velocity was measured using the same transducer at a frequency of 5 MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insolation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel. Arterial diameter was measured, and mean velocity (Vmean) (angle corrected, and intensity-weighted area under the curve) was calculated with proprietary software (Logic 7). Using arterial diameter and Vmean, BF in the popliteal artery was calculated as BF = Vmean·π(vessel diameter/2)^2 ±60, where BF is in milliliters per minute. Arterial O2 content (CaO2) was calculated as the sum of bound (1.34·Hb·SaO2) and dissolved O2 (0.0033·PO2) assuming a constant SaO2 = 93.4% and PO2 = 79.2 mmHg (young) or SaO2 = 94.0% and PO2 = 70.8 mmHg (old), based on typical values for the laboratory altitude and a normal Hb association curve (28). O2 delivery was then calculated as the product of CaO2 and popliteal artery BF.

**Muscle Oxygenation and Capillary BF**

Muscle oxygenation was assessed using the NIRS technique, which provides continuous, noninvasive measurements of oxygenated (HbO2), deoxygenated (HHb), and total (Hbtot) hemoglobin levels and tissue oxygenation (i.e., HbO2/Hbtot). Because of identical spectral characteristics, hemoglobin and myoglobin are not separated using NIRS. However, considering the ratio of Mb to Hb concentrations in human muscle, the signal is usually considered as being derived mainly from Hb (29). In the current study, changes in muscle oxygenation of the right gastrocnemius muscle were continuously monitored at 2 Hz using a spatially resolved spectroscopy oximeter (Oxiplex TS, ISS, IL). The probe was positioned at the level of the largest circumference of the medial gastrocnemius and secured with Velcro straps and biadhesive tape.

**Physical Activity Level**

Physical activity was assessed by both a subjective physical activity recall interview and objective accelerometer data. The physical activity interview determined the average duration, frequency, intensity, and type of physical activity in any given week. After receiving standardized operating instructions, participants wore the accelerometer (GT1M; ActiGraph, Pensacola, FL) during waking hours for seven consecutive days (30), with adherence assessed by the data collected. Average daily physical activity was expressed as both steps per day and total accelerometer counts per minute.

**Data Analysis**

The PCr and HHb recovery kinetics were determined by fitting the time-dependent changes during the recovery period to a single exponential curve described by the following equation:

\[ Y(t) = Y_{sol} + Y_{imm}(1 - e^{-(t - TD)/TP}) \]

in which \( Y_{sol} \) is the level of [PCr] and HHb measured at end-of-exercise and \( Y_{imm} \) refers to the amount of PCr resynthesized or the resaturation during the recovery. Unlike HHb, there is no time delay (TD) in the resynthesis of PCr, and therefore, TD was fixed to 0 for PCr kinetics. Then, the initial rate of PCr resynthesis (\( V_{PCr} \)) was calculated as follows:

\[ V_{PCr} = k \cdot [PCr]_{imm} \]

in which \([PCr]_{imm}\) represents the amount of PCr resynthesized during the recovery and the rate constant \(k\) is 1/t.\(\phi\).

Muscle oxidative phosphorylation capacity (\( V_{max} \) in mM/min) was calculated using the initial rate of PCr synthesis (\( V_{PCr} \)) during the recovery period and [ADP] obtained at the end of exercise as described previously (31):

\[ V_{max-ATP} = V_{PCr}(1 + (K_a / [ADP]_{imm})) \]

in which \(K_a\) (the [ADP] at half-maximal oxidation rate) is ~30 µM in skeletal muscle (32). Of note, both \(V_{PCr}\) and \(V_{max}\) are documented to be independent of work rate and alterations in pH (33). The initial rate of ATP demand (\( ATP_{imm}\) in mM/min) at the offset of the exercise was determined from the initial PCr resynthesis rate during the first 6 seconds of the recovery because ATP synthesis is almost entirely oxidative at this point (34,35):

\[ ATP_{imm} = dPCr / dt \]

Model variables were determined with an iterative process by minimizing the sum of squared residuals between the fitted function and the observed values. Goodness of the fit was assessed by visual inspection of the residual plot and the frequency plot distribution of the residuals; chi-square values and the coefficient of determination \((r^2)\) are calculated as follows (36):

\[ r^2 = 1 - (SS_{res} / SS_{imm}) \]

in which \(SS_{res}\) is the sum of squares of the residuals from the fit and \(SS_{imm}\) is the sum of squares of the residuals from the mean. In a subset of participants demonstrating the greatest drop in pH \((n = 6)\), we determined that the PCr recovery kinetics was best fitted with a single exponential function rather than a double exponential function.

**Statistical Analysis**

Differences in all measured variables between young and old were determined with either independent t tests or nonparametric Mann–Whitney tests, where appropriate (Statsoft, version 5.5; Statistica, Tulsa, OK). Potential between variable relationships was assessed using the Pearson test or the nonparametric Spearman rank-order correlation. A preliminary analysis performed with a two-factor analysis of variance, using gender and age as factors, revealed no evidence of a significant gender-specific effect on the main variables of this study. Therefore, the main effect of age on the metabolic and vascular responses was the focus of all subsequent analyses. Statistical significance was accepted at \(p < .05\). Results are presented as mean ± SD in the text and tables, whereas mean ± SEM are used in the figures for clarity.
Results

Participant Characteristics
Participant characteristics are displayed in Table 1. Plantar flexion WR_max, determined by an incremental exercise test, was 44% higher in the young compared with the old participants (p < .05). Young and old were well matched for BMI, blood glucose, blood lipids, hematocrit, and white blood cell differentials, revealing no age-related changes despite an approximate 50-year age difference between groups. All participants, with the exception of one old participant who was taking a statin, were free of medications. In addition, by experimental design, physical activity was similar between groups.

High-Energy Phosphates and Intracellular pH
Pre-exercise resting, PCr, Pi, pH, and ADP were not different between the young and old, whereas phosphodiesterase was 2.25-fold greater in the old (p < .05) (Table 2). Representative curve fittings for postexercise PCr resynthesis from the young and old are displayed in Figure 1. The PCr resynthesis kinetics postexercise were similar between groups, as determined by the Tau (Table 2). The percent decrease in intracellular pH from rest to end-exercise (Table 2) was significantly greater in the young compared with the old (p < 0.05). Maximal oxidative ATP synthesis rate postexercise, assessed by V_max, was not significantly different between the young (26.7 ± 8.2 mM/min) and the old (24.6 ± 8.6 mM/min) (Figure 2). The 31P MRS data collected in three young and three old participants were not included in these analyses because of poor signal-to-noise ratio during the exercise and the recovery.

Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young (n = 20)</th>
<th>Old (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (female/male)</td>
<td>20 (10/10)</td>
<td>20 (10/10)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>22 ± 2</td>
<td>73 ± 7*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>172 ± 10</td>
<td>169 ± 9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69 ± 13</td>
<td>75 ± 13</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 3</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Muscle volume (L)</td>
<td>2.1 ± 0.6</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Muscle mass (kg)</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Maximum plantar flexor work rate (W)</td>
<td>13 ± 5</td>
<td>9 ± 5*</td>
</tr>
<tr>
<td>Step (count/d)</td>
<td>6331 ± 1575</td>
<td>6282 ± 2735</td>
</tr>
<tr>
<td>Physical activity (count/min)</td>
<td>159 ± 47</td>
<td>151 ± 85</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>79 ± 14</td>
<td>70 ± 8</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>197 ± 30</td>
<td>178 ± 41</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>120 ± 59</td>
<td>107 ± 73</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>54 ± 13</td>
<td>54 ± 10</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>125 ± 25</td>
<td>110 ± 33</td>
</tr>
<tr>
<td>WBC (K/µL)</td>
<td>5.5 ± 1.2</td>
<td>5.9 ± 0.9</td>
</tr>
<tr>
<td>RBC (M/µL)</td>
<td>4.8 ± 0.3</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44 ± 2</td>
<td>45 ± 3.3</td>
</tr>
<tr>
<td>Neutrophil (K/µL)</td>
<td>3.3 ± 1</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>Lymphocyte (K/µL)</td>
<td>1.6 ± 0.4</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Monocyte (K/µL)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
</table>

Notes: BMI = body mass index; HDL = high-density cholesterol; LDL = low-density cholesterol; RBC = red blood cells; WBC = white blood cells. Values expressed as mean ± SD.

* Significant difference between groups (p < .05).

BF and Microcirculatory Measurements
Postexercise
At rest, popliteal BF was not significantly different between the young (55 ± 21 mL/min) and the old (45 ± 30 mL/min) (p > .05). Postexercise popliteal BF (p = .06) and convective O₂ delivery tended to be higher (p = .07) in the old participants compared with the young (Figure 3), although there were no statistically significant differences between the groups. BF data collected in two young and two old participants were not included in these analyses because of poor signal-to-noise ratio during the recovery. The NIRS assessment of microvascular deoxygenation, deoxyhemoglobin/myoglobin kinetics during recovery, revealed no significant differences between the young and old groups (Table 3).

Discussion

Previous conclusions regarding the effect of aging on skeletal muscle oxidative capacity may have been confounded by differences in O₂ delivery, physical activity, and small numbers of study participants. Therefore, this investigation evaluated skeletal muscle oxidative capacity and O₂ delivery after submaximal plantar flexion exercise in a relatively large cohort of young and old participants matched for physical activity. The main results of this study were that oxidative capacity, as evidenced by similar PCr recovery Tau and V_max, and postexercise convective O₂ delivery were not different in the young and old. Together, these findings reveal that, when participants are matched for physical activity and O₂ delivery is not different between groups, age, per se, does not seem to influence oxidative capacity in the plantar flexor muscles. These findings reveal that diminished skeletal muscle oxidative capacity is not an obligatory accompaniment to the aging process.

Oxidative Capacity With Age

By assessing the PCr recovery Tau and V_max, this study revealed no effect of age on oxidative capacity in the plantar flexor muscles when the level of physical activity and O₂ availability were similar in young and old. Therefore, the results of the current study support investigations

Table 2. Metabolic Parameters Assessed by Magnetic Resonance Spectroscopy at Rest and Postexercise in the Young and Old

<table>
<thead>
<tr>
<th></th>
<th>Young (n = 17)</th>
<th>Old (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>33 ± 5</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>Pi (mM)</td>
<td>2.6 ± 0.9</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>PME (mM)</td>
<td>1.5 ± 1.5</td>
<td>2.2 ± 1.6</td>
</tr>
<tr>
<td>ADP (mM)</td>
<td>9.5 ± 1.6</td>
<td>9.5 ± 1.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.03 ± 0.08</td>
<td>7.03 ± 0.07</td>
</tr>
<tr>
<td>PDE (mM)</td>
<td>1.2 ± 1.0</td>
<td>2.7 ± 1.1*</td>
</tr>
<tr>
<td>End-exercise and recovery parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>20 ± 6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Pi (mM)</td>
<td>12.3 ± 5.4</td>
<td>13.3 ± 4.8</td>
</tr>
<tr>
<td>ADP (µM)</td>
<td>42 ± 17</td>
<td>53 ± 20</td>
</tr>
<tr>
<td>pH</td>
<td>6.86 ± 0.16</td>
<td>6.98 ± 0.08*</td>
</tr>
<tr>
<td>Tau (s⁻¹)</td>
<td>33 ± 16</td>
<td>30 ± 11</td>
</tr>
<tr>
<td>IC 95</td>
<td>9 ± 7</td>
<td>11 ± 5</td>
</tr>
</tbody>
</table>

Notes: ADP = adenosine diphosphate; AMP = amplitude; IC 95 = 95% confidence interval; PCr = phosphocreatine; PDE = phosphodiester; Pi = inorganic phosphate; PME = phosphomonoester; Tau (s⁻¹) = PCr recovery time constant. Values expressed as mean ± SD.

* Significant difference between groups (p < .05).
that have documented a preserved oxidative capacity with age in vivo (4,6). Supporting the validity of the current in vivo measurements, the PCr recovery Tau observed in the young participants (33 ± 5 s⁻¹) was comparable to values documented by Haseler and colleagues (13), who reported a Tau of 30 ± 2 s⁻¹ in the plantar flexors of similarly young and untrained participants. In addition, in the current study, the V_{max} of the young participants (26.7 ± 8.2 mM/min) was within the range of previously reported values (20–59 mM/min) in the gastrocnemius of untrained young adults (11,37,38). However, this study also recorded a PCr recovery Tau in the old participants (30 ± 11 s⁻¹) that was notably faster than the range of previously reported values (46–51 s⁻¹) in the plantar flexors of several smaller samples of old, untrained, participants (4,6,39). Taken together, the current shorter than typical PCr recovery Tau in the old and the absence of any difference between the values exhibited by the young and old implies the potential influence of additional factors, other than age, on skeletal muscle oxidative capacity, for example, physical activity.

Oxidative Capacity, Aging, and Physical Activity
It should be acknowledged, that prior in vivo studies (39,40), including work from our own group (18), have provided evidence against a preservation of oxidative capacity in the plantar flexor muscles...
with age. Such reports may be attributed to the need to carefully match young and old participants for physical activity, a factor that has been more strongly associated with changes in skeletal muscle oxidative capacity than simply the aging process itself (41,42). Furthermore, accumulating evidence suggests that mitochondrial function assessed in vitro, including oxidative enzyme activity and respiration, are more strongly influenced by physical activity than chronological age (43,44). For instance, Lanza and colleagues (43) revealed an age-related decline in mitochondrial ATP production rates in sedentary participants which was restored when young and old participants were matched for endurance training.

Considering the impact of habitual physical activity on oxidative capacity, it was essential to accurately characterize the activity level of the participants in this study. Indeed, studies using questionnaires alone do not provide an equally accurate estimation of physical activity as the accelerometry assessments used in the current study, particularly in older adults (45). The accelerometry data revealed that both young and old attained approximately 6,000 steps per day, which is more closely aligned with the criteria to be considered moderately physically active (7,000 steps per day) than the cut off to be considered sedentary (<4,700 steps per day) (46). Thus, the direct assessment of physical activity and the use of these data to select participants resulted in a comparison of well-matched, moderately active, young and old participants that exhibited no age-related differences in muscle oxidative capacity of the plantar flexor muscles. Overall in vitro and in vivo evidence, including the current data, support the finding of a preserved skeletal muscle oxidative capacity and mitochondrial function with age when physical activity is taken into account.

**WR\textsubscript{max} and Oxidative Capacity**

Although the current results document an ~30% lower plantar flexor WR\textsubscript{max} in the old compared with the young, a classic indirect assessment of oxidative capacity, in contrast, the \textsuperscript{31}P MRS assessment of oxidative capacity by PCr recovery and V\textsubscript{max}\textsuperscript{2} revealed no such difference. Although not measured in the current study, this decline in WR\textsubscript{max} of the plantar flexor muscles could be the consequence of the age-associated increase in the ATP cost of contraction (47) or the transition from glycolytic type II to more oxidative type I skeletal muscle fibers in the gastrocnemius. Using an animal model, Picard and colleagues (48) demonstrated that, unlike muscle force generating capacity, such a fiber type change may not influence oxidative capacity assessed at the mitochondrial level. Therefore, although WR\textsubscript{max} in the plantar flexors seems to decline with age, this decrement does not seem to influence, or be a result of, a decreased mitochondrial oxidative capacity within this muscle group, as assessed by PCr recovery and V\textsubscript{max}\textsuperscript{2}.

**Skeletal Muscle Oxygen Delivery and Oxidative Capacity With Age**

In this study, the old exhibited preserved popliteal artery BF, thus the assessment of muscle oxidative capacity and inferences about mitochondrial function could be achieved without the confounding effect of altered O\textsubscript{2} availability. This preservation of BF in the distal segment of the lower limb with age contrasts starkly with studies of the quadriceps that have documented a 20–30% age-associated decline in femoral BF (15,49,50). However, BF in the upper extremity also seems unaffected with age (51–53). In support of these anatomic variations in age-related changes in BF, translational studies have postulated that aging results in diverse changes in vascular function across tissues and throughout the arterial tree (54). This lends credence to the current finding of a preserved popliteal artery BF despite previously reported age-associated decrements in femoral artery BF.

PCr recovery kinetics and V\textsubscript{max}\textsuperscript{2} are known to be sensitive to alterations in O\textsubscript{2} supply and convective O\textsubscript{2} delivery (13,18,55). Given this well-documented association, in the current study, it was critical to examine both oxidative capacity of the plantar flexor muscles and popliteal artery BF in the same participants to gain clear insight into the effects of age on mitochondrial function in vivo. The similarity between both ATP demand and O\textsubscript{2} delivery in the old and young participants (Figure 4) suggests that in healthy participants, matched for physical activity, O\textsubscript{2} transport adequately meets skeletal muscle needs.

**Figure 2.** Individual peak data for the rate of in vivo mitochondrial respiration (V\textsubscript{max}) data from postexercise phosphocreatine recovery assessed with 31 phosphorous magnetic resonance spectroscopy in young (n = 17) and old (n = 17) participants. Horizontal bars represent the group mean.
ATP demand. Although a mismatch between muscle metabolism and perfusion at a local level cannot be entirely ruled out with the current model (56), this scenario is unlikely, given recent evidence implying improved homogeneity in BF distribution to the muscle tissue in older individuals (57). In addition, the microvascular \( O_2 \) extraction, assessed with NIRS in the current study, did not reveal any differences between the young and old, suggesting no age-related effect on microcirculatory function of the plantar flexors. However, these data conflict somewhat with prior results from our group that documented an age-related attenuation in skeletal muscle perfusion accompanied by similar PCr recovery \( T_{au} \)s in young and old participants (6). Although different methodological approaches (skeletal muscle perfusion with arterial spin labeling vs limb BF with Doppler ultrasound) may potentially account for the disparate observations in terms of \( O_2 \) delivery, as recently suggested (57), the incongruent findings may also be attributable to the increased number of participants in the current study in combination with a more precise matching of participants based on physical activity.

Figure 3. Postexercise popliteal blood flow assessed with Doppler ultrasound and the calculated convective \( O_2 \) delivery (panels A and B, respectively) in young and old participants. Data presented as mean ± SE.
The concept that O₂ demand exhibits a greater influence on BF than age has been highlighted in the lower limb by recognizing that there is a single common relationship between resting femoral BF and estimated single-leg O₂ consumption in both young and old participants (58). During plantar flexion exercise, recent studies from our group have revealed a significantly increased ATP cost of contraction in old participants compared with their younger counterparts (47). In agreement with these previous findings, despite different absolute workloads, the current data reveal strikingly similar end-exercise ATP synthesis rates and subsequently similar metabolic demand in the plantar flexor muscles of the young and old participants. As illustrated in Figure 4, this similar level of metabolic demand was well matched by O₂ delivery after submaximal exercise in both the young and old, additionally supporting the undeniable link between metabolic demand and O₂ delivery even in the face of large age differences.

Experimental Considerations
In the current study, the young and old participants were matched for physical activity using accelerometry rather than the assessment of physical fitness by a whole body VO₂ max test. Although a VO₂ max would have provided additional descriptive characteristics, this evaluation is not without risk and, just as with accelerometry, is a global assessment that does not focus upon the calf muscles, which were the emphasis of this study.

The assessment of BF in the current study was limited to bulk flow in the popliteal artery, measured by ultrasound Doppler. There is emerging evidence that differences or similarities in bulk BF may not translate to skeletal muscle perfusion and this is a limitation of the current study, which should be followed up by investigations that use positron emission tomography or arterial spin labeling with MRI to confirm the present findings.

Although O₂ delivery was not significantly different between the young and old, the strong tendency for a higher BF and O₂ delivery in the old should not be ignored. In fact, because \( V_{\text{max}} \) integrates the contribution of muscle mitochondrial density, the integrative function of the respiratory chain complexes, and O₂ availability, it is difficult with the current experimental design to discern whether \( V_{\text{max}} \) is primarily limited by O₂ availability or intrinsic mitochondrial capacity. However, there are at least three lines of reasoning that support the conclusion that the current data predominantly reflect intrinsic mitochondrial function and were not influenced by O₂ delivery: (i) as just acknowledged, although tending to be different, O₂ delivery was not significantly different between the young and old, (ii) it has previously been documented that higher O₂ availability does not necessarily translate into a faster postexercise PCr resynthesis kinetics or \( V_{\text{max}} \) in individuals who are not exercise trained (10,29), a description that fits the old participants in this study, and (iii) there was no evidence of a relationship between O₂ delivery and PCr recovery Tau or \( V_{\text{max}} \) in the young, the old, or when both groups were combined. We acknowledge that the PCr recovery Tau in the old participants was more rapid compared with what has been previously documented (3,6,39). This, along with the absence of medication use in all but one participant, suggests that the old participants in

![Figure 4](image-url)

**Figure 4.** A comparison of postexercise adenosine triphosphate (ATP) demand and convective O₂ delivery, assessed in the first 6 seconds of recovery, in young and old participants. Data presented as mean ± SE.

<table>
<thead>
<tr>
<th>Table 3. Deoxyhemoglobin/Myoglobin Kinetics Assessed by Near-Infrared Spectroscopy After Submaximal Plantar Flexion Exercise</th>
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<td>Resting Deoxy[Hb/Mb] (µM)</td>
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<td>TD (s)</td>
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<td>Tau (s)</td>
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Notes: Amp = amplitude; Deoxy[Hb/Mb] = resting concentration of deoxyhemoglobin/myoglobin; IC 95 = 95% confidence interval; MRT = mean response time; TD = time delay. Values expressed as mean ± SD.
the current study were healthier than the average elderly population, 
a likely consequence of matching the groups for physical activity. 
Although this may limit the generalizability of the current findings 
the population at large, such an approach does allow the effect of 
age alone to be isolated.

Conclusion
In conclusion, skeletal muscle oxidative capacity, assessed by PCR 
recovery Tau and $V_{\text{max}}$, after submaximal plantar flexion exercise 
was preserved with age in a relatively large group of young and old 
participants matched for physical activity. Both postexercise pop 
liteal artery BF and convective O$_2$ delivery were also not affected 
by age. Overall, these findings suggest that mitochondrial function 
in the plantar flexor muscles is not affected by aging per se and that 
diminished skeletal muscle oxidative capacity is not an obligatory 
accompaniment to the aging process.

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