Age-related changes in ATP-producing pathways in human skeletal muscle in vivo

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Submitted 13 May 2005; accepted in final form 4 July 2005

The aging process in humans is accompanied by a variety of changes to the neuromusculature; in particular, muscle mass and strength decrease with age (10). Despite these declines, there are numerous reports that older adults are more resistant to muscle fatigue than young (24, 25, 27). The development of muscle fatigue is associated with the accumulation of metabolic by-products within muscle during contraction (9). It is plausible that alterations in intramuscular energy metabolism that occur with age may contribute to the age-related fatigue resistance. A recent study using phosphorus magnetic resonance spectroscopy (31P-MRS) to investigate muscle metabolism in vivo reported greater fatigue in young subjects compared with older, which was associated with greater accumulation of diprotonated inorganic phosphate (P1) (24). Furthermore, muscles of young subjects experienced greater acidosis during exercise than older subjects, suggesting greater utilization of anaerobic glycolysis, despite working at equivalent contraction intensities. The potential for oxidative phosphorylation, evaluated by increases in the ratio of P1 to phosphocreatine (PCr) during incremental steady-state exercise, was similar in young and older subjects (24). These data suggest that, despite similar skeletal muscle oxidative potentials in young and old, there may be an age-related difference in the reliance on anaerobic glycolysis for ATP production during exercise that could explain the enhanced fatigue resistance of old age.

During muscle activity, ATP is derived from three major pathways: 1) oxidative phosphorylation, 2) anaerobic glycolysis, and 3) the net hydrolysis of PCr through the creatine kinase (CK) reaction, which acts to buffer the concentrations of ATP (43). Some in vivo studies suggest that mitochondrial function declines with age (7, 31), although others report similar mitochondrial function in young and older subjects when physical activity patterns are accounted for (5, 23). The effects of old age on anaerobic glycolysis are unclear; biopsy studies have revealed similar (6) or lower (28) glycolytic enzyme activities in older compared with young adults. However, we are unaware of studies that have evaluated the effects of old age on glycolytic flux in vivo. The effect of old age on the CK reaction is also poorly studied, although one group found no age-related impairment in CK flux during low-intensity contractions (14).

The purpose of the present study was to determine whether there are differences between healthy young and older men in ATP generation via the net PCr hydrolysis in the CK reaction, oxidative phosphorylation, and anaerobic glycolysis during maximal intensity muscle contractions. We hypothesized that 1) oxidative capacity would be similar in young and older men, 2) glycolytic flux would be lower in older than young men, and 3) older men would derive a greater proportion of their ATP from oxidative metabolism. The results of this study extend earlier biopsy and 31P-MRS studies and provide novel information related to the effects of old age on the in vivo functionality of the major ATP-producing pathways in human skeletal muscle.

Experimental procedures

Experimental design. Concentrations of PCr, P1, phosphomonoesters (PME), and ATP were measured in vivo by use of 31P-MRS during two maximal contractions (16 s, 60 s) of the ankle dorsiflexor muscles in young and older men. A 16-s maximal voluntary isometric contraction (MVIC) was used to assess oxidative capacity. The short

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duration but high intensity of this contraction allows moderate depletion of PCr in the absence of appreciable acidosis. Recovery from the 16-s MVIC was used to determine oxidative capacity (7, 32). A longer and more metabolically demanding 60-s MVIC allowed the contributions of the CK reaction, oxidative phosphorylation, and anaerobic glycolysis to ATP generation to be determined under conditions that generate high flux through each of these pathways.

Changes in pH and the concentrations of phosphorus metabolites during contraction and recovery were used to calculate oxidative capacity and the rates of ATP synthesis by the CK reaction, oxidative phosphorylation, and anaerobic glycolysis by using established methods (4, 17, 19, 45).

All subjects were tested on two occasions separated by at least 48 h. The first session took place at the University of Massachusetts, where subjects were familiarized with the exercise protocol and the capability of each subject to fully activate the dorsiflexor muscles by a voluntary contraction was verified. The second session took place at the Magnetic Resonance Research Center at Yale University, where subjects repeated the exercise protocol with simultaneous measurement of intramuscular energy metabolism using 31P-MRS. Exercise consisted of two MVICs of the right ankle dorsiflexor muscles sustained for 16 s and 60 s, respectively, separated by 15 min of recovery.

Subjects. Eight young (22 ± 1 yr) and eight older (75 ± 5 yr) nonsmoking men in good health and free from medications affecting muscle function or blood flow (e.g., antihypertensives, Acetylcholine esterase inhibitors, calcium channel blockers, statins) were enrolled in this study. Written, informed consent was obtained for the protocol, which was approved by the University of Massachusetts, Amherst and the Yale University School of Medicine human subjects review boards and conformed to the standards set by the Declaration of Helsinki. Women were excluded from this study because gender differences have been observed in muscle metabolism (24, 41) and fatigue-resistance (36, 37). To avoid the confounding effect of training status on our results, we recruited subjects who were relatively sedentary or minimally active. To confirm comparable activity patterns between groups, physical activity of each subject was assessed by a uniaxial accelerometer (Manufacturing Technology, Fort Walton Beach, FL). Subjects were instructed to maintain their customary level of physical activity while wearing the monitor; activity counts were averaged over a 5-day period.

Preliminary testing. Subjects were positioned supine with the right foot secured in a custom-built device that allowed measurements of ankle dorsiflexor force, electromyography, and electrical stimulation (37). Analog signals corresponding to force and EMG were acquired and digitized by use of customized LabVIEW software (National Instruments, Austin, TX). To assess maximal voluntary strength, subjects performed three brief (3–4 s) separated by 2 min of rest to establish MVIC force for that day and provide a warm-up period to allow for the potentiation of blood flow in response to muscle contractions (46). Two minutes after baseline MVICs were completed, subjects performed two exercise protocols: 1) 60-s resting baseline, 16-s MVIC, 10-min recovery; and 2) 60-s resting baseline, 60-s MVIC, 10-min recovery. 31P-MR spectra (100 μs hard pulse, nominal 60° flip angle, 2-s repetition time, 2,048 data points, 8,000 Hz spectral width) were obtained continuously throughout both contraction protocols. The acquired spectra were then averaged (number of averages = 3) to yield 6-s temporal resolution. Verbal encouragement and visual force feedback were provided to subjects, as described earlier.

Spectral analysis. Exponential multiplication corresponding to 10-Hz line broadening was applied to the free-induction decays before being converted to the frequency domain. After phasing and baseline correction, peaks corresponding to PCr, phosphomonoesters (PME), P₆, and γ, α, and β ATP were identified on the basis of their chemical shifts. The underlying broad peak due to the phosphorus in bone marrow was removed by calculating a fifth order polynomial fit of the baseline region. Commercially available line-fitting software (Acorn NMR, Livermore, CA) was used to fit Lorentzian-shaped curves to each spectral peak to quantify its area. Representative spectra from one young and one older subject are shown in Fig. 1. Six young subjects demonstrated distinct splitting of the P₆ peak during the 60-s MVIC, reflecting heterogeneous regions of pH within the muscle (33). In these cases, two Lorentzian-shaped lines were used to fit P₆. Intracellular pH was calculated as a weighted average of the two P₆ peaks, taking into account the proportion of the total P₆ area that each peak encompassed (see Eq. 2). To ensure accurate estimations of intramuscular concentrations, corrections for partial saturation of the 31P-MRS peaks due to the rapid repetition time of the MR protocol were determined from a subset of subjects (6 young, 3 older). Partially saturated and fully relaxed 31P-MR spectra of the resting muscle were collected at repetition times of 2 and 30 s, respectively. Millimolar concentrations of phosphorus metabolites were calculated by assuming that resting [PCr] + [Pi] = 42.5 mM and resting [ATP] = 8.2 mM (12) (brackets denote concentration). Intramuscular pH was calculated on the basis of the chemical shift (δ) of P₆ relative to PCr in parts per million:

\[
\text{pH} = 6.75 + \log[(\sigma - 3.27)/(5.69 - \sigma)]
\] (1)

When P₆ splitting was evident, the pH corresponding to each P₆ 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

\[
\text{pH} = \text{pH}_1(\text{area P}_6/\text{total P}_6 \text{ area}) + \text{pH}_2(\text{area P}_6/\text{total P}_6 \text{ area})
\] (2)

ADP concentrations were calculated on the basis of the equilibrium of the CK reaction and the stoichiometry of free creatine and P₆ (29):

\[
\text{[ADP]} = \frac{[\text{ATP}][\text{P}_6]}{K_{\text{ck}}[\text{PCr}]}
\] (3)

The equilibrium constant of the CK reaction (K₉) was assumed to be 1.66 × 10⁻⁷ (41).
Metabolic calculations. Oxidative capacity was determined from the recovery of PCr after the 16-s contraction. PCr recovery approximates a single exponential curve, from which the rate constant of recovery can be determined (42):

$$PCr(t) = \Delta PCr(1 - e^{-kt}) + PCr_{es}$$  \hspace{1cm} (4)

$PCr_{es}$ is the concentration of PCr at the end of exercise, and $\Delta PCr = (PCr_{rest} - PCr_{es})$, where $PCr_{rest}$ is the concentration of PCr at rest. The rate constant of PCr recovery ($k_{PCr}$) reflects the rate of oxidative phosphorylation under these conditions (23). Oxidative capacity ($Q_{max}$, mM ATP/s) was estimated as the product of $k_{PCr}$ and the resting [PCr] (7, 32).

Rates of ATP synthesis from the net PCr hydrolysis in the CK reaction, oxidative phosphorylation, and anaerobic glycolysis were measured throughout the 60-s MVIC. Because the ATP synthesis rates are expressed in units that are independent of muscle volume (mM ATP/s), these calculations are not confounded by intersubject differences in the quantity of active muscle tissue.

The rate of ATP synthesized from the net breakdown of PCr via the CK reaction ($ATP_{CK}$, mM ATP/s) was determined from the rate of decrease in PCr at any time point during the contraction (4):

$$ATP_{CK} = dPCr/dt$$  \hspace{1cm} (5)

Oxidative ATP production ($ATP_{OX}$, mM ATP/s) during the 60-s contraction was calculated on the basis of the assumption that phosphate potential ($[Pi][ADP]/[ATP]$) controls the rate of oxidative phosphorylation with a $K_m$ of 0.11 (44):

$$ATP_{ox} = Q_{max}/(1 + 0.11/[Pi][ADP]/[ATP])$$  \hspace{1cm} (6)

This “phosphate potential” control model assumes that oxygen delivery to the mitochondria is not limited, which may not be the case during “mixed” exercise (19) such as used in the present study. Previous work from our laboratory in subjects with a wide range of strengths showed that blood flow to the working muscles is fully occluded during isometric ankle dorsiflexion generating greater than ~117 N (46). During a significant portion of the 60-s contraction used in this study, this threshold for blood flow occlusion was exceeded in all subjects. Therefore, the phosphate potential control model likely overestimated the rates of oxidative phosphorylation in the present study. We corrected for this overestimation by examining PCr change during the first 12 s of recovery, which reflects the rate of oxidative phosphorylation during the last few seconds of exercise (2, 4, 19). A correction factor was calculated by comparing the rate of oxidative phosphorylation measured from the first 12 s of PCr recovery to the value calculated using Eq. 3. The difference between the two values was used as a correction factor, which was then subtracted from the oxidative phosphorylation rate calculated at each time point throughout the 60-s MVIC. A similar approach has been used previously to quantify rates of oxidative ATP production (17).

Glycolytic flux ($ATP_{GLY}$, mM ATP/s) was calculated during the 60-s MVIC from exercise-induced changes in intramuscular pH and metabolites, after correction for consumption of protons by the CK reaction, proton efflux, and buffering capacity (19):

$$ATP_{GLY} = 1.5(-\beta(dpH/dt) + \theta(dPCr/dt) - v_{net})$$  \hspace{1cm} (7)

where $dpH/dt$ is change in pH during exercise, $dPCr/dt$ is change in [PCr] during exercise, and $\theta$ represents a correction factor for the consumption of protons in the CK reaction:

$$\theta = 1/[1 + 10^{(pH-6.75)})]$$  \hspace{1cm} (8)

$\beta$ represents the pH buffering capacity (slykes) of the muscle, which takes into account the inherent buffering capacity of the muscle ($\beta_i$) and buffering due to muscle bicarbonate ($\beta_{CO_2}$), P, ($\beta_p$), and PME ($\beta_{PME}$), which are calculated throughout exercise as discussed elsewhere (44):

$$\beta = \beta_i + \beta_{CO_2} + \beta_p + \beta_{PME}$$  \hspace{1cm} (9)

Inherent buffering capacity was determined from changes in pH and PCr during the first 6 s of exercise, when glycolysis and proton efflux are assumed to have a negligible effect on pH changes (19, 44):
PCr ratio. Proton efflux rate during exercise \( v_{\text{eff}} \) was estimated on the basis of rates calculated during recovery from the 60-s contraction \( v_{\text{eff}} \) was plotted against \( \text{pH}_{\text{rest}} - \text{pH}_{\text{observed}} \), the slope of which (\( \lambda \)) was used to calculate \( v_{\text{eff}} \) (44):

\[
\text{v}_{\text{eff}} = \lambda (\text{pH}_{\text{rest}} - \text{pH}_{\text{observed}})
\]

(13)

Where \( \text{pH}_{\text{observed}} \) is pH observed at each time point. In addition to calculating glycolytic flux throughout the 60-s contraction, we also estimated on the basis of rates calculated during recovery from the end-exercise breakdown of PCr via the CK reaction across age groups. The central slope of which (\( \lambda \)) was used to calculate \( v_{\text{eff}} \) (44):

\[
\text{v}_{\text{eff}} = \beta (\text{dpH}/\text{dt}) + mQ + \theta (\text{dPCR}/\text{dt})
\]

(12)

When these major factors contributing to the change in pH during recovery are accounted for, the remaining \( \text{dpH}/\text{dt} \) represents the rate of proton efflux. \( v_{\text{eff}} \) was plotted against \( \text{pH}_{\text{rest}} - \text{pH}_{\text{observed}} \), the slope of which (\( \lambda \)) was used to calculate \( v_{\text{eff}} \) (44):

\[
\text{v}_{\text{eff}} = \lambda (\text{pH}_{\text{rest}} - \text{pH}_{\text{observed}})
\]

(13)

Where \( \text{pH}_{\text{observed}} \) is pH observed at each time point. In addition to calculating glycolytic flux throughout the 60-s contraction, we also determined peak glycolytic flux for each subject during this protocol. The metabolic cost of the 60-s contraction (mM ATP/s) was estimated for each subject as the sum of the rate of ATP synthesized from the net breakdown of PCr via the CK reaction, oxidative ATP production, and glycolytic flux and any net ATP hydrolysis. The relative contribution of each pathway during the 60-s MVIC was determined by expressing rate of ATP synthesized from the net breakdown of PCr via the CK reaction, oxidative ATP production, and glycolytic flux as proportions of total ATP turnover.

Statistical analyses. Student’s unpaired t-tests were used to compare MVIC, 16-s fatigue, 60-s fatigue, oxidative capacity, peak rate of ATP synthesized from the net breakdown of PCr via the CK reaction across age groups. The central activation ratio and \( K_{\text{PCr}} \) data exhibited skewed distributions, necessitating analysis using the nonparametric Wilcoxon rank sum test to examine differences across age groups in these variables. A two-factor (age, time) repeated-measures ANOVA was used to examine age-related differences in [PCR] and pH during the 16- and 60-s contractions. Rate of ATP synthesized from the net breakdown of PCr via the CK reaction, oxidative ATP production, and glycolytic flux during the 60-s contraction as well as the percentage of total ATP derived from each pathway were assessed with a two-factor (age, time) repeated-measures ANOVA, using Tukey’s procedure to make pairwise comparisons where significant age × time interactions were present. Group means, 95% confidence intervals, and precise values for young and older groups are presented as means ± SE. 95% confidence intervals are presented for age-group differences in each variable.

### Table 2. Selected variables from the 16-s MVIC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Older</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue (MVICpost/pre)</td>
<td>0.76±0.03</td>
<td>0.76±0.03</td>
<td>-0.09, 0.09</td>
<td>0.97</td>
</tr>
<tr>
<td>End-exercise pH</td>
<td>6.99±0.02</td>
<td>7.09±0.02</td>
<td>0.05, 0.14</td>
<td>0.0003</td>
</tr>
<tr>
<td>End-exercise PCR, mM</td>
<td>20.7±1.1</td>
<td>22.4±0.8</td>
<td>-1.41, 4.76</td>
<td>0.26</td>
</tr>
<tr>
<td>End-exercise ATP, mM</td>
<td>8.65±0.50</td>
<td>7.66±0.40</td>
<td>-2.36, 0.38</td>
<td>0.64</td>
</tr>
<tr>
<td>( K_{\text{PCr}} ), mM ATP/s (^{-1} )</td>
<td>0.036±0.006</td>
<td>0.040±0.004</td>
<td>-0.01, 0.02</td>
<td>0.60</td>
</tr>
<tr>
<td>( Q_{\text{max}} ), mM ATP/s (^{-1} )</td>
<td>1.40±0.23</td>
<td>1.51±0.19</td>
<td>-0.53, 0.75</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Values for young and older groups are presented as means ± SE. 95% confidence intervals are presented for age-group differences in each variable. \( K_{\text{PCr}} \), rate constant of PCr recovery; \( Q_{\text{max}} \), oxidative capacity; MVIC post/pre, ratio of MVIC after to MVIC before exercise.

**RESULTS**

Young and older men were similar in height (young = 175 ± 6 cm, older = 172 ± 8 cm, \( P = 0.76 \)), mass (young = 85 ± 21 kg, older = 83 ± 16 kg, \( P = 0.64 \)), and activity levels (Table 1). The difference in strength (MVIC) across age groups did not reach statistical significance, although there was a trend for lower strength in older men (Table 1). All subjects, regardless of age, were able to achieve full voluntary activation of the unfatigued ankle dorsiflexors, as evidenced by the CAR values (Table 1). Fatigue was similar in young and older men during the 16-s (Table 2) and 60-s contractions (Table 3).

**Phosphorus metabolites and pH.** Young and older men had similar resting [PCr] and Pi-to-PCr ratios (Table 1). During the 16-s MVIC, PCr depletion was similar in both age groups (Table 2, Fig. 2A), although the rate of PCr hydrolysis during the first 6 s of exercise was faster in young men (Table 2, Fig. 2A). After an initial alkalosis, there was a modest decline in intramuscular pH (Fig. 2B), which was greater in young compared with older men (Table 2). There was no change in [ATP] during the 16-s contraction in either age group (\( P = 0.34 \)), nor were there age group differences in end-exercise [ATP] (Table 2).

During the 60-s MVIC, [PCr] was depleted to a greater extent in young compared with older men (Table 3, Fig. 3A).

### Table 3. Selected variables from the 60-s MVIC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Older</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue (MVICpost/pre)</td>
<td>0.51±0.06</td>
<td>0.56±0.04</td>
<td>-0.11, 0.21</td>
<td>0.50</td>
</tr>
<tr>
<td>End-exercise pH</td>
<td>6.67±0.03</td>
<td>6.97±0.02</td>
<td>0.22, 0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>End-exercise PCR, mM</td>
<td>8.00±1.39</td>
<td>13.82±1.41</td>
<td>1.57, 10.08</td>
<td>0.011</td>
</tr>
<tr>
<td>End-exercise ATP, mM</td>
<td>7.94±0.39</td>
<td>8.04±0.53</td>
<td>-1.13, 1.52</td>
<td>0.88</td>
</tr>
<tr>
<td>End-exercise PME, mM</td>
<td>7.04±1.08</td>
<td>8.57±2.55</td>
<td>-4.49, 7.56</td>
<td>0.68</td>
</tr>
<tr>
<td>End-exercise ADP, mM</td>
<td>0.16±0.04</td>
<td>0.09±0.01</td>
<td>-0.15, 0.02</td>
<td>0.14</td>
</tr>
<tr>
<td>End-exercise AMP, mM</td>
<td>2.92±1.21</td>
<td>1.22±0.42</td>
<td>-4.72, 1.32</td>
<td>0.24</td>
</tr>
<tr>
<td>Peak ATP(_{\text{CK}}), mM</td>
<td>1.30±0.17</td>
<td>0.88±0.15</td>
<td>-0.90, 0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>ATP(_{\text{GLY}}), mM</td>
<td>1.41±0.15</td>
<td>0.84±0.17</td>
<td>-1.06, -0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>Metabolic cost, mM ATP(_{\text{GLY}})(^{-1} )</td>
<td>1.52±0.18</td>
<td>1.19±0.20</td>
<td>-0.64, -0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values for young and older groups are presented as means ± SE. 95% confidence intervals are presented for age-group differences in each variable. ATP\(_{\text{CK}}\), rate of ATP synthesized from net breakdown of PCr via the creatine kinase reaction. ATP\(_{\text{GLY}}\), glycolytic flux.
Young men experienced greater Pi accumulation and acidosis (Table 3, Fig. 3B) compared with older men. There was no significant change in [ATP] in either group (P = 0.38, Table 3), nor were there age group differences in end-exercise [ATP] (Table 3). Concentrations of PME, ADP and AMP were similar in young and older men at rest (Table 1) and at the end of the 60-s MVIC (Table 3).

Metabolic calculations. The k$_{PCr}$ after the 16-s MVIC was similar, as was the calculated capacity for oxidative phosphorylation (Table 2).

High rate of ATP synthesized from the net breakdown of PCr via the CK reaction rates (mM ATP/s) were observed at the onset of the 60-s MVIC in both groups (Fig. 4A). The peak rate of PCr hydrolysis during the initial 6 s of contraction tended to be higher in young compared with older men, although this difference did not reach statistical significance (Table 3). After the first 12 s of exercise, ATP synthesis via PCr hydrolysis diminished to a similar extent in both age groups (Fig. 4A).

Oxidative ATP production (mM ATP/s) was similar in young and older men over the entire duration of the 60-s MVIC (P = 0.93, Fig. 4B). The contribution of this pathway to the total ATP synthesis rate increased steadily until ~36 s, then remained relatively constant until the end of the contraction (Fig. 4B).

Glycolytic flux (mM ATP/s) was minimal during the first 6 s of exercise in both groups but rose sharply from 12 s of exercise onward (Fig. 4C). Overall, glycolytic rates were higher in the muscles of the younger compared with older men (P < 0.001, Fig. 4C). In addition to higher overall glycolytic rates in the young, peak glycolytic flux was also higher in young than older men (Table 3). The inherent buffering capacity was similar in young (31.3 ± 8.3 slykes) and older (19.4 ± 9.8 slykes, P = 0.37).

Metabolic cost, averaged over the entire 60-s MVIC, was higher in young compared with older men (Table 3). When considered on a relative basis (Fig. 5), the young and older men derived a similar proportion of total ATP production from the CK reaction (P = 0.08, Fig. 5). However, younger men relied more on glycolytic ATP synthesis during muscle contraction (P < 0.001), whereas older men derived a greater proportion of ATP from oxidative sources (P = 0.014, Fig. 5).

DISCUSSION

In this study, we demonstrate that, despite similar capacity for oxidative phosphorylation in both age groups, peak and overall glycolytic fluxes were lower in the skeletal muscle of older men during a maximal contraction sustained for 60 s. Furthermore, older men utilized oxidative ATP synthesis to a
greater extent than young to accomplish this task. These data provide a novel demonstration of age-related differences in ATP production during muscular work.

We observed a tendency for lower maximal isometric strength in older men, as previously reported in the ankle dorsiflexor muscles of healthy young and older individuals (20, 26, 30, 47). Fatigue during the sustained 16- and 60-s muscle contractions was similar in young and older men. Others have reported similar fatigability across these age groups when contractions are sustained or have relatively short rest intervals (1, 13, 39).

The noninvasive MRS methodology used in the present study allows determination of in vivo metabolic pathway function during muscular contractions. An advantage of this approach over the biopsy method is that continuous measures of ATP synthesis rates are possible, rather than peak activities of selected enzymes. Here, we have applied this technique to address age-related changes in muscle energy metabolism. Our results are consistent with the results of earlier biopsy studies (28, 34) and extend these studies by examining the pathways of ATP synthesis in vivo.

PCr resynthesis kinetics after the 16-s MVIC were similar in young and older men, which translated to similar oxidative capacity, indicating that the maximum capacity of mitochondrial ATP synthesis was not diminished with old age. Other investigators have reported reductions in mitochondrial function with age using similar techniques (7, 31). However, these studies did not include controls for physical activity levels of the subjects and so the results may be related to lifestyle changes with aging, rather than the aging process itself (38). The similarity in oxidative capacity that we observed in the present study confirms our earlier observations in the same muscles from a different group of healthy young and older men and women (23) and is consistent with results from other researchers who have accounted for the health and physical activity levels of their subjects (5). Interestingly, Petersen et al. (35) recently demonstrated that resting mitochondrial oxidative and phosphorylation activities were reduced in older subjects even after controlling for physical activity levels. Although the study suggested that resting mitochondrial activity may be lower with age, the present data demonstrated that the maximal capacity for mitochondrial ATP production was unaffected by old age in the ankle dorsiflexor muscles of healthy adults.

Fig. 4. Rates of ATP production by the creatine kinase reaction (ATP\text{CK}), oxidative phosphorylation (ATP\text{OX}), and anaerobic glycolysis (ATP\text{GLY}) (means ± SE) during 60-s MVIC. There were no effects of age or age-by-time interactions for the creatine kinase reaction or oxidative phosphorylation. There was a significant effect of age on ATP production by anaerobic glycolysis, with young men having higher glycolytic ATP production than older men.

Fig. 5. Relative contributions of the creatine kinase reaction, oxidative phosphorylation, and anaerobic glycolysis to total ATP turnover during exercise, expressed as percentage of total ATP flux at each time point. Young and older men derived similar proportions of total ATP from the creatine kinase reaction. There were significant effects of age and age-by-time interactions for the proportions of ATP derived from oxidative and glycolytic pathways, such that young men derived more of their ATP from glycolytic sources than older men, who relied more on oxidative phosphorylation.
In addition to assessing the capacity for oxidative phosphorylation, we also determined the contribution of the CK reaction, oxidative phosphorylation, and anaerobic glycolysis to ATP generation during a sustained maximal muscle contraction. In all subjects, regardless of age, ATP supplied from net PCr hydrolysis diminished as the contraction progressed (Figs. 4A and 5). Our observations agree with reports of ATP production via CK during voluntary plantar flexion (4, 44). This behavior supports the concept that PCr is a direct source of energy that is hydrolyzed to maintain ATP concentration until other pathways can meet the metabolic demand of muscular work. PCr breakdown was similar over the course of the 60-s contraction in young and older men, although a trend for greater rate of PCr hydrolysis was apparent during the first 6 s of contraction in young men (Fig. 4A, Table 3).

Blunted PCr hydrolysis during the initial portion of the 60-s contraction in older men may reflect lower flux through the CK reaction. This possibility is unlikely, however, because previous investigators have demonstrated similar CK reaction kinetics during exercise in young and older subjects using steady-state saturation transfer techniques (14). An alternative interpretation is that the increased rate of PCr hydrolysis in young men reflects a greater metabolic demand of the exercise compared with older subjects. In support of this notion, we observed higher ATP demand in young compared with older men over the course of the 60-s MVIC. Because young and older men were contracting at the same relative intensity and showed similar fatigue profiles, the difference in metabolic cost is unlikely to be due to different amounts of active muscle tissue. Because oxidative and glycolytic pathways require several seconds to increase their rates of ATP synthesis, it is likely that the greater ATP demand of the younger subjects may be met by increased PCr hydrolysis during the first few seconds of exercise.

Unlike the CK reaction, ATP generated by oxidative phosphorylation is not immediately available to meet the energy demand of the working muscle. As demonstrated in Fig. 4B, the rate of mitochondrial ATP production increased during the first 30 s of exercise and remained relatively constant over the latter half of the contraction. Our values for oxidative ATP production are similar to values reported in the literature using similar techniques (4, 44). Rates of oxidative ATP production were comparable in young and older men over the duration of the muscle contraction, consistent with the similarity in oxidative capacity observed across age groups. These results agree well with recently published data suggesting that the potential for oxidative ATP production during exercise is comparable in young and older adults (24). Throughout an incremental exercise protocol, changes in the P_i-to-PCr ratio during the steady-state portion of the exercise were similar in young and older subjects, suggesting that oxidative phosphorylation was equally capable of providing ATP across age groups (22).

Glycolytic flux was negligible during the first 6 s of exercise but quickly increased and on average reached a peak rate at 18 s of exercise in both young and older adults (Fig. 3C). We observed an age-related decline in peak glycolytic rate, which may be partially explained by the reduction in lactate dehydrogenase and hexokinase activities with old age (28, 34), or by the decreased total area of type II muscle fibers (6, 28). After 18 s, glycolytic rate decreased gradually throughout the remainder of the contraction. This rapid increase and modest decline in glycolytic ATP production is consistent with data from other investigators (4, 44). Overall, the rates of glycolytic ATP production were consistently lower in older compared with younger subjects during the 60-s MVIC. We also observed greater acidosis in young men (Fig. 2), resulting predominantly from proton production during glycolytic ATP turnover. Our pH data are in agreement with recent work demonstrating greater acidosis in young compared with older adults during fatiguing muscle contractions (24). Collectively, our results indicate that the absolute rates of ATP production from the CK reaction and oxidative phosphorylation are similar in young and older men but that young men demonstrate higher glycolytic flux throughout the 60-s contraction.

To assess the relative contribution of each pathway during exercise, we compared each pathway as a fraction of the total rate of ATP production at each time point (Fig. 5). It is apparent that during the initial phase of the contraction the CK reaction provided more than 90% of total ATP synthesis, in agreement with earlier reports (44). Within several seconds, the contribution of CK to ATP production declined to ~15–20% in young and older subjects. Despite similar absolute rates of oxidative ATP production (Fig. 3B), older adults generated a greater proportion of total ATP from this pathway and thus demonstrated a smaller contribution of glycolytic metabolism compared with young men.

The mechanisms for lower glycolytic flux and greater reliance on oxidative metabolism in the older group remain to be determined. P_i, a potent stimulator of glycolysis, increased more in young than older adults, although other purported activators of glycolysis (i.e., ADP, AMP) accumulated to a similar degree in both age groups. At present, several possibilities can be suggested as potential mechanisms for the lower glycolytic flux with old age. First, reduced glycolytic flux with old age may reflect reduced type II fiber area, which has been reported in older humans (6, 28, 34). Biopsies from the tibialis anterior muscle (a prime dorsiflexor) showed that type II fibers account for 63.5% of total fiber area in young (type I area: 5,840 ± 1,450 μm², type II area: 10,170 ± 2,950 μm²) and 54.7% in older subjects (type I area: 5,850 ± 940 μm², type II area: 7,160 ± 2,540 μm²) (15, 16). In a separate study (11), tibialis anterior type IIa fibers were shown to have higher anaerobic glycolytic enzyme activity (alpha-glycerol phosphate dehydrogenase activity, GPDH) than type I fibers (67.5 vs. 38.7 μM glyceral-3-phosphate/min, respectively). By multiplying the GPDH activity of each fiber type (from Gregory et al., Ref. 11) by the respective proportions of type I and II fibers in the tibialis anterior muscles of young and old (from Jakobsen et al., Refs. 15 and 16), we predict that the modest age-related reduction in type II fiber area reported in this muscle would lead to only a 4.5% reduction in anaerobic ATP production. Although it is reasonable to expect that this fiber-type shift impacts glycolytic ATP production to some extent, it is unlikely to account for the ~40% reduction in peak glycolytic flux that we observed in older men.

Alternatively, oxygen delivery may be limited in young but not older men during exercise, necessitating greater reliance on anaerobic glycolysis. The stronger muscles of young men generate higher intramuscular pressure on the vasculature, which may occlude blood flow during a sustained MVC. On the basis of our recent observation of full occlusion of perfusion above contraction intensities of ~117 N in this muscle.
group (46), it is possible that the young men were relatively more occluded during the latter part of the 60-s contraction compared with the older men, a situation that might require the young to generate more ATP anaerobically. On average, muscle force was ~118 N at the end of the 60-s contraction in the young and 105 N in the old, which is consistent with the possibility of relatively lower oxygen availability in the young group. This would not, however, explain the lower peak glycolytic rates observed in the older subjects, because these occurred early in the contraction, when force was >117 N in all subjects.

Finally, it is tempting to attribute the higher glycolytic flux of young men to their greater metabolic cost of contraction. As discussed earlier, the greater ATP turnover in young compared with older men during the 60-s MVC is unlikely due to differences in the quantity of active muscle tissue because both age groups were contracting at the same relative intensity and demonstrated similar declines in voluntary force during the contraction. It is also unlikely that differences in central activation failure could account for the differences in metabolic cost, because all subjects were able to fully activate their muscles in the unfatigued state and previous work has demonstrated no central activation failure in older adults during fatiguing contractions of the tibialis anterior muscle. (24, 25).

It is possible that the higher metabolic cost of contraction in type II fibers (40) could contribute to higher glycolytic rates in the young. However, the small impact of the reported changes in fiber types across age groups, as discussed above, makes it unlikely that fiber-type differences in economy could explain the entirety of our results. Neural mechanisms related to reduced maximal discharge rates (8, 18) may also have an impact on metabolic cost during MVCs, because older muscle would experience a lower “driving force” compared with the young. Thus it is unclear at this time whether glycolytic flux is reduced in old age due to changes in metabolic cost, the capacity for anaerobic glycolysis, or an alternative mechanism.

In conclusion, we have applied noninvasive MRS methodology to the study of the effects of old age on the pathways of ATP production in human skeletal muscle in vivo. Our results demonstrate for the first time that, despite a similar capacity for oxidative ATP production in young and older men, healthy older men demonstrated lower glycolytic flux and derived a greater proportion of their ATP from oxidative sources during a sustained maximal contraction. At this time, explanations for the age-related reductions of glycolytic ATP production are unclear. The possibilities that reduced glycolytic flux with old age represent either a metabolic “preference” for oxidative metabolism or a reduced capacity for anaerobic ATP production await further investigation.

ACKNOWLEDGMENTS

We thank Douglas Rothman for support and insightful comments throughout the study. We also thank David Russ, John Buonaccorsi, Peter Brown, Terry Nixon, Stephen Foulis, Linda Chung, and the subjects for participation.

GRANTS

This research was supported by National Institute on Aging Grant R01 AG21094 (J. Kant-Braun) and the American Federation for Aging Research Doctoral Student Fellowship (I. R. Lanza).

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