

Reduced muscle oxidative capacity is independent of O₂ availability in elderly people

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Abstract Impaired O₂ transport to skeletal muscle potentially contributes to the decline in aerobic capacity with aging. Thus, we examined whether (1) skeletal muscle oxidative capacity decreases with age and (2) O₂ availability or mitochondrial capacity limits the maximal rate of mitochondrial ATP synthesis in vivo in sedentary elderly individuals. We used ³¹P-magnetic resonance spectroscopy (³¹P-MRS) to examine the PCr recovery kinetics in six young (26±10 years) and six older (69±3 years) sedentary subjects following

4 min of dynamic plantar flexion exercise under different fractions of inspired O₂ (FiO₂, normoxia 0.2; hyperoxia 1.0). End-exercise pH was not significantly different between old (7.04±0.10) and young (7.05±0.04) and was not affected by breathing hyperoxia (old 7.08±0.08, *P*>0.05 and young 7.05±0.03). Likewise, end-exercise PCr was not significantly different between old (19±4 mM) and young (24±5 mM) and was not changed in hyperoxia. The PCr recovery time constant was significantly longer in the old (36±9 s) compared to the young in normoxia (23±8 s, *P*<0.05) and was not significantly altered by breathing hyperoxia in both the old (35±9 s) and young (29±10 s) groups. Therefore, this study reveals that the muscle oxidative capacity of both sedentary young and old individuals is independent of O₂ availability and that the decline in oxidative capacity with age is most likely due to limited mitochondrial content and/or mitochondrial dysfunction and not O₂ availability.

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Introduction

Reductions in exercise tolerance and maximal O₂ consumption during whole-body exercise are among the most consistent hallmarks of aging (Poole et al. 2006). Although the mechanistic basis for this age-related

decline in physical performance is complex, alterations in the capacity to transport, exchange, and utilize O₂ are believed to play a major role. Indeed, it is well known that aging is associated with reduced maximal heart rate (Fleg et al. 1995) and cardiac output (Hossack and Bruce 1982), as well as peripheral hemodynamic alterations such as reduced limb blood flow, and vascular conductance (Lawrenson et al. 2003; Proctor and Parker 2006; Russell et al. 2003). In combination, these age-related changes likely alter both convective and diffusive components of O₂ transport to skeletal muscle and contribute to a reduction in exercise capacity (Poole et al. 2003).

Unlike O₂ transport, the question whether skeletal muscle oxidative capacity itself declines as a consequence of aging is still unclear. For instance, several studies have documented that *in vivo* measurements of PCr recovery rate, an index of muscle oxidative capacity, using ³¹P magnetic resonance spectroscopy (³¹P-MRS) was either slowed (Taylor et al. 1997; McCully et al. 1993; Conley et al. 2000) or similar (Wray et al. 2009a; Lanza et al. 2005; Kent-Braun and Ng 2000) in young and elderly subjects, suggesting impaired or preserved muscle oxidative capacity with age. In light of these studies, it is interesting to note that PCr recovery kinetics can be very sensitive to O₂ availability (Haseler et al. 1999; Haseler et al. 2007) and, likely as a consequence of the aforementioned reduction in O₂ transport, the postexercise O₂ partial pressure in the microvasculature appears to be lower with age (Hirai et al. 2009). Therefore, in terms of *in vivo* assessment of muscle oxidative capacity, questions remain as to the relative role of O₂ supply and the capacity of the mitochondria to generate ATP adequately in older subjects. Interestingly, the combination of PCr recovery measurements with ³¹P-MRS under different fractions of inspired O₂ (FiO₂) has previously been successfully applied to differentiate O₂ supply limitation from the limited mitochondrial capacity to generate ATP in young sedentary and exercise-trained subjects (Haseler et al. 1999, 2007) and could, therefore, provide new insight into the role of these potential limitations with age.

Based upon previous research, there are two equally plausible hypotheses regarding the role of O₂ supply and demand in limiting metabolic capacity in the elderly. Specifically, as O₂ supply appears to slow pulmonary O₂ consumption kinetics at the onset of contractions in older individuals (DeLorey et al. 2007), it could be hypothesized that O₂ availability will also limit the recovery kinetics of PCr. Therefore, unlike their sedentary younger counterparts (Haseler et

al. 2004), an improved O₂ availability induced by breathing a hyperoxic gas mixture could accelerate the PCr recovery time constant in the older sedentary subjects. Alternatively, it could be hypothesized that, if despite the reduction in O₂ transport, intracellular PO₂ in older adults is maintained above a critical value (Wilson et al. 1977; Haseler et al. 2007), PCr recovery kinetics could be limited by mitochondrial capacity to generate ATP such that hyperoxia will not affect the PCr recovery time constant. The validity of these two, quite different, conceptual scenarios has yet to be tested.

Therefore, the purpose of the present study was to utilize ³¹P-MRS to explore skeletal muscle PCr recovery kinetics following plantar flexion exercise in both normoxia (FiO₂ 0.21) and hyperoxia (FiO₂ 1.0) in a group of old and young sedentary subjects. Although the literature is mixed on this topic, we hypothesized that (1) PCr recovery time constant in normoxia will be slower in the elderly compared to young sedentary individuals and (2) based upon previous work from our group, revealing substantial metabolic reserve in young sedentary individuals (Haseler et al. 2007), hyperoxia will not alter PCr recovery in either young or old sedentary subjects. This would indicate that the decline in oxidative capacity with age is most likely a consequence of limited mitochondrial content and/or mitochondrial dysfunction rather than O₂ availability.

Method

Subjects

Six young (mean ± SD, age 26±10 years; height, 176±6 cm; weight, 76±4 kg) and six older male subjects (age 69±3 years; height, 180±3 cm; weight, 83±4 kg) volunteered to participate in this study and gave written informed consent. The subjects were recruited based upon no evidence of regular or occasional physical activity above that required for activities of daily living (self-report and interview). All the subjects included in the young and older groups were male, nonsmoker, and free of diabetes and known cardiovascular, peripheral vascular, neuromuscular, or pulmonary disease. Additionally, all the subjects were not taking any medications known to affect muscle function or blood flow. The study was approved by the Human Research Protection Program of the University of California, San Diego.

Exercise protocol

Subjects were familiarized with supine plantar flexion exercise in the whole-body MRI system (GE 1.5 T Medical Systems, Milwaukee, WI). Individual maximum work rate (WR_{max}) was determined by performing incremental dynamic plantar flexion exercise until exhaustion. On a separate day, subjects performed constant-load submaximal plantar flexion at ~50 % of WR_{max} (frequency of 1 Hz) under condition of normoxia (FiO₂ 0.21) or hyperoxia (FiO₂ 1.0) in the scanner. Specifically, after 5 min of rest, subjects exercised for 4 min followed by 5 min of recovery. The sequence of the two treatments, which were separated by 45 min, was varied to minimize any potential ordering effects and the subjects were blinded to the treatment.

³¹P MRS

MRS was performed using a clinical 1.5 T General Electric Signa system (LX 8.3 version) operating at 25.86 MHz for ³¹P. ³¹P MRS data were acquired with a dual frequency flexible array spectroscopy coil (Medical Advances Inc., Milwaukee, Wisconsin) positioned around the calf at its maximum diameter. The phosphorus coil was an 11.5-cm square, centered between two 14 × 15.5 cm Helmholtz-type proton coils. The centering of the coil around the leg was confirmed by T₁-weighted ¹H localizing images obtained in the axial plane and the coil was repositioned if the majority of the gastrocnemius muscle was not encompassed. For all subjects, a similar ratio between the volumes of gastrocnemius/soleus muscles was maintained within the coil. Magnetic field homogeneity was optimized by shimming on the proton signal from tissue water and the ³¹P MRS signal was optimized by prescan transmitter gain adjustment. Then, MR data were acquired throughout the graded exercise protocol with the following parameters (radiofrequency hard pulse duration=500 μs, repetition time=4 s, number of excitation=1, sweep width=2.5 kHz, data points=1,024, nominal flip angle=90°) so that the corresponding time resolution was 4 s.

Data analysis

Data were processed using SAGE/IDL software on a Silicon Graphics Indigo workstation. Each FID was processed with 5 Hz exponential line broadening prior to zero filling and Fourier transformation. All spectra

were manually phased using zero- and first-order phase corrections. The levels of PCr determined from the intensity of that peak were normalized to 100 % using the average value obtained from the last 40 s of rest acquired for each subject as a reference. Muscle intracellular pH was calculated from the chemical shift difference (δ) of the Pi peak relative to the PCr peak using the following equation: $\text{pH} = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$ (Taylor et al. 1983).

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

$$[\text{PCr}](t) = [\text{PCr}]_{\text{end}} + [\text{PCr}]_{\text{cons}} \left(1 - e^{-(t/\tau)}\right)$$

where $[\text{PCr}]_{\text{end}}$ is the concentration of [PCr] measured at end-of-exercise and $[\text{PCr}]_{\text{cons}}$ refers to the amount of PCr consumed at the end of the exercise session. The initial rate of PCr resynthesis (V_{iPCr}) was calculated as follows:

$$V_{\text{iPCr}} = k \cdot [\text{PCr}]_{\text{cons}}$$

in which $[\text{PCr}]_{\text{cons}}$ indicates the amount of PCr consumed at the end of exercise and the rate constant, $k=1/\tau$.

Model variables were determined with an iterative process by minimizing the sum of squared residuals (RSS) 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) calculated as follows (Motulsky and Christopoulos 2003):

$$r^2 = 1 - (\text{SS}_{\text{reg}}/\text{SS}_{\text{tot}})$$

with SS_{reg} , the sum of squares of the residuals from the fit and SS_{tot} , the sum of squares of the residuals from the mean.

Statistical analysis

Difference in PCr recovery time constant between young and old in normoxia was analyzed using the Mann–Whitney test (Statsoft, version 5.5; Statistica, Tulsa, Oklahoma). The effect of hyperoxia on the PCr recovery time constant within each group was analyzed using the Wilcoxon test. Statistical significance was accepted at $P < 0.05$. Results are presented as

mean \pm SD in the tables and for clarity, mean \pm SEM are illustrated in the figures.

Results

Phosphorylated compounds and intracellular pH

End-exercise pH following constant load exercise was not significantly different from baseline in normoxia and hyperoxia for both groups (Table 1). Similarly, end-exercise PCr, Pi, and ADP were not significantly different in normoxic and hyperoxic conditions within either group (Table 1). With the exception of ADP, which was significantly higher in old subjects compared to young in normoxia ($P < 0.05$), end-exercise PCr, Pi, and pH were not significantly between these groups.

PCr offset kinetics

As illustrated with exemplary data in Fig. 1, the PCr recovery time constant was significantly longer in old subjects compared to young in normoxia (Table 2). The group mean PCr recovery responses in normoxia and hyperoxia for both old and young are illustrated in Fig. 2. During the postexercise period, there was no difference in the normoxic and hyperoxic PCr recovery time constant (Fig. 3) and the corresponding amplitude (Table 2) for either the old or the young groups. As a result, the initial PCr resynthesis rate was also not significantly different in normoxia and hyperoxia in either group (Table 2). Likewise, the minimum intracellular pH reached during recovery was not significantly different under conditions of normoxia or hyperoxia within or between the groups (Table 2).

Discussion

We sought to examine whether skeletal muscle oxidative capacity in vivo was limited by O₂ supply or the mitochondrial capacity to generate ATP in untrained humans with a special interest in the effect of age. The main findings were that (1) the PCr recovery time constant in normoxia was slower in the elderly group compared to young sedentary subjects and (2) the PCr recovery time constant was unaffected by breathing a hyperoxic gas mixture in both groups of sedentary individuals. Together, these findings indicate that the documented decline in maximal rate of mitochondrial ATP synthesis in the skeletal muscle with age is likely due to reduced mitochondrial content and/or mitochondrial dysfunction rather than limited O₂ supply.

Evidence of age-related decline in oxidative capacity

An important finding of this study was the ~50 % slower PCr recovery kinetics in the elderly compared to the young subjects in normoxic conditions (Fig. 3), documenting a reduced muscle oxidative capacity with age. This decrease in oxidative capacity is of a similar magnitude to previous values reported in the plantar flexor muscles of elderly individuals (~46–48 %) (Taylor et al. 1997; McCully et al. 1993). However, unlike the present study, the groups of older and younger subjects in these prior studies were not sedentary and/or included subjects who were late–middle aged. It should, however, be noted that the current findings do contrast with other studies from our group (Wray et al. 2009b; Wray et al. 2009c), documenting a preserved oxidative capacity in the plantar flexor muscles of older subjects. Unfortunately, it is unclear to what extent the contrasting level of end-exercise PCr between young and older individuals,

Table 1 End-exercise phosphorylated compounds and intracellular pH

	Old		Young	
	Normoxia	Hyperoxia	Normoxia	Hyperoxia
PCr (mM)	19 \pm 4	19 \pm 4	24 \pm 5	25 \pm 5
Pi (mM)	16 \pm 4	15 \pm 3	12 \pm 3	12 \pm 3
ADP (mM)	67 \pm 14*	79 \pm 27	44 \pm 18	38 \pm 15
pH	7.04 \pm 0.10	7.08 \pm 0.08	7.05 \pm 0.04	7.05 \pm 0.03

All values are expressed as mean \pm SD

* $P < 0.05$ (statistically significant difference from young)

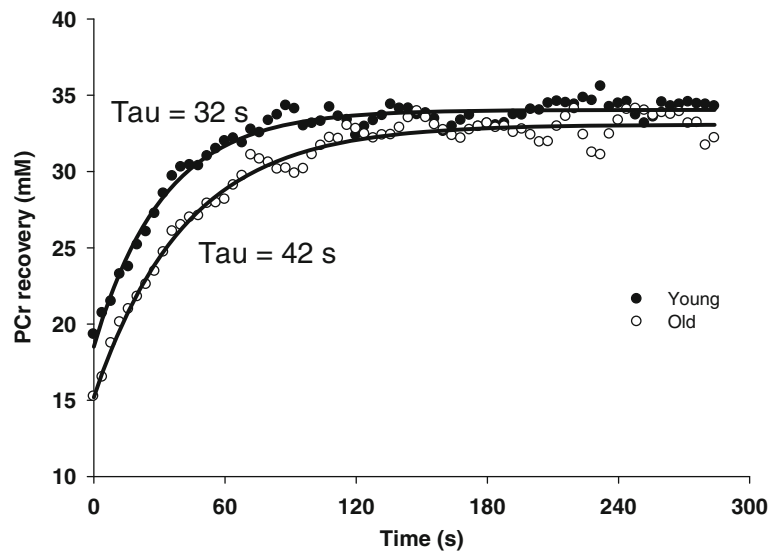


Fig. 1 Example of young (filled circle) and old (unfilled circle) PCr recoveries with respect to time following exercise in normoxia

which may affect the PCr recovery kinetics (Roussel et al. 2000), could have affected these prior results by Wray et al. (2009b; 2009c). In contrast, in the present study, the end-exercise conditions were remarkably similar between groups, which rules out any confounding effects of pH or PCr level on our conclusions.

Evidence that mitochondrial ATP production is not O₂ supply-dependent in normoxia

In agreement with our hypothesis, the PCr recovery time constant was unchanged when breathing hyperoxia in both young and old sedentary subjects (Fig. 2). These results are consistent with the unaltered postexercise PCr recovery kinetics (Haseler et al. 2004) and leg VO_{2max} (Cardus et al. 1998) in hyperoxia compared to normoxia previously reported in young sedentary

subjects and extend these findings to the elderly. These findings, therefore, reveal that the maximal rate of mitochondrial ATP synthesis in the calf muscle is determined by mitochondrial capacity rather than O₂ supply in both young and elderly sedentary individuals. Somewhat in contrast to the present study, Wray et al. (2009b) has recently reported that enhanced tissue perfusion induced by acute antioxidant administration in elderly subjects improved oxidative capacity, again assessed by ³¹P-MR spectroscopy. However, this apparent discrepancy is likely related to the quite different methods used to improve O₂ availability (hyperoxia versus enteral antioxidant administration resulting in hyperemia). Indeed, while hyperoxia enhanced O₂ availability by increasing blood O₂ content, antioxidant administration induced an increase in muscle blood flow by enhanced scavenging of free radicals and a

Table 2 PCr recovery kinetics parameters

	Old		Young	
	Normoxia	Hyperoxia	Normoxia	Hyperoxia
Amplitude (mM)	14±4	14±3	9±4	8±4
Tau (s)	36±9*	35±10	23±8	29±10
IC 95	1.2±0.4	1.0±0.5	2.3±1.7	2.6±2.4
V _i (mM min ⁻¹)	24±6	24±4	23±7	24±4

All values are expressed as mean ± SD. Amplitude refers to the amount of PCr resynthesized during the recovery

V_i initial PCr resynthesis rate, IC 95 95 % confidence interval

* $P < 0.05$ (statistically significant difference from young)

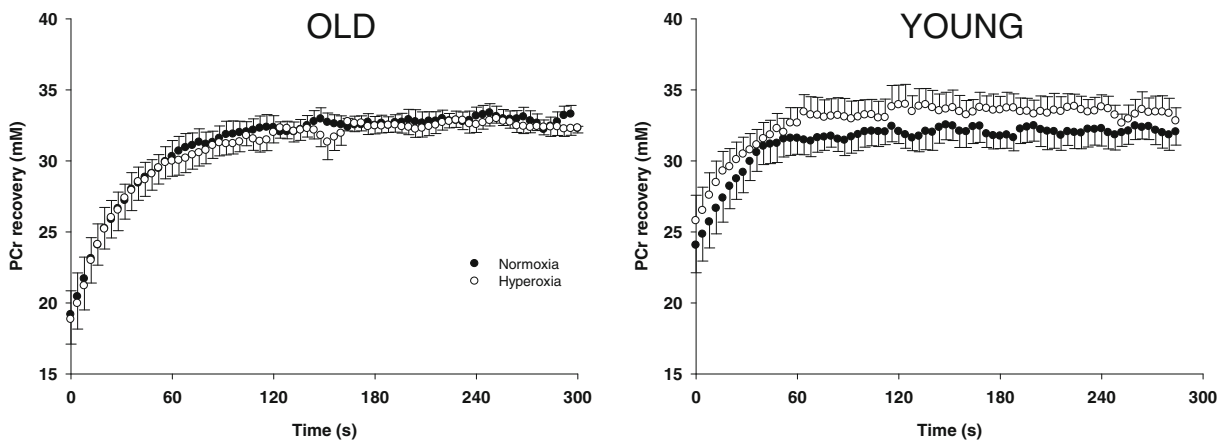


Fig. 2 PCr resynthesis kinetics in old (*left panel*) and young humans (*right panel*) following submaximal plantar flexion exercise in normoxia and hyperoxia

subsequent increase in nitric oxide (NO) bioavailability in the elderly subjects (Wray et al. 2011). Although such an intervention has consistently been documented to increase muscle blood flow in old sedentary subjects by restoring endothelium-dependent vasodilation (Donato et al. 2006; Kirby et al. 2009), an acute increase in NO bioavailability has also been recognized to decrease O_2 cost (Larsen et al. 2010) and improve mitochondrial efficiency (Larsen et al. 2011). Therefore, while an improved O_2 supply may contribute to some extent to the faster PCr recovery in the study of Wray et al. (2009b), other factors related to mitochondrial function could also play a role when antioxidant administration alters NO bioavailability.

The unchanged PCr recovery time constant from normoxia to hyperoxia in elderly individuals (Fig. 3) also suggests that, in normoxia, the diffusion of O_2 from capillary to mitochondria is able to compensate for the reduction in convective O_2 delivery commonly associated with aging (Proctor and Parker 2006). If true, this increased O_2 diffusion during exercise in the elderly would then result in a preserved intracellular O_2 pressure (iPO_2), which drives mitochondrial respiration (Wilson et al. 1977; Richardson et al. 1999; Richardson et al. 1995). In line with this possibility, O_2 diffusing capacity appears to be well preserved in the elderly as the capillary to fiber area ratios is maintained (Chilibeck et al. 1997; Proctor et al. 1995) and

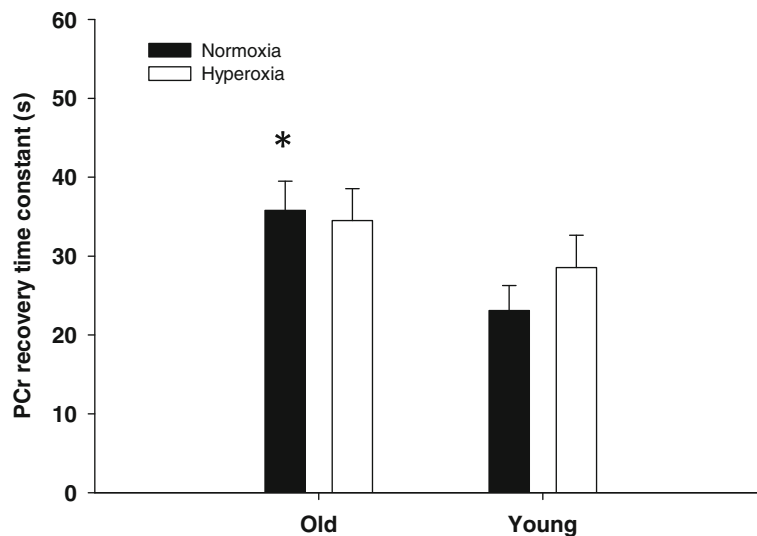


Fig. 3 PCr recovery time constants, an index of maximal oxidative ATP synthesis, in old and young humans following submaximal plantar flexion exercise in normoxia and hyperoxia. *Asterisk* indicates significant difference from young in normoxia ($P < 0.05$)

may actually be increased when capillary to fiber area is normalized for mitochondrial volume (Mathieu-Costello et al. 2005). In addition, the reduction in muscle blood flow does not necessarily translate into a reduced O₂ supply to the mitochondria as, in this scenario, the erythrocyte transit time through the capillary is prolonged, which allows higher O₂ extraction by the tissues (Richardson 1998). Accordingly, it has previously been reported that O₂ extraction was increased in the elderly during steady-state submaximal cycling exercise (Poole et al. 2003; Lawrenson et al. 2003; Proctor et al. 2003). Likewise, during the transition from rest to cycling exercise, duManoir et al. (2010) recently documented a greater O₂ extraction, inferred from near-infrared spectroscopy measurements, to compensate for the slower adjustment of muscle blood flow to exercise in older people (duManoir et al. 2010). Thus, there may be significant age-related peripheral adaptations that tend to offset the potential consequences of attenuated O₂ delivery to skeletal muscle.

An alternative explanation for the unaltered PC recovery kinetics in conditions of hyperoxia in elderly sedentary subjects is that, despite the probable reduction in convective O₂ transport, iPO₂ does not reach a critical level, such that O₂ supply still exceeds mitochondrial capacity for ATP synthesis. Indeed, it has been demonstrated both *in vitro* (Wilson et al. 1977) and *in vivo* (Richardson et al. 1999; Richardson et al. 1995) that the relationship between respiration rate and iPO₂ is not linear, but hyperbolic. For this reason, mitochondrial ATP synthesis is independent of changes in O₂ supply unless intracellular iPO₂ falls below ~2–5 mmHg during exercise (Richardson et al. 1999; Richardson et al. 1995). Given the concomitant reduction in mitochondrial capacity with age (see section below), it is possible that some reduction in convective O₂ delivery and iPO₂ in elderly individuals may take place before O₂ supply limits mitochondrial respiration rate. This possible scenario is also further supported by our prior finding that O₂ supply was in excess relative to mitochondrial capacity in young sedentary subjects during normoxia and even mild hypoxia (Haseler et al. 2007).

One could notice that while the PCr recovery time constant was almost similar in normoxia and hyperoxia in elderly subjects, young subjects exhibited a nonsignificant increase in hyperoxia (~29 s) compared to normoxia (~23 s). A potential explanation for this slower PCr recovery in the young is the development of hyperoxic vasoconstriction with the use of high

fraction of inspired O₂. Indeed, by using a high O₂ gas mixture, we reasoned that it would provide a larger O₂ gradient between red blood cell and mitochondria thereby facilitating O₂ diffusion. This increase in the PO₂ gradient is induced by a greater arterial O₂ saturation and O₂ delivery, provided that muscle blood flow is unchanged. However, it has been suggested that hyperoxia can reduce muscle blood flow during exercise thereby reducing O₂ delivery to the muscle to a level similar to normoxic conditions (Welch et al. 1977; Pedersen et al. 1999). For instance, Pedersen et al. (1999) reported diminished leg blood flow and a trend for a lower maximal O₂ consumption during one-leg knee extension exercise in hyperoxia compared to normoxia (Pedersen et al. 1999). Interestingly, similar to the present study, Haseler et al. (2004) also reported a slight increase in the PCr recovery time constant in hyperoxia compared to normoxia in young untrained individuals, although this difference was less marked (Haseler et al. 2004). It cannot be ruled out that breathing hyperoxic gas mixture during our plantar flexion exercise may have resulted in hyperoxic vasoconstriction, thus compromising to some extent O₂ availability in the young untrained subjects. However, this explanation is unlikely to be accurate as it has been demonstrated that O₂ availability had to be dramatically reduced before affecting the PCr recovery kinetics (Haseler et al. 2007). A more likely explanation is that the parameters of the PCr recovery kinetics typically exhibit some variability (~10–20 %), due to the procedure of fitting and biological variability (Layec et al. 2009), which can probably explain most of this nonsignificant difference between the normoxic and hyperoxic results in the young.

Mechanism of age-related decline in muscle oxidative capacity

Together, the impaired oxidative capacity in elderly subjects (Fig. 3) and unchanged PCr recovery rate in hyperoxia (Fig. 2) indicate that this was likely the result of a decrease in mitochondrial capacity rather than O₂ supply limitation. Possible mechanisms accounting for this decrease in mitochondrial capacity in older individuals include a reduction mitochondrial content and/or mitochondrial dysfunction. For instance, there is some evidence that mitochondrial density in skeletal muscle declines with age as measured by electron microscopy (Conley et al. 2000; Bailey et al. 2010) or citrate

synthase activity (Short et al. 2005; Coggan et al. 1993; McCully et al. 1993). However, it has been suggested that physical inactivity rather than aging per se may be an underlying cause of the reduced mitochondrial density (Russ and Lanza 2012) as these effects can be reversed by exercise training (Coggan et al. 1993; Short et al. 2003; Konopka et al. 2010). Interestingly, both young and elderly subjects in the present study were not involved in any regular physical activity. Therefore, if we assume no mitochondrial dysfunction, our findings that oxidative capacity was reduced in the sedentary elderly compared to the sedentary young could be interpreted to suggest that the activities of daily living were not sufficient to maintain mitochondrial capacity in the older group.

Using a combination of *in vivo* and *in vitro* measurements, Conley et al. (2000) previously reported a 50 % lower oxidative capacity in adults compared to elderly subjects, a result similar to the current study. This group interpreted their results to indicate that half of this impairment was due to reduced mitochondrial density and the remaining half due to reduced mitochondrial function (Conley et al. 2000). In particular, these authors documented a greater *in vivo* respiration uncoupling (P/O) at rest with age in muscles with high type II fiber content, indicative of an intrinsic defect in mitochondrial machinery (Amara et al. 2007). In this line, we observed that despite similar PCr resynthesis rate in normoxic conditions, the old subjects displayed a higher [ADP] at the end of exercise in comparison to the young subjects suggestive of a disturbance in the metabolic control of respiration rate by ADP (Chance and Williams 1955; Erecinska and Wilson 1982). Given its dimension, the coil used in the current study interrogated both the soleus, mostly composed of type I fibers, and the gastrocnemius, which is comprised of the same proportion of type I and type II fibers in the human adult (Johnson et al. 1973). Therefore, despite the progressive loss of type II fibers typically observed in older individuals (Lexell 1995), it cannot be ruled out that part of the reduction in oxidative capacity reported here was induced by greater respiration uncoupling during exercise in the elderly subjects.

Conclusion

In summary, this study has revealed that elderly subjects exhibit an impaired muscle oxidative capacity in

comparison to sedentary young controls. PCr recovery kinetics were unaffected by breathing a hyperoxic gas mixture in both groups of sedentary individuals, thereby indicating that the maximal rate of mitochondrial ATP synthesis was limited by mitochondrial capacity rather than O₂ supply. Together, these findings reveal that reduced mitochondrial content and/or mitochondrial dysfunction rather than limited O₂ supply likely contributes to the decline in muscle oxidative capacity of sedentary elderly individuals.

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