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Effect of age on in vivo oxidative capacity in two locomotory muscles of the leg

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Abstract To determine the effects of age and sex on in vivo mitochondrial function of distinct locomotory muscles, the tibialis anterior (TA) and medial gastrocnemius (MG), of young (*Y*; 24±3 years) and older (*O*; 69±4) men (M) and women (W) of similar overall physical activity (PA) was compared. In vivo mitochondrial function was measured using phosphorus magnetic resonance spectroscopy, and PA and physical function were measured in all subjects. Overall PA was similar among the groups, although *O* (n=17) had fewer daily minutes of moderate-to-vigorous PA (p=0.001), and slowed physical function (p<0.05 for all variables), compared with *Y* (n=17). In TA, oxidative capacity (V_{max}; mM s^{-1}) was higher in *O* than *Y* (p<0.001; *Y*=0.90±0.12; *O*=1.12±0.18). There was no effect of age in MG (p=0.5; *Y*=0.91±0.17; *O*=0.96±0.24), but women had higher oxidative capacity than men (p=0.007; *M*=0.84±0.18; *W*=1.03±0.18). In vivo mitochondrial function was preserved in healthy *O* men and women, despite lower intensity PA and physical function in this group. The extent to which compensatory changes in gait may be responsible for this preservation warrants further investigation. Furthermore, women had higher oxidative capacity in the MG, but not the TA.

Keywords Oxidative phosphorylation · Bioenergetics · Mobility · Physical function

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Introduction

Despite intensive investigation in recent years, the impact of old age on skeletal muscle mitochondrial function remains unclear. For example, oxidative capacity, which is one measure of mitochondrial function, has been reported by some to decline in older (*O*) adults (Conley et al. 2000; Short et al. 2005; Coggan et al. 1992; McCully et al. 1991; Pastoris et al. 2000; Karakelides et al. 2010), while others report no change with aging (Lanza et al. 2005; Larsen et al. 2012b; Hutter et al. 2007; Rasmussen et al. 2003; Tevald et al. 2010; Chilibeck et al. 1998). A number of factors, including the means of assessing mitochondrial function, the health and physical activity (PA) status of the study participants, and the muscle group studied, apparently contribute to these conflicting findings (Larsen et al. 2012b).

Various in vitro (i.e., enzyme activities, isolated mitochondria assays, and permeabilized fiber assays) and in vivo (phosphorus magnetic resonance spectroscopy) approaches have been employed to assess the impact of age on the capacity of skeletal muscle mitochondria to produce ATP (Johnson et al. 2013). Although there is evidence of a correlation between the results of certain

in vitro and in vivo approaches (McCully et al. 1993; Lanza et al. 2011), these approaches likely assess different aspects of mitochondrial function, which may contribute to the discrepancies in the literature and should be considered when comparing results of individual studies. Furthermore, the methods used to isolate mitochondria, which is necessary for some in vitro approaches, appear to exaggerate age-related differences in mitochondrial function (Picard et al. 2010). Although in vitro methods provide powerful mechanistic information, in vivo approaches, which assess the function of the intact system, have an important role for investigating the impact of aging on overall mitochondrial function.

Several studies using a variety of approaches have shown that skeletal muscle mitochondrial function is sensitive both to increases (Forbes et al. 2008; Gollnick et al. 1973; Jacobs and Lundby 2013; Short et al. 2003; Konopka et al. 2014; Larsen et al. 2012a) and decreases (Hikida et al. 1989) in PA. Recent studies report a direct, if modest, association between habitual PA and several markers of skeletal muscle mitochondrial function (den Hoed et al. 2008), including in vivo oxidative capacity (Larsen et al. 2009; Tartaglia et al. 2000). Although PA generally declines across the lifespan (Troiano et al. 2008), many studies lack specific, objective measures of this important lifestyle variable. It has been proposed that differences in PA, rather than aging per se, are responsible for losses in skeletal muscle oxidative capacity with aging (Brierley et al. 1996, 1997; Russ and Kent-Braun 2004). Furthermore, several studies (Kent-Braun and Ng 2000; Lanza et al. 2005, 2007; Larsen et al. 2012b) have shown preserved in vivo oxidative capacity in the tibialis anterior (TA) muscle of *O* adults compared to young (Y) adults with similar, sedentary PA.

In contrast with studies of the TA, other studies have shown age-related declines in oxidative capacity of the plantarflexor muscles using both in vivo (McCully et al. 1991) and in vitro approaches (e.g., enzyme activities; Coggan et al. 1992; Houmard et al. 1998). In the quadriceps muscles, which are most commonly studied, in vivo (Conley et al. 2000; Larsen et al. 2012b) and in vitro (Short et al. 2005) oxidative capacities are both lower in *O* adults. Additionally, diminished mitochondrial content (Conley et al. 2000), mitochondrial protein content (Short et al. 2005) and synthesis (Rooyackers et al. 1996), and diminished markers of mitochondrial biogenesis and quality control (Konopka et al. 2014), have all been reported (see Konopka and Nair 2013; Johnson et al. 2013 for recent reviews).

While the finding of reduced mitochondrial function in the quadriceps with age is not universal (Rasmussen et al. 2003), these results raise the possibility that the effects of aging on oxidative capacity may vary depending on the muscle under investigation, as has been shown with other aspects of muscle function. For example, several studies have documented greater age-related declines in the strength (Hasson and Caldwell 2012; Winegard et al. 1996; Simoneau et al. 2005) and size (Hasson et al. 2011) of the plantarflexors than the ankle dorsiflexors. The mechanisms for these muscle-specific effects are still under investigation but may include age-related changes in the patterns of use of the muscles (Schmitz et al. 2009; Benjuya et al. 2004), as well as different susceptibilities of the different fiber types to age-related changes in mitochondrial function (Jacobs et al. 2013; Proctor et al. 1995). The previously reported selective atrophy of type II fibers with age (Proctor et al. 1995) may also contribute to intermuscular variation in the effects of aging.

Studies of the oxidative capacity of multiple muscle groups in the same individual, which are ideal for investigating intermuscular variation in the effects of aging, are rare. However, the studies that we are aware of all show evidence of intermuscle variation in the impact of aging on oxidative capacity. Pastoris et al. (2000) reported lower citrate synthase activity, an in vitro marker of oxidative capacity, of the vastus lateralis, but not the rectus abdominus or gluteus medius, in *O* sedentary subjects. By contrast, Houmard et al. (1998) found an age-related decline in the gastrocnemius, but not the vastus lateralis, using the same technique. Finally, Larsen et al. (2012b) found that oxidative capacity was preserved in *O* TA, but not VL, muscles.

The results of these studies prevent simple generalizations about the impact of age on muscle oxidative capacity and make clear that further study of additional muscle groups is necessary to fully understand the impact of aging on human muscle. Therefore, the primary purpose of this study was to investigate the effect of aging on skeletal muscle in vivo oxidative capacity in two leg muscles in the same individuals. *Y* and *O* men and women with similar overall PA, determined using accelerometry, were studied. Oxidative capacity was measured by phosphorus magnetic resonance spectroscopy (^{31}P-MRS). The TA and medial gastrocnemius (MG) muscles were selected for study because, while

both are critical to physical function, gait, and fall prevention in *O* adults (Hasson et al. 2014; Wolfson et al. 1995), they exhibit a variety of differences. Functionally, the TA operates largely eccentrically during the stance phase of the gait cycle, while concentric activation of the MG is critical to provide forward propulsion. The TA is largely composed of type I fibers (75 % type I; Gregory et al. 2001), while the MG is mixed in fiber-type composition (50 % type I; Edgerton et al. 1975). Furthermore, while most studies of the TA do not report lower oxidative capacity in *O* adults (Kent-Braun and Ng 2000; Lanza et al. 2005, 2007; Larsen et al. 2012b; Tevald et al. 2010), reports on the MG differ, with studies reporting either lower (McCully et al. 1991; Coggan et al. 1992; Houmard et al. 1998) or similar (Chilibeck et al. 1998; Wray et al. 2009) oxidative capacity in *O* compared to Y adults. Based on the previously discussed evidence that age-related changes in several aspects of muscle performance are smaller in the TA than the MG, we hypothesized that oxidative capacity would be preserved in the TA, but not the MG, of *O* subjects. The results of this study will advance our understanding of the variation in age-related effects on muscle oxidative capacity.

Methods

Subjects

Thirty-four *Y* (21–29 years) and *O* (65–77 years) men (M) and women (W) participated in this study (see Table 1 for distribution). All participants gave written informed consent prior to participation. All procedures were approved by the appropriate institutional review boards at Yale University School of Medicine and the University of Massachusetts, Amherst, and conformed to the standards set by the Declaration of Helsinki.

Table 1 Subject characteristics

	Y (17)	*O* (17)	*p* value
Female (*n* (%))	8 (47 %)	9 (53 %)	–
Age (years)	24±3	69±3	–
Height (cm)	167.8±76	165.9±90	0.515
Body mass (kg)	66.9±12.7	79.7±15.5	0.013
BMI	23.7±3.9	28.8±4.3	0.001

Data presented as mean±SD, except where indicated

All participants were nonsmokers, generally healthy, and sedentary (<60 min of structured PA per week, by self-report). They were free from neurological or neuromuscular disease, stroke, diabetes, or known coronary artery disease and had ankle-brachial index ≥1, suggesting the absence of peripheral vascular disease. Medications used by subjects included ace inhibitors (two OM and one OW), angiotensin receptor blockers (one OW), and diuretics (two OW) for the control of hypertension and statins (two OM and two OW) for the treatment of hypercholesterolemia. One OW was on estrogen replacement therapy. Participants were instructed to take their medications as prescribed during the course of the study.

Procedures and activity assessment

The study consisted of two testing sessions, separated by at least 48 h. The first session was conducted in the Muscle Physiology Lab at the University of Massachusetts, Amherst, and consisted of physical function testing. The second session was the metabolic testing session, which was conducted at the Magnetic Resonance Research Center at the Yale University School of Medicine. In addition, all subjects wore a uniaxial accelerometer (Actigraph GT1M; Pensacola FL) during waking hours for 7–10 days to quantify PA. Total PA (sum of all counts over the day), as well as the time spent in low-intensity PA (LPA; 1–1,951 counts min^{-1}) and moderate-to-vigorous intensity PA (MVPA; >1,952− counts min^{-1}), were calculated based on a modification of established thresholds for Actigraph accelerometers (Freedson et al. 1998; Matthews et al. 2008). Accelerometer data were verified against an activity diary maintained by the subjects. Days during which the participant's activity patterns could not be reconciled with the diary, or the subject did not wear the monitor for at least 10 h, were excluded (Matthews et al. 2008). Each participant's daily average for all variables was used to determine group averages.

Physical function testing

Physical function testing was performed to more fully characterize the mobility status of the participants. A battery of commonly used tests was selected to provide a measure of the general functional status of the

individuals. The tests including the following: seated foot-tap speed over 10 s (Kent-Braun and Ng 1999; Kent-Braun et al. 1998), ten timed chair rises, timed climb, and descent of eight steps, and a 400-m walk (Simonsick et al. 2001). Foot tap speed, chair rises, and stair climb and descent were each performed twice, and the fastest time was used for analysis. The 400-m walk was performed by laps around a straight 20-m track, separated by cones. Participants were instructed to walk at the quickest pace that they could maintain for the entire walk.

Muscle metabolic testing

Participants were instructed to avoid caffeine for 6 h and strenuous activity for 24 h prior to testing due to their potential effects on neuromuscular function. All testing was performed while participants lay supine in a 4.0-Tesla whole-body superconducting magnet (Bruker Biospin, Rheinstetten, Germany). The TA and the MG muscles were tested in separate sessions on the same day, with a minimum of 30 min between sessions. For all participants, the TA was tested first, followed by the MG. The apparatus and protocol used to test the TA muscles has been described previously (Tevald et al. 2010). Procedures for the MG were similar, although the knee was positioned in approximately 15° of flexion, and the foot position at 90° with respect to the tibia. Velcro straps were used to secure the foot, ankle, and shank, and shoulder straps were used to minimize upper body movement during contractions. To verify that the source of our ^{31}P signal was from MG only, we measured the distance from the surface of the skin to the border between MG and soleus. This distance was 2.8±0.4 and 2.6±0.4 cm in *O* and *Y* groups, respectively. Because the maximum depth for signal detection by a surface coil corresponds to its smallest radius (1.5 cm), signal contamination from soleus was not an issue.

The contraction protocols were initiated with two "warm-up" maximal voluntary contractions (MVC), each lasting 3–4 s. Following 2 min of rest, ^{31}P-MRS data acquisition was initiated. After an additional 1 min of rest, the subject performed a 16-s (TA) or 20-s (MG) MVC. ^{31}P-MRS data acquisition continued during and for 10 min following each contraction. Contraction durations were chosen, based on pilot data, to deplete PCr by ~40 %, without intracellular acidosis.

Phosphorus spectral analysis and metabolic calculations

Spectral analysis and metabolite concentrations were performed as previously described (Tevald et al. 2010). To calculate metabolite concentrations from ^{31}P-MRS spectra, peak areas were corrected for partial saturation effects. Correction factors for partial saturation effects for the MG were obtained in a subset of subjects (n=8; PCr=1.69, P_i=2.16, ATP=1.62), as previously described (Tevald et al. 2010), while values from a previous study (Tevald et al. 2010) were used for TA (PCr=1.97, P_i=1.89, ATP=1.83). Resting and end-exercise metabolite concentrations were calculated by assuming that resting (ATP)=8.2 mM (23), and that (PCr)+(P_i)=42.5 mM, which is based on the assumptions that (PCr)+(creatine)=42.5 (Harris et al. 1974), and P_i and creatine exhibit a 1:1 stoichiometry (Kemp and Radda 1994). Intramuscular pH was calculated from the chemical shift of P_i relative to PCr (Taylor et al. 1986).

Assessment of in vivo oxidative capacity

To quantify in vivo *oxidative capacity*, the recovery of PCr following the 16-s and 20-s MVCs was fit with a monoexponential function, and oxidative capacity (V_{max}; mM ATP·s^{-1}) was calculated as the product of the rate constant (k_{PCr}) and resting (PCr) (Lanza et al. 2005; Meyer 1989). This analysis provides a robust measure of oxidative capacity in the intact system (Lanza et al. 2011).

Statistical analysis

Subject characteristics (body height, mass, body mass index (BMI), PA, and physical function variables) and metabolic variables (V_{max}, k_{PCr}, and metabolites at rest and the end of contraction) were compared between the *Y* and *O* groups using separate two-tailed t tests. To evaluate the potential effects of the use of medications (blood pressure medications and statins, respectively), oxidative capacity among *O* adults who did and did not take these drugs was compared with separate nonparametric tests (Mann–Whitney *U*). Because the effects of age may vary by sex, our secondary purpose regarding the influence of sex on oxidative capacity was evaluated with a two-factor (age and sex) ANOVA and post-hoc testing using Tukey's HSD. Additional analysis included a two-tailed paired t test to determine if there were

differences in oxidative capacity between the TA and MG, as well as Pearson and partial correlation to evaluate the strength of the relationships between oxidative capacity, BMI, and PA. Statistical analyses were performed using IBM SPSS 19 (IBM Corp, Armonk, NY), and α was set at 0.05. All data are presented as mean± SD.

Results

Descriptive data for the groups are summarized in Table 1. There was no age difference in height, but the *O* adults were heavier and had higher BMI. The PA data are summarized in Table 2. There were no differences in the total time the subjects wore the monitors each day. Daily accelerometer counts were not different between the age groups, indicating that overall PA was not different. The total amount of time that the participants engaged in PA did not differ between the groups nor did the amount of time spent in LPA. However, the *O* adults spent less time in MVPA than the younger participants. Data from one *O* subject is missing due to technical issues.

Performance on physical function tests by each of the groups is also summarized in Table 2. Despite the fact that the participants were reasonably healthy and of similar overall PA level, *O* subjects were slower on all tests of physical function. Foot tap data from one younger subject and 400 m walk time from one O subject are missing due to investigator error.

Table 2 Physical activity and physical function

	Y	*O*	*p* value
Physical activity	*n*=17	*n*=16	
Wear time[a] (min day^{-1})	865±185	858±72	0.887
Active[b] (min day^{-1})	464±128	209±88	0.257
PA (ct day$^{-1,000}$)	267±114	220±63	0.156
LPA (min day^{-1})	422±114	487±90	0.085
MVPA (min day^{-1})	42±24	21±15	0.001
Physical function			
Foot tap[c] (no. in 10 s)	56±7	45±7	<0.001
Stair climb[d] (s)	2.8±0.4	3.8±0.8	<0.001
Stair descend[d] (s)	2.6±0.4	3.6±1.1	0.001
Chair rise×10[e] (s)	11.8±3.1	14.9±4.1	0.022
400-m walk[f] (s)	222±20	274±30	<0.001

Data presented as mean±SD. Physical activity data from one older subject missing for technical reasons; 400-m walk data missing from one older and one younger subject

PA total physical activity by accelerometer, *LPA* time spent in sedentary-to-low PA, *MVPA* time spent in moderate-to-vigorous PA

[a] Time spent wearing accelerometer

[b] Time spent in nonzero PA

[c] Number of foot taps in 10 s

[d] Time to climb and descend, respectively, eight steps

[e] Time to rise from a chair ten times

[f] Time to walk 400 m

Age and oxidative capacity

Metabolic variables for the TA are summarized in Table 3 and Fig. 1, while those for the MG are summarized in Table 4 and Fig. 2. Metabolic data from the MG of one *Y* and two *O* subjects was not available for technical reasons. *At rest*, no differences in (PCr) or pH were apparent in either muscle. *During contraction*, there were no group differences in the extent of the decline in PCr. As a result of the consumption of protons by the CK reaction, intracellular pH increased briefly at the onset of contraction, as expected (Fig. 1). In the TA, but not the MG, pH at the end of contraction was higher in *O* than *Y*, but the minimum pH observed during recovery was not significantly different for either muscle. *During recovery*, both k_{PCr} and V_{max} were higher in *O* than *Y* in the TA, but there were no differences in the MG.

V_{max} was not different in *O* adults who took statins and those who did not, for either the TA (*n*=4; 1.07± 0.17 μM/s vs. *n*=13; 1.01±0.17; *p*=0.102) or for the

Table 3 Metabolic variables from the tibialis anterior muscle

	Y (*n*=17)	*O* (*n*=17)	*p* value
Rest			
PCr (mM)	38.1±1.3	38.6±1.5	0.234
pH	7.02±0.03	7.03±0.04	0.519
Contraction			
End PCr[a] (% rest)	61.3±6.2	58.0±7.5	0.170
End pH[a]	7.03±0.04	7.10±0.05	<0.001
Min pH[b]	6.86±0.06	6.89±0.04	0.066
k_{PCr} (s^{-1})	0.024±0.003	0.029±0.004	<0.001
V_{max} (mM ATP s^{-1})	0.90±0.12	1.12±0.18	<0.001

k_{PCr} rate constant of PCr recovery, V_{max} oxidative capacity

[a] PCr and pH at the end of contraction

[b] Lowest pH achieved during recovery

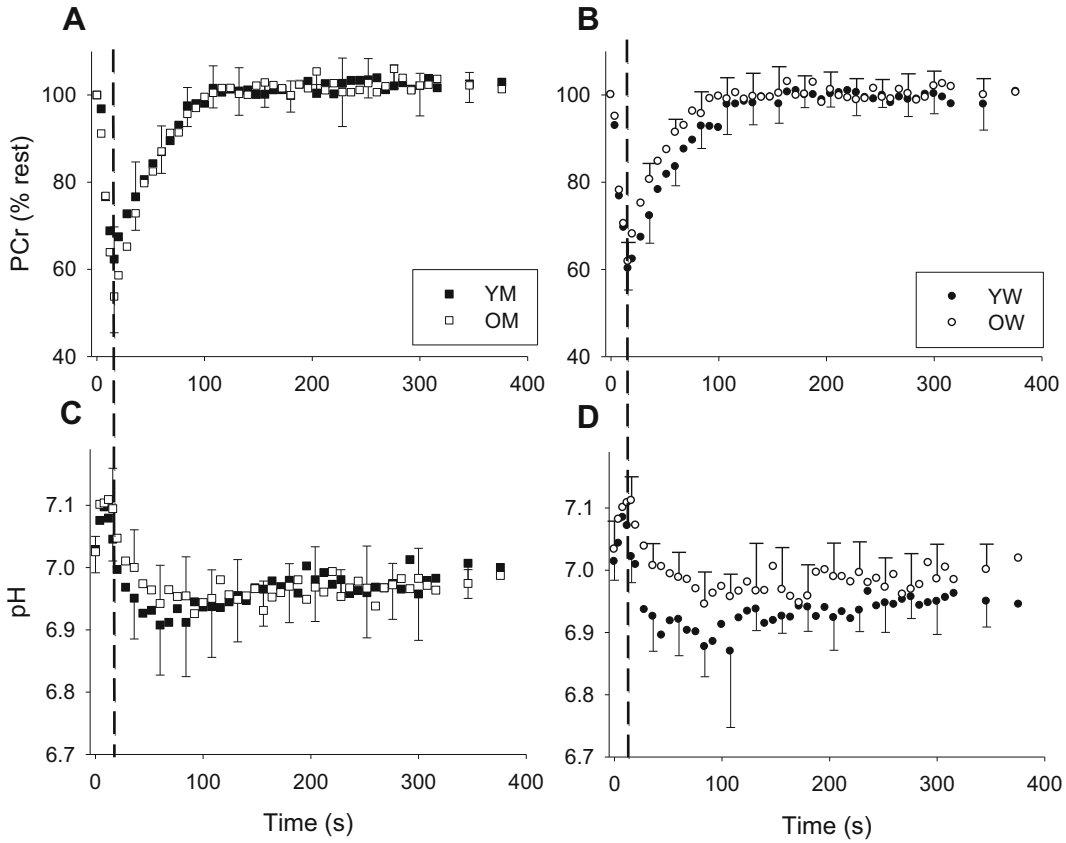

Fig. 1 Metabolic changes in tibialis anterior PCr (**a**, **b**) and pH (**c**, **d**) during contraction and recovery in young and older men (**a**, **c**) and women (**b**, **d**). PCr is expressed relative to resting values. The end of the 16-s contraction is indicated by the *dashed vertical line* on each plot. Following contraction, the recovery of PCr was well approximated by a monoexponential function in all groups (r^2= 0.90±0.06, 0.90±0.04, 0.91±0.03, and 0.87±0.04 for YM, OM, YW, and OW, respectively). *Error bars* indicating SD are provided every 24 s during recovery, for clarity

Table 4 Metabolic variables from the medial gastrocnemius muscle

	Y (*n*=16)	*O* (*n*=15)	*p* value
Rest			
PCr (mM)	37.9±1.2	38.1±1.2	0.593
pH	7.02±0.03	7.02±0.04	0.707
Contraction			
End PCr[a] (% rest)	65.4±10.7	59.4±9.3	0.112
End pH[a]	7.07±0.06	7.08±0.04	0.605
Min pH[b]	6.88±0.07	6.89±0.08	0.965
k_{PCr} (s^{-1})	0.022±0.004	0.025±0.006	0.551
V_{max} (mM ATP s^{-1})	0.91±0.17	0.96±0.24	0.500

k_{PCr} rate constant of PCr recovery, V_{max} oxidative capacity

[a] PCr and pH at the end of contraction

[a] Lowest pH achieved during recovery

MG (*n*=3; 1.01±0.17 μM/s vs. *n*=12; 0.76±0.42; *p*= 0.365). Similarly, V_{max} was not different among *O* adults who took blood pressure medications and those who did not for the TA (*n*=5; 1.18±0.21 μM/s vs. *n*=12; 1.09± 0.19 μM/s; *p*=0.57) or MG (*n*=4; 0.85±0.39 μM/s vs. *n*=11; 0.88±0.17 μM/s; *p*=0.57).

Sex and oxidative capacity

A secondary analysis was used to investigate the combined effects of age and sex on in vivo oxidative capacity. The results are displayed in Fig. 3. For the TA, there was a trend (*p*=0.066) for an age by sex interaction, along with a significant effect of age. Post-hoc analysis revealed that V_{max} in the OW (*n*=9; 1.27±0.11 mM ATP s^{-1}) was significantly higher than in the YM (*n*= 9; 0.83±0.11) and YW (*n*=8; 0.86±0.18) but not the OM (*n*=8; 1.09±0.28). In the MG, there was no

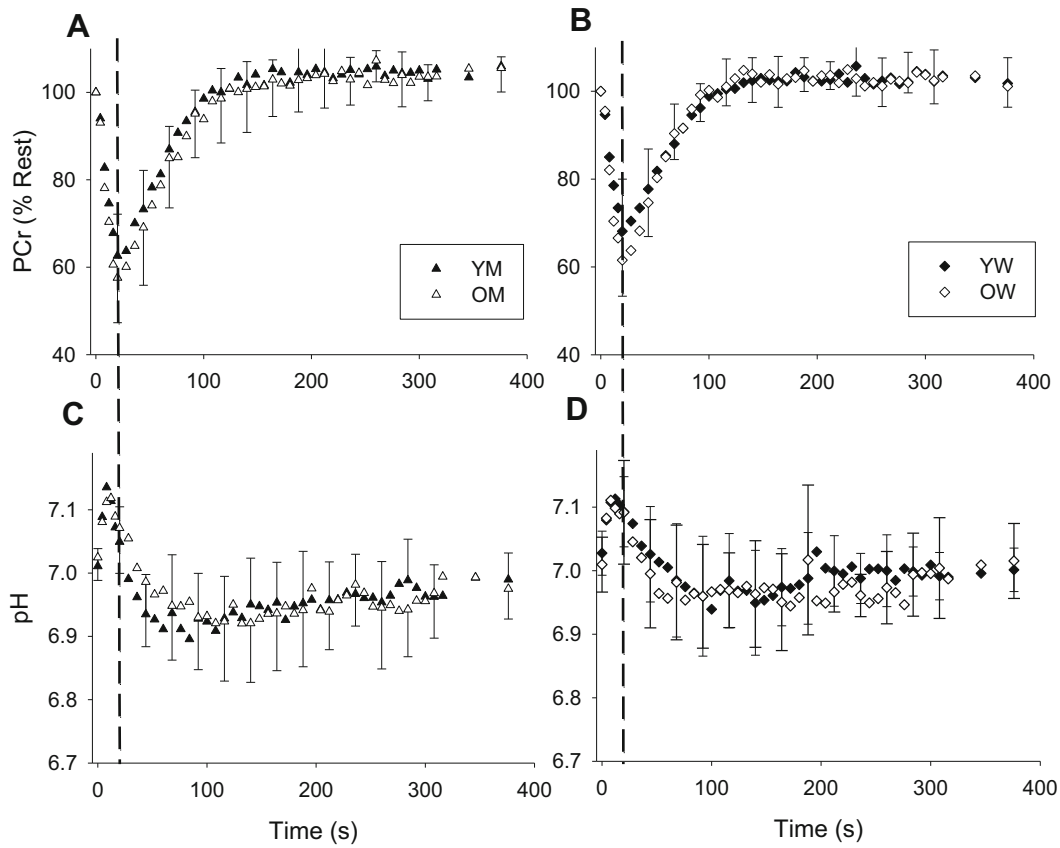

Fig. 2 Metabolic changes in medial gastrocnemius PCr (**a**, **b**) and pH (**c**, **d**) during contraction and recovery in young and older men (**a**, **c**) and women (**b**, **d**). PCr is expressed relative to resting values. The end of the 20-s contraction is indicated by the *dashed vertical line*. Recovery of PCr following contraction in MG was well approximated by a monoexponential function (r^2=0.89±0.09, 0.92±0.02, 0.86±0.09, and 0.89±0.06 for YM, OM, YW, OW, respectively). *Error bars* indicating SD are provided every 24 s during recovery, for clarity

interaction (p=0.171) or effect of age (p=0.367), but V_{max} for women (n=15; 1.03±0.18) was significantly higher than men (n=16; 0.84±0.18; p=0.007).

V_{max} of the TA (n=31; 0.99±0.03) was not significantly different from the MG (n=31; 0.93±0.04; p=0.23) overall, or within the *O* (n=15; 1.10±0.17 vs. 0.96±0.062; p=0.118) or *Y* (n=16; 0.89±0.13 vs. 0.91±0.17; p=0.791) age groups.

Correlations

Neither k_{PCr} nor V_{max} in the TA or MG were associated with any PA variables (PA, LPA, or MVPA, r=−0.28 to 0.23; p>0.12 for all comparisons). V_{max} of the TA (r=0.376; p=0.029) but not the MG (−0.255; p=0.167), was correlated with BMI. BMI was also associated with age (r=0.549; p=0.001), and removing the influence of age eliminated the relationship between BMI and V_{max} in the TA (r=0.095; p=0.6).

Discussion

The results of this study provide evidence of well-preserved in vivo mitochondrial function in both the TA and MG of healthy *O* men and women, even though the *O* adults showed signs of diminished physical function. Thus, while previous studies have shown that the MG appears to be more sensitive than the TA to age-related changes in size (Hasson et al. 2011) and strength (Hasson and Caldwell 2012; Winegard et al. 1996; Simoneau et al. 2005), the same does not appear to be true for oxidative capacity when overall PA is similar among groups. We also found higher oxidative capacity in women than men for the MG muscle, and no

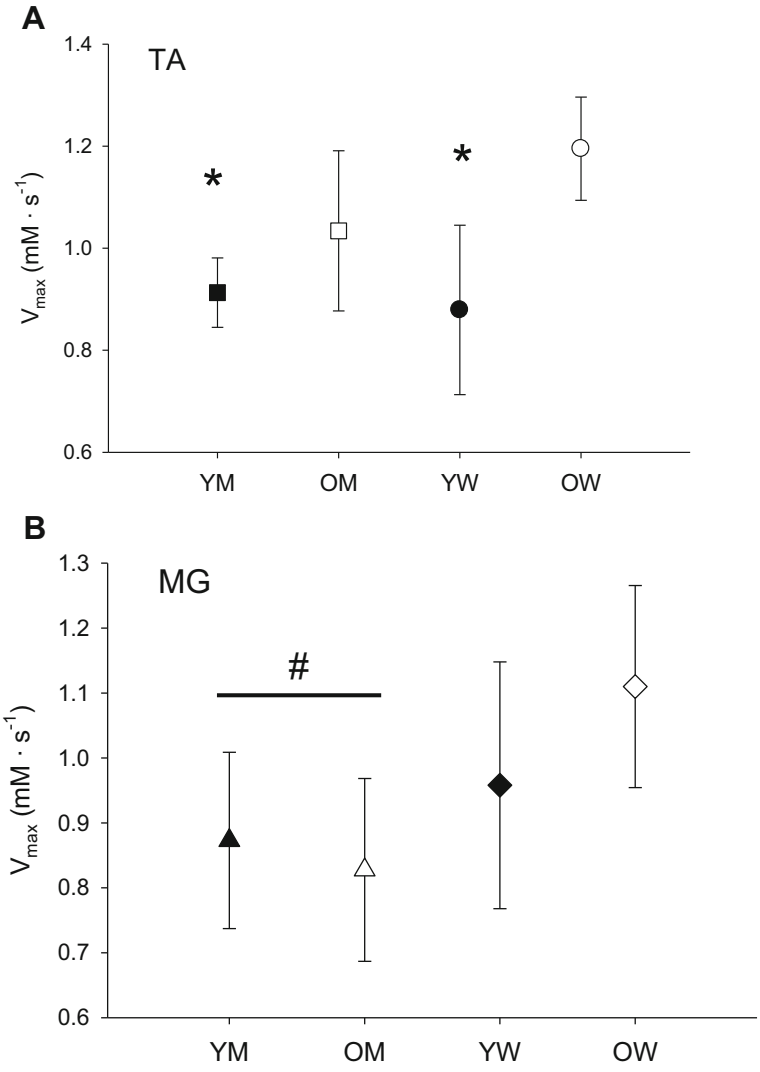

Fig. 3 Muscle oxidative capacity V_{max} (mM s^{-1}) for tibialis anterior (*TA*, **a**) and medial gastrocnemius (*MG*, **b**) muscles; *asterisk*, significantly lower than OW; *number sign*, main effect of sex

difference in oxidative capacity between the MG and TA muscles. These results support the concept that PA may be a more important determinant of in vivo muscle mitochondrial function than aging, per se.

Age and oxidative capacity

The finding of preserved oxidative capacity in this study would appear to be counter to a number of studies that have identified age-related deficits in a variety of markers of mitochondrial function, content, and biogenesis in human muscle (reviewed in Konopka and Nair 2013; Johnson et al. 2013). However, they are consistent with the concept that the effects of age on oxidative capacity depend on the muscle being investigated and on the level of habitual PA of the individual. Several studies from this lab have reported well-preserved in vivo oxidative capacity in the TA (Kent-Braun and Ng 2000; Lanza et al. 2005; Tevald et al. 2010), including one recent study showing higher oxidative capacity in the TA of *O* adults but lower oxidative capacity in the quadriceps (Larsen et al. 2012b). We are not aware of studies that have used in vitro techniques to examine the effect of age on oxidative capacity of the TA.

While some of the *O* subjects in the current study took statin or blood pressure medications, these did not have a significant effect on oxidative capacity in either muscle. Although the small number of individuals taking these medications preclude us from drawing strong conclusions about the impact of these drugs on oxidative

capacity, they did not appear to influence the results of this study. In this context, our results are consistent with previous reports.

With regard to in vivo oxidative capacity of the MG, the present results are consistent with two prior studies (Chilibeck et al. 1998; Wray et al. 2009). They are not consistent with the results of one in vivo study (McCully et al. 1991) and two in vitro (enzyme activities) studies (Coggan et al. 1992; Houmard et al. 1998), all of which showed lower oxidative capacity in the *O* subjects. In the first study (McCully et al. 1991), the lack of control for PA and health status of the *O* subjects likely contributed to lower oxidative capacity in that group. The discrepancy between the present results and those of the two in vitro studies is less clear, as both only studies included only people who reported that they were sedentary. However, self-reported means of assessing PA typically overestimate actual PA (Tucker et al. 2011) and may not have been sensitive enough to detect a difference in PA among the age groups. Additionally, the differences in the technique used to assess oxidative capacity (in vivo vs. in vitro) may contribute to the different results. Although in vitro approaches provide powerful mechanistic information, in vivo approaches assess the integrated function of the entire intact system in vivo, and therefore play a critical role in the investigation of the effects of age and disease on human physiology.

Indeed, although overall PA was similar among the groups in the current study, the *O* adults spent less time in moderate-to-vigorous PA (Table 2). Given the sensitivity of muscle oxidative capacity to PA, one might expect this to lead to lower oxidative capacity in the *O* subjects. However, Larsen et al. (2009, 2012b) found that time spent in MVPA was more strongly associated with oxidative capacity of the quadriceps (r=0.64) than the TA (r=0.38), in a sample that included a wide range of PA levels. Thus, it appears that age-related difference in the intensity or pattern of PA has greater effects on some muscles than others, and it may be that both the TA and MG are less affected than the quadriceps by these differences in PA. There was no association between PA and oxidative capacity in either the TA or MG in the current study, although the narrow range of PA levels (all sedentary) limits our ability to assess this potential relationship, and further study is needed to evaluate this hypothesis.

Additionally, age-related changes in muscle activation during daily activities may lead *O* adults to use certain muscles more than younger adults while performing the same task. *O* adults exhibit greater co-contraction during standing (Benjuya et al. 2004) and walking (Schmitz et al. 2009; Hortobagyi et al. 2011), leading to greater activation of the ankle dorsiflexors and plantarflexors during these tasks. These differences in muscle activation contribute to the reduced mechanical efficiency of gait in *O* adults (Hortobagyi et al. 2011). They also provide a potential explanation for preserved, or even enhanced, oxidative capacity in certain muscles, such as that observed here. Together, these results suggest that, unlike the quadriceps, age-related changes in the *pattern* of PA do not have detrimental effects on the oxidative capacity of the TA and MG when the overall amount of PA is similar in *Y* and *O* subjects.

Sex and mitochondrial function

In the current study, we found that oxidative capacity of the MG was higher in women than men. Furthermore, in the TA, the *O* women exhibited the highest oxidative capacity of all the groups. These results are consistent with one prior study from our lab (Kent-Braun and Ng 2000) and those of Essen-Gustavsson and Borges (1986), who found higher citrate synthase activity in the vastus lateralis of women in their sixties than age-matched men. However, other studies have shown similar (Grimby et al. 1982; Lanza et al. 2007; Russ et al. 2005) or lower (Coggan et al. 1992) oxidative capacity activity in women compared with men. The explanations for these discrepant results are not clear, although they are broadly consistent with studies of the effects of sex on fatigue (Hunter et al. 2004) and energetics (Kent-Braun et al. 2002; Ruby and Robergs 1994; Russ et al. 2005). Specifically, women are less reliant on nonoxidative glycolysis during whole body (Ruby and Robergs 1994) and small muscle mass (Russ and Kent-Braun 2003) exercise. During muscle contraction, women experience less fatigue (Hunter et al. 2004), less acidosis (Russ et al. 2005), and smaller changes in (PCr) and (P_i) (Kent-Braun et al. 2002).

Muscle differences

Overall, we found that oxidative capacity in the TA was not different from oxidative capacity in the MG in our sedentary subjects. This contrasts with two recent studies of active *Y* individuals, which found higher oxidative

capacity in the MG than in the TA (Forbes et al. 2009b; Gregory et al. 2001), despite the higher percentage of type I fibers in the TA. The authors of both previous studies reasoned that the athletic events the active subjects participated in likely involved frequent concentric contractions of the gastrocnemius (Gottschall and Kram 2003), which are more energetically costly than the largely eccentric contractions of the TA (Ryschon et al. 1997). Regular participation in these activities may be a strong stimulus for increasing oxidative capacity in the MG. By contrast, the largely eccentric action of the TA during these activities may be a less potent stimulus for adaptation, leading to comparatively modest increases in the TA. The discrepancy between these two studies and the current one may also be explained by the fact that the sedentary subjects in the present study may not have had sufficient exposure to these types of activities to produce high oxidative capacities in the MG. Indeed, the similar overall PA in *Y* and *O* here suggests that the TA was activated during walking to a similar extent in both age groups, thus maintaining its activation overall.

Methodological considerations

The use of PCr recovery kinetics to investigate mitochondrial function is widespread and based on a concept that, after a brief contraction, PCr recovers along a monoexponential time course due to aerobic processes (Meyer 1988, 1989). Recent studies have demonstrated that, during high intensity contractions such as those used here, nonoxidative processes can contribute to the recovery of PCr in some people (Forbes et al. 2009a; Lanza et al. 2006). However, the fast, nonoxidative component of recovery does not appear to affect the accuracy of the estimate of the recovery kinetics from a monoexponential fit (Forbes et al. 2009a). Furthermore, Forbes et al. (2009a) found no evidence for higher-order recovery kinetics in the human plantar flexors under conditions that are similar to this study (e.g., approximately 40 % PCr depletion, pH >6.8). Therefore, the rate constant from a monoexponential fit are still valid indices of mitochondrial function under these conditions. Additional studies have shown that the recovery of PCr is slowed by acidosis. Although we found age- and sex-related differences in end-exercise pH, pH during recovery stayed above 6.8, suggesting that any effect of pH on the recovery of PCr was small. Finally, a recent comparison between in vivo estimates of oxidative capacity, similar to those used here, and in vitro measures of mitochondrial function demonstrated the validity and robustness of these measures (Lanza et al. 2011).

The in vivo approach used here assesses the integrated function of multiple systems that contribute to muscle oxidative capacity but provides limited information about mechanisms. Furthermore, it cannot assess the relative impacts of other physiological changes that are associated with aging. For example, *O* muscle can exhibit preferential atrophy of type II fibers (Proctor et al. 1995) and express a greater number of hybrid fiber types (Purves-Smith et al. 2014), which may influence the metabolic profile of the muscle. While this may be considered a limitation, the integrated nature of the approach used here provides information that is highly relevant to physiological functioning and plays an important role in the investigation of aging and disease on muscle energetics. Furthermore, the recent findings of Larsen et al. (2012b) showing higher oxidative capacity in the VL than TA in *Y* (but not *O*) adults, indicate that oxidative capacity may be a function more of usage than of fiber-type composition, per se.

We used PA monitors, worn during waking hours, to objectively quantify PA. Based on previous studies (Matthews et al. 2008), we considered a day valid if at least 10 h of data was recorded. However, some activity, including nighttime activity, was not included in our estimates of PA. If systematic differences between the groups in nighttime activity were present, these would not be reflected in our data. However, the magnitudes of these differences, if present, are likely small relative to activity during waking hours, and thus are unlikely to change the interpretation of our results.

It is well accepted that whole-body aerobic capacity declines with age (Proctor and Joyner 1997), even when overall PA level is similar (Kent-Braun and Ng 2000). For this reason, as well as the risks and costs associated with maximal exercise testing, we did not measure maximal aerobic capacity in the current study. This prevents the use of aerobic capacity as a means to compare our sample to those in other studies. However, we chose to characterize physical function with a battery of tests that are commonly used in large-scale studies of *O* adults (Simonsick et al. 2001) to allow comparison to those studies.

Conclusions

We found no evidence for age-related declines in in vivo oxidative capacity in two distinct locomotory muscles in

sedentary, relatively healthy individuals. Our results are consistent with the concept that differences in PA, rather than aging per se, play a substantial role in the oxidative function of muscles in *O* adults. The extent to which age-related alterations in gait pattern may contribute to this preservation of energetics in the face of declining physical function remains to be determined.

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