Impact of age on exercise-induced ATP supply during supramaximal plantar flexion in humans

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Am J Physiol Regul Integr Comp Physiol 309: R378–R388, 2015. First published June 3, 2015; doi:10.1152/ajpregu.00522.2014.—Current physiological changes in aerobic and anaerobic muscle activity and pulmonary oxygen kinetics in old people are poorly understood. The purpose of this study was to quantify the capacity for muscle to maintain aerobic ATP synthesis (P/O ratio) during supramaximal isometric plantar flexion exercise at the onset of whole body exercise. Despite a significant reduction in heart rate and blood pressure between the young (22 ± 2 yr) and old (74 ± 8 yr) activity-matched subjects, we assessed the impact of age on: 1) the relative contribution of the three main pathways of ATP synthesis (oxidative ATP synthesis, glycolysis, and the creatine kinase reaction) and 2) the ATP cost of contraction during high-intensity exercise. Specifically, during supramaximal plantar flexion (120% of maximal aerobic power), to stress the functional limits of the skeletal muscle energy systems, we used 31P-labeled magnetic resonance spectroscopy to assess metabolism. Although glycolytic activation was delayed in the old, ATP synthesis from the main energy pathways was not significantly different between groups. Similarly, the inferred peak rate of mitochondrial ATP synthesis was not significantly different between the young (25 ± 8 mM/min) and old (24 ± 6 mM/min). In contrast, the ATP cost of contraction was significantly elevated in the old compared with the young (5.1 ± 2.0 and 3.7 ± 1.7 mM·min⁻¹·W⁻¹, respectively; P < 0.05). Overall, these findings suggest that, when young and old subjects are activity matched, there is no evidence of age-related mitochondrial and glycolytic dysfunction. However, this study does confirm an abnormal elevation in muscle metabolism, small muscle mass modalities such as plantar- or dorsiflexion exercise have been used (13, 49, 64, 88). However, even with this approach, conflicting results have still been reported in terms of aging and the interplay between the energy pathways during exercise. For instance, several studies have reported a higher P/O-to-PCr ratio for a given dynamic plantar flexion exercise work rate in the old, suggesting higher metabolic demand and/or a greater reliance on oxidative ATP synthesis, compared with their younger counterparts (13, 64, 88) while, contrasting with these findings, Chilibeck et al. (8) have consistently reported similar PCr and pulmonary VO₂ kinetics in young and old subjects at the onset of submaximal plantar flexion exercise (7–9). These differences in findings are likely multifactorial (discrepancies in exercise paradigm, use of differing absolute or relative exercise intensities between age groups, assessment techniques, etc.); however, it is highly likely that variations in physical activity, both within and between studies, a factor accepted to affect the relative contribution of aerobic and anaerobic processes, may have also played an important role.

Interestingly, a reduced contribution from anaerobic glycolysis to ATP synthesis (49) has been proposed as the explanation for the smaller reduction in pH (44, 49) in the tibialis anterior muscle of the old during isometric exercise. Indeed, because the rates of ATP synthesis from anaerobic glycolysis were comparable between old and young subjects under ischemic conditions (50), it has been suggested that the lower glycolytic flux during isometric exercise is related to a preferential reliance upon oxidative phosphorylation rather than impaired glycolytic function. However, an alternative explanation for these and other alterations in muscle energetics documented in older individuals during dynamic exercise (13, 64, 88) may be an increased cost of contraction rather than a change in the ATP synthesis pathways. However, this possibility has rarely been investigated in human locomotor muscles (15, 58). In this context, the supramaximal model (i.e., above maximal aerobic power) during a small muscle mass exercise, in which all the skeletal muscle energy systems are stressed to their functional limit, represents an appealing approach to quantitatively evaluate the mechanisms that couple ATP synthesis to metabolic demand with age.
In a quest to unveil the physiological factors responsible for exercise intolerance and bioenergetic alterations with age, our group has recently demonstrated that, unlike muscle oxidative phosphorylation capacity, which appears to be preserved during the process of aging when physical activity is controlled (30), the ATP cost of contraction during submaximal exercise is significantly higher in older individuals (58). Mechanistically, we suggested that this is likely a result of greater metabolic demand from noncontractile processes (57). Building upon these prior investigations, the purpose of the present study was, therefore, in activity-matched young and old subjects, to quantitatively assess the impact of age on the contribution of the major energy pathways to muscular work and the ATP cost of these contractions utilizing 31P-labeled-magnetic resonance spectroscopy (MRS) during high-intensity exercise. Specifically, we hypothesized that, during supramaximal plantar flexion exercise: 1) the old would exhibit preserved oxidative and glycolytic function such that the relative contribution from the main pathways of ATP synthesis would be similar between groups, however, 2) the ATP cost of contraction would be higher in the old compared with their younger counterparts.

METHODS

Subjects. Following informed consent procedures, 40 subjects (20 older and 20 young) with activity levels similar to the estimates of U.S. adults’ habitual physical activity levels (84) participated in this study (Table 1). Because of poor 31P-MRS signal-to-noise ratio or an inability to maintain the required power output during the exercise protocol, two young and two older subjects were excluded from the study, and their data were therefore not analyzed further nor included in the current results. Healthy subjects were recruited based on age (18–25 yr for the young and >65 yr for the old) and no evidence of regular physical activity, above that required for activities of daily living, which was assessed by both questionnaire and accelerometer. All subjects were nonsmokers, free of diabetes, and diagnosed cardiovascular, peripheral vascular, neuromuscular, or pulmonary disease. Premenopausal women were studied during days 1–7 of their menstrual cycle to standardize the influence of female hormones. Women taking hormone replacement therapy were excluded from the study. Other experimental data from this cohort of subjects have already been published elsewhere (30, 58). The study was approved by the Human Research Protection Program of the University of Utah and the Salt Lake City Veterans Affairs Medical Center.

Exercise protocol. After familiarization, individual maximum dynamic plantar flexion work rate (WRmax) was determined by performing incremental exercise to exhaustion (0.5- to 2-watt increments/min) to determine the maximal power corresponding to the peak aerobic capacity of the calf muscle. On a separate day, subjects performed constant-load supramaximal plantar flexion at 120% of WRmax (frequency of 1 Hz) in the whole body MR system (TimTrio 2.9T; Siemens Medical Systems, Erlangen, Germany), with the thigh and hip secured to the patient bed to isolate power production from the plantar flexors and minimize movement. Specifically, after 1 min of resting baseline assessment, subjects exercised for 1 min, followed by 5 min of recovery. The intensity and the duration of the exercise protocol were chosen based upon preliminary work to ensure that the majority of the participants would be very close to exhaustion at the end of the exercise, but would still be able to maintain the required power output throughout the protocol. An inability to maintain the same range of motion, the contraction frequency, or evidence of PCr resynthesis during the exercise were each used as criteria to exclude a subject’s data from the analysis. On a different day, blood samples were collected to perform a complete blood cell count. All experimental trials were performed with participants in an overnight fasted state, and having refrained from any physical activity for 24 h.

31P-MRS. MRS was performed using a clinical 2.9T MRI system (TimTrio; Siemens Medical Solutions) operating at 49.9 MHz for 31P resonance. 31P-MRS data were acquired with a 31P-1H dual-surface coil with linear polarization (Rapid Biomedical, Rimpar, Germany) positioned around the calf at its maximum diameter. The 31P single-loop coil diameter was 125 mm surrounding a 110-mm 1H coil loop. After a three-plane scout image was acquired, advanced localized volume shimming (9 cm × 9 cm × 9 cm) was performed. Before each experiment, two fully relaxed spectra were acquired at rest with three averages per spectrum and a repetition time (TR) of 30 s. Next, MRS data acquisition was performed throughout the rest-exercise-recovery protocol using a free induction decay pulse sequence with a 2.56-ms adiabatic-half-passage excitation radio frequency pulse and the following parameters: TR = 2 s, receiver bandwidth = 5 kHz, 1,024 data points, and 3 averages/spectrum. Saturation factors were quantified by the comparison between fully relaxed (TR = 30 s) and partially relaxed (TR = 2 s) spectra.

As previously described (55), relative concentrations of phosphocreatine ([PCr]), inorganic phosphate ([Pi]), phosphomonoester ([PME]), and ATP ([ATP]) were obtained by a time-domain fitting routine using the AMARES algorithm (85) incorporated into the CSIAPD software (60). Intracellular pH was calculated from the chemical shift difference between the P, and PCr signals. The free cytosolic ADP concentration ([ADP]) was calculated from [PCr] and pH using the creatine kinase equilibrium constant (CK1 = 1.66 × 1010 M−1), with the assumption that phosphocreatine represents 85% of the total creatine content (37). The resting concentrations were calculated from the average peak areas of the two relaxed spectra (TR = 30 s; n = 3) recorded at rest and assuming an 8.2 mM ATP concentration at rest (29). When Pi splitting was evident, the pH corresponding to each Pi pool was calculated separately as $\text{pH}_1$ (area $\text{Pi}_1$/total $\text{Pi}$ area) + $\text{pH}_2$ (area $\text{Pi}_2$/total $\text{Pi}$ area). Most PME generated during exercise are hexose phosphate, i.e., glycolytic intermediates such as glucose 6-phosphate (−80%) and fructose 6-phosphate (−15%) (4, 5). Therefore, as previously suggested (19, 20), changes in

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
</tr>
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<tbody>
<tr>
<td>n (female/male)</td>
<td>18 (9/9)</td>
<td>18 (9/9)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27 ± 6</td>
<td>74 ± 8.1*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172 ± 10.1</td>
<td>170 ± 9.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69 ± 12.2</td>
<td>74 ± 15.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23 ± 3.2</td>
<td>25 ± 4.1</td>
</tr>
<tr>
<td>Muscle volume, liters</td>
<td>2.0 ± 0.6</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>Peak work rate, watts</td>
<td>13 ± 5</td>
<td>9 ± 4*</td>
</tr>
<tr>
<td>Step, count/day</td>
<td>5,927 ± 2,252</td>
<td>6,883 ± 2,766</td>
</tr>
<tr>
<td>Physical activity, count/min</td>
<td>157 ± 46</td>
<td>165 ± 89</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>71.4 ± 6.4</td>
<td>80.1 ± 13.8*</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>177.2 ± 43.3</td>
<td>198.7 ± 28.3</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>107.4 ± 78.0</td>
<td>122.1 ± 63.0</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>53.9 ± 11.0</td>
<td>54.6 ± 13.8</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>109.3 ± 35.4</td>
<td>125.7 ± 25.3</td>
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<tr>
<td>WBC, K/µl</td>
<td>5.8 ± 0.9</td>
<td>5.7 ± 1.1</td>
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<tr>
<td>RBC, Mµl</td>
<td>51 ± 0.4</td>
<td>4.8 ± 0.3*</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>15.3 ± 1.5</td>
<td>14.8 ± 1.0</td>
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<tr>
<td>Hematocrit, %</td>
<td>45.0 ± 3.6</td>
<td>44.3 ± 2.4</td>
</tr>
<tr>
<td>Neutrophil, K/µl</td>
<td>3.2 ± 0.8</td>
<td>3.4 ± 1.0</td>
</tr>
<tr>
<td>Lymphocyte, K/µl</td>
<td>2.0 ± 0.5</td>
<td>1.6 ± 0.5*</td>
</tr>
<tr>
<td>Monocyte, K/µl</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
</tbody>
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Values expressed as means ± SD; n, no. of subjects; BMI, body mass index; HDL, high-density cholesterol; LDL, low-density cholesterol; WBC, white blood cells; RBC, red blood cells. *P < 0.05, significantly different from young.
in [PME] were considered equivalent to changes in hexose phosphate such that accumulation of [PME] reflects additional glycogenolytic flux that has not passed through the glycolytic pathway (19, 20). Changes in pH and in the concentration of phosphorus metabolites during contraction and recovery phases were used to calculate oxidative capacity and the rates of ATP synthesis through the creatine kinase reaction, oxidative phosphorylation, and anaerobic glycolysis as previously described (40) and are detailed in the APPENDIX. The data during the exercise were averaged over 12 s for the flux analysis.

Lower leg volume. Lower leg volume was calculated based on lower leg circumference (three sites: distal, middle, and proximal), lower leg length, and skinfold measurements (39). This method has recently been confirmed to provide a valid estimate for muscle volume across a spectrum of individuals with normal muscle mass and severe muscle atrophy (59).

Physical activity level. Physical activity level (PAL) was assessed using both a subjective PAL recall questionnaire and objective accelerometer data. The PAL questionnaire included items regarding the average type, frequency, intensity, and duration of physical activity in any given week. After receiving standardized operating instructions, subjects wore an accelerometer (GT1M; Actigraph, Pensacola, FL) for seven continuous days, with adherence automatically assessed. Average daily physical activity was expressed as both steps per day and total accelerometer counts per minute.

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Statistical analysis. Because there was no evidence of a significant sex effect on the main variables of the study, assessed in an initial preliminary analysis, only a main effect of age on the variables, irrespective of sex, was assessed in this study. For variables evolving with respect to time during exercise, the effect of age on the overall time course was determined using a two-way ANOVA with repeated measurements (group × time) (Statistica software; Statsoft). For each time point, post hoc comparisons (Bonferroni test) were used to compare values between the young and the old. Specifically, the first data point significantly greater than zero (i.e., baseline) was considered to reflect the onset of glycolytic flux (19). The assessment of differences between the young and the old for the other variables was performed with either independent t-tests or nonparametric Mann-Whitney tests, where appropriate (Statsoft, version 5.5; Statistica, Tulsa, Oklahoma). Statistical significance was accepted at P < 0.05. Results are presented as means ± SD in Table 1 and means ± SE in Figs. 1–5 for clarity.

RESULTS

Subject characteristics and physical activity assessment. Apart from age, the young and old subjects only differed significantly in terms of peak plantar flexion work rate, resting blood glucose levels, red blood cells, and lymphocytes (Table 1). By experimental design, the young and old subjects did not differ from each other in terms of the PAL as assessed by steps per day and total accelerometer counts per minute (Table 1).

Muscle volume and plantar flexion exercise. Although muscle volume was not different between young and old (P > 0.05), plantar flexion WRmax was significantly lower in the old compared with the young group (Table 1, P < 0.05). Thus, the corresponding power output used for supramaximal exercise was 16 ± 6 and 11 ± 5 W in the young and old subjects, respectively (P < 0.05). Also, skinfold thickness was not significantly different between groups (young: 7.4 ± 2.4 mm; old: 7.7 ± 2.9 mm, P > 0.05).

High-energy phosphate compounds and intracellular pH. Example MR spectra acquired from the plantar flexor muscles during the protocol for both young and old subjects are illustrated in Fig. 1. The effects of supramaximal dynamic plantar flexion exercise on phosphorylated compounds and pH in the young and old are illustrated in Fig. 2. [ATP] did not change significantly during the exercise in either group (P > 0.05).

ATP synthesis rates and energy cost. Neither absolute nor relative rates of ATP synthesis (i.e., oxidative ATP synthesis, glycolysis, and the creatine kinase reaction) were significantly different between the young and old (P > 0.05, Fig. 3). Thus, the corresponding total ATP synthesis rate during supramaximal exercise was also not significantly different between groups (P > 0.05, Fig. 4A). Because these were nonsignificant findings, as hypothesized, it should be noted that post hoc power calculations confirmed a very small effect size (d = 0.2). Indeed, these calculations revealed the need for a very large sample size (~1,300 subjects) to affirm this as a significant difference between groups, supporting the conclusion that

Fig. 1. Representative examples of rapid 1H imaging transverse slices, illustrating the spatial sensitivity of the coil, and 31P spectra acquired from the plantar flexor muscles of a young and old subject at rest and at the end of the 1-min exercise bout. The signal-to-noise ratio from phosphocreatine (PCr) was 61 in the old and 95 in the young with a time resolution of 6 s. A double inorganic phosphate (Pi) peak was detected in both subjects. PME, phosphomonoester; ATP, adenosine triphosphate (α, β, γ).
there was actually no effect of age. PME accumulation, which is related to glycogenolytic activation, occurred after ~12 s in both groups (Fig. 3C), whereas glycolytic activation occurred after ~24 s in the young and ~36 s in the old (Fig. 3A). Despite similar total ATP synthesis rates, but as a result of the lower power output developed during the exercise, the energy cost of contraction was significantly elevated in the old subjects compared with the young ($P < 0.05$, Fig. 4B). In this case, post hoc power analysis revealed $1 - \beta = 0.6$.

$\text{PCr kinetics at the offset of exercise and muscle oxidative capacity.}$ Representative examples of the PCr dynamics during the postexercise recovery period in the young and old are displayed in Fig. 5. The PCr recovery time constant was $45 \pm 18$ s in the young and $41 \pm 12$ s in the old ($P > 0.05$). The initial PCr resynthesis rate in the old ($22 \pm 6$ mM/min) was akin to that of the young subjects ($22 \pm 8$ mM/min, $P > 0.05$). Similarly, the inferred peak rate of mitochondrial ATP synthesis ($V_{\text{max}}$) was not significantly different between the young and old ($P > 0.05$, Fig. 5C).

**DISCUSSION**

With the use of $^{31}$P-MRS, this study sought to determine the contribution of the three major energy pathways during dynamic supramaximal plantar flexor exercise in young and old physical activity-matched subjects. The principal novel findings of this study were that 1) both oxidative and glycolytic function were preserved with age such that the relative contribution from the main ATP synthesis pathways in the old were not different from the young, 2) glycolytic activation was delayed in the old, and 3) the ATP cost of contraction was substantially higher in the old compared with their younger counterparts. Overall, these findings imply that an abnormally elevated metabolic demand from skeletal muscle, rather than the capacity of the three main energy pathways to generate ATP, may play a role in the decline in skeletal muscle exercise capacity with advancing age.

**Oxidative ATP synthesis.** A novel and important finding of the present study was the comparable oxidative ATP synthesis, expressed either as absolute or relative rates, during dynamic supramaximal exercise in old and young subjects (Fig. 3). Therefore, aging does not compromise the intrinsic aerobic capacity of skeletal muscle to adequately support a sudden increase in ATP demand caused by muscle contraction. Consistent with the results obtained during exercise, the peak rate of mitochondrial ATP synthesis ($V_{\text{max}}$) inferred from the postexercise recovery period was also not significantly different between the young and old subjects (Fig. 5C), which further supports the suggestion that age per se does not impair mitochondrial function in vivo (30) and ex vivo (72).

Of note, the peak rates measured during the supramaximal exercise were close to the $V_{\text{max}}$ calculated during the recovery period in both young and old (~24 mM/min), supporting both the maximal nature of our protocol and the low-activity status of our subjects. Indeed, the peak rates of oxidative ATP synthesis during dynamic plantar flexion (~22 mM/min) were consistent with those of previous studies conducted in untrained individuals (2, 34, 69). For instance, Hunter et al. (34) and Newcomer and Bosca (69) reported a rate of ~18–19 mM/min during a 90-s maximal isometric contraction of the calf muscle in untrained adults. With moderately active or trained sprinters as subjects, which can dramatically increase the peak rate of mitochondrial respiration (54), Walter et al. (86) reported much higher values (~40 mM/min) during similar supramaximal dynamic plantar flexion exercise.

Consistent with the current study, Chilibeck et al. (7–9) have previously reported similar PCr and pulmonary $\text{VO}_2$ kinetics in
young and old subjects during the transition from rest to submaximal plantar flexion exercise. However, unlike these former studies, an advantage of the \(^{31}\)P-MRS approach employed here is that skeletal muscle energy systems were stressed to their functional limit during a supramaximal exercise, and all three major ATP synthesis pathways (oxidative phosphorylation, glycolysis, and creatine kinase reaction) were examined quantitatively. Our findings do, however, somewhat

Fig. 3. The rate of ATP synthesis through anaerobic glycolysis (ATP\(_{\text{gly}}\), A), oxidative phosphorylation (ATP\(_{\text{ox}}\), B), and the creatine kinase reaction (ATP\(_{\text{CK}}\), D) with respect to time during supramaximal plantar flexion exercise. The time course of PME (C) illustrates the glycogenolytic flux that has not passed through the glycolytic pathway. Relative contribution of the anaerobic glycolysis, oxidative phosphorylation, and the creatine kinase reaction to total ATP synthesis during exercise (E). Values are presented as means ± SE. None of the ATP synthesis rates were significantly different between the young and old (\(P > 0.05\)). *First data point that is significantly greater than 0 (glycolysis) or baseline (PME).
contrast with a previous study documenting a greater reliance upon oxidative ATP synthesis during a 60-s maximal isometric voluntary contraction in the tibialis anterior of older individuals (49). However, the discrepancy between this study and the current results may be explained by the differences in force generation between the young and old subjects. Indeed, the young men in the study by Lanza et al. (49) generated an ~50% greater force during the isometric contraction, which likely generated higher intramuscular pressure and potentially compromised blood flow (87). Accordingly, the lower oxidative ATP synthesis reported in the young subjects might, in fact, be attributable to a compromised O2 availability rather than a greater reliance on oxidative phosphorylation in the elderly (49). This confounding factor was likely less of an issue in the current study, since subjects performed dynamic contractions, likely preserving peripheral hemodynamics, even at the higher work rates.

A similar finding to the study by Lanza et al. (49) (i.e., a greater reliance on oxidative phosphorylation) was recently reported by the same group in old compared with young subjects matched for maximum voluntary contraction (10). However, it should be underscored that the muscle investigated by both Lanza et al. (49) and Christie et al. (10), the tibialis anterior, exhibits some unique features, most notably a preserved mitochondrial efficiency with age (1) and a maintained or even increased oxidative capacity in the elderly (10, 51, 80), perhaps as a result of a greater activation of this muscle for postural control in this population (52). These potentially unique age- and muscle-specific adaptations render the comparison with these studies more difficult. Other studies have used dynamic plantar flexion to investigate the effects of age on muscle energetics (13, 64, 88). Although not quantitative, these studies suggested a greater reliance on oxidative phosphorylation in older individuals as inferred from a higher P-to-PCr ratio for a given work rate. However, in these cases, either physical activity was not controlled (13, 64) or relative exercise intensity between the age groups was different (88), which may explain the discrepancy with the current results.

**ATP synthesis from glycolysis and the creatine kinase reaction.** There is accumulating evidence that glycolysis is activated according to a “dual control” model (14, 16, 19, 20). Accordingly, both a feedforward signal related to muscle contraction (e.g., intracellular Ca2+) and a feedback signal related to metabolic demand/metabolite accumulation [ADP, AMP, and P, affecting phosphofructokinase activity (17) or glycogen phosphorylase activity via P, and H+ (73)] regulates glycolytic rate. In light of this, a major goal of the present study was to comprehensively compare and contrast the anaerobic ATP synthesis flux within the skeletal muscle of young and old subjects in response to supramaximal dynamic plantar flexion.
exercise. Interestingly, although the relative ATP synthesis rates from glycolysis and the creatine kinase reaction averaged over the entire exercise bout were similar in the young and old, glycolytic activation was delayed in the old subjects (~36 s) compared with the young (~24 s, Fig. 3A).

In terms of metabolic control, it has been suggested that a threshold of metabolite accumulation must be achieved before contraction-related signals can increase glycolytic flux (19). However, it is unlikely that the longer delay in glycolysis activation in the old subjects can be explained by different time to reach this threshold, since the levels of [Pi] (old: ~15 mM; young: ~13 mM) and [ADP] (old: ~93 μM; young: ~61 μM) were actually higher, not lower, in the old at the activation of glycolysis. In addition, the levels of these metabolites are well above the K_m for phosphofructokinase activation in vitro (1 mM and 30 μM for Pi and ADP, respectively) (17). A shortage in substrate such as glucose 6-phosphate and fructose 6-phosphate can also be ruled out since [PME] levels significantly increased after only ~12 s in both groups, implying that hexokinase was activated and glycogenolysis generation of substrate was actually in excess, compared with glycolytic flux, in both the young and old (Fig. 3C).

A mechanism that may explain the longer delay for the onset of glycolysis rate in the elderly is a different intracellular Ca^{2+} level between groups. Indeed, based on in vitro experiments (62, 63) and computer simulation (47, 77), it has been suggested that Ca^{2+}-calmodulin activation of the phosphofructokinase enzyme has a dominant role in controlling the glycolytic flux during skeletal muscle contraction, although metabolite feedback and H^+ concentration accumulation are also required (19, 20, 41, 47). In the present study, although the total rate of ATP turnover was similar between the old and young (Fig. 4A), elderly subjects exercised at a lower absolute power output. This implies that the Ca^{2+} release from the sarcoplasmic reticulum and the intracellular Ca^{2+} may have been lower in the old compared with the young. This would lead to a lower feedforward signal requiring greater level of metabolites (Pi, ADP, and AMP) and lower pH to activate an increase in glycolysis rate. In support of this theory, as already indicated, compared with the young subjects, the old tended to exhibit higher levels of [Pi] and [ADP] at the activation of glycolysis.

Another interesting observation from the present study was the similar rates of ATP synthesis from glycolysis and the creatine kinase reaction over the entire exercise in the young and old subjects. This was the case whether expressed as absolute or relative total ATP synthesis (Fig. 3E) and is in agreement with a previous study demonstrating an unchanged creatine kinase reaction flux using the magnetization transfer technique (33).

**ATP cost of contraction.** In agreement with a previous study investigating the same muscle group but at a lower exercise intensity (58), the ATP cost of contraction during dynamic supramaximal plantar flexion exercise was substantially elevated in the old (~37%) compared with the young (Fig. 4B). Interestingly, previous studies have reported that the ATP cost of contraction was improved (80), unchanged (10, 15, 56), or impaired (56) during exercise localized to the tibialis anterior and the quadriceps muscles. Taken together, these results provide support for the hypothesis that age-related alterations in muscle efficiency may vary among muscles depending on the chronic activity of the muscle itself. Additionally, the rate of contraction employed during the task can substantially affect conclusions regarding muscle metabolism, which could also explain some of the discrepancies in this field of study, since older individuals have been documented to exhibit an impaired ATP cost of contraction during intermittent contractions, but not during a continuous isometric contraction (56).

Although very interesting and innovative, some methodological concerns have been raised (57) regarding a recent study reporting a preserved cost of contraction in the quadriceps with age by Conley and colleagues (15). Specifically, the combined analysis of data from different exercise modalities, and assumptions about muscle recruitment during exercise in both young and old, had some bearing on the interpretation of the findings. Despite these limitations, the suggestion in this study (15) that mitochondrial dysfunction can contribute to a reduction in muscle efficiency should not be ruled out, since these two mechanisms (contractile and/or mitochondrial inefficiency) are not mutually exclusive and, in fact, may both explain the increase in the cost of locomotion and greater fatigability associated with aging (12, 76).

The mechanisms underlying this age-related increase in ATP cost of muscle contraction are likely numerous, but an increase in the noncontractile processes of ion transport (Ca^{2+}-ATPase and Na^+-K^+-ATPase) may account for much of the present findings. Indeed, an excessive energy demand from ion pumping in the skeletal muscle of older individuals has recently been implied, by our group (57), to contribute to the decline in muscle efficiency with age. In line with this concept, slower rates of relaxation after contractions evoked by electrical stimulation have been consistently documented in older subjects (3, 28, 36, 74) and associated with lower Ca^{2+} uptake and Ca^{2+}-ATPase activity measured in vitro (36, 45). This impairment in Ca^{2+} sequestration by the sarcoplasmic reticulum would further contribute to the slowing of muscle fiber cross-bridge dissociation, prolonging relaxation and total contraction time, further elevating the metabolic cost of dynamic exercise.

Additionally, an age-related slowing of the contractile properties specific to the plantar flexor muscle has previously been documented (22, 24, 81), likely affecting skeletal muscle metabolic demand in older individuals during dynamic exercise. This is potentially the consequence of a slower rate of myosin attachment and detachment to actin (66), which may eventually decrease fiber power output, although this has yet to be confirmed. In addition, there is a growing appreciation that older individuals rely more on torque-generating capacity for power production due to an impaired velocity of contractile function (21) and impaired membrane excitability (24), thus impeding rapid muscular contraction and relaxation. Therefore, it is likely that, in combination, the slower contractile properties and excessive energy demand from the noncontractile processes of ion transport may all contribute to exacerbate the energy cost of dynamic contractions with age.

Aging is generally associated with a shift in fiber type toward slow-twitch oxidative fibers (61), which, conceptually, should result in improved muscle efficiency (18, 35) not a reduction in contractile efficiency, as reported here. However, it should be noted that conflicting results, again in the tibialis anterior, have both supported (80) and challenged (10) a role for fiber type in age-related changes in muscle efficiency. Therefore, because fiber type composition was not assessed in...
the present study, it is, at present, unclear to what extent a shift in fiber type may have influenced the current findings.

As previously discussed in more detail (58), to some extent, mechanical factors, for example, antagonist muscle coactivation, may have contributed to the greater ATP cost of contraction in the elderly. However, the use of a rather simple task (plantar flexion exercise) and experimental evidence questioning the importance of enhanced antagonist muscle coactivation during plantar flexion exercise in elderly subjects (70, 78) suggests that this effect may be minor.

The ATP cost of contraction may also increase in the elderly as a consequence of motor neuron loss with age (67, 75). However, this hypothesis does not seem to pertain to the soleus muscle (22, 23), and the dynamic process of fiber reinnervation, through collateral sprouting, appears to successfully compensate for neuronal loss in the early stage of aging (65, 81). Also, the age-related deficit in the ability to finely control force or movement during a motor task appears to be limited to low-intensity contraction and can be restored to the level of the young by a single-practice session (11). Together, it appears unlikely that age-related alteration in neural activation of agonist muscles and greater motor output variability contributed substantially to the greater ATP cost of contraction documented in the current study.

Experimental considerations. Careful monitoring of the movement of the weight used for resistance during plantar flexion ensured that subjects exercised within the full range of motion throughout the exercise and maintained a consistent power output. However, the lack of a direct measurement of the power output is a limitation of the current study.

It could be expected that the skeletal muscle fat infiltration that occurs with aging (27) may affect the quantification of muscle volume using the current anthropometric approach. However, although this phenomenon is of clinical interest, it is unlikely that fat infiltration resulted in a significant error in our estimation of muscle volume. Indeed, the validity of this anthropometrically based method has been demonstrated multiple times in both young (46, 82, 83) and old subjects (6, 26), as well as in a wide spectrum of individuals including those with a spinal cord injury (59), a group recognized to exhibit significant muscle atrophy and fat infiltration. In addition, a recent MR imaging study comparing the dorsi- and plantar flexor muscles in two very distinct groups with regard to their anthropometric characteristics documented a limited increase in the relative proportion of the noncontractile tissues from ~2–3% in normal-weight active young to ~8–10% in overweight to obese old (31). Together, these findings therefore suggest that, in the current, normal-weight and activity-matched subjects, such a bias in terms of the estimation of muscle volume induced by the infiltration of fat into the muscle was likely negligible.

Perspectives and Significance

Here our findings indicate that the intrinsic ability of the skeletal muscle energy system to cope with a given ATP demand does not appear to be affected by chronological age but rather by changes in physical activity. We also identified contractile inefficiency as a key factor in the age-related reduction in exercise capacity, and this phenomenon should be the target for future intervention to reverse this source of debilitation in the elderly.

In conclusion, this study reveals that when young and old subjects are activity matched, apart from delayed glycolytic activation in the old, there is no evidence of age-related mitochondrial and glycolytic dysfunction. However, this study does reveal an abnormal elevation in exercise-induced skeletal muscle metabolic demand in the old (~37%) that may contribute to the decline in exercise capacity with advancing age.

APPENDIX

ATP production from PCr breakdown. The rate of ATP production from the breakdown of PCr (ATPck) through the creatine kinase (CK) reaction (mM/min) was calculated from the change in PCr for each time point of the exercise period (40):

$$\text{ATP}_{\text{CK}} = \frac{d\text{PCR}}{dt}$$

ATP production from oxidative phosphorylation. Based on the sigmoid relationship between the oxidative ATP production rate (ATPox, mM/min) and free cytosolic ADP concentration ([ADP]), the rate of mitochondrial ATP production was calculated as follows:

$$\text{ATP}_{\text{OX}} = V_{\text{max}} \left(1 + \frac{K_m}{[\text{ADP}]^{1/2}}\right)$$

in which $K_m$ (the [ADP]) at half-maximal oxidation rate is ~30 μM in skeletal muscle (40), 2.2 is the Hill coefficient for a sigmoid function (38), and $V_{\text{max}}$ is the inferred peak rate of mitochondrial respiration in vivo.

$V_{\text{max}}$ (in mM/min) was calculated using the initial rate of PCr resynthesis ($V_{\text{PCR}}$) during the recovery period, and [ADP] was measured at the end of exercise:

$$V_{\text{PCR}} = V_{\text{PCR}} \left(1 + \frac{K_m}{[\text{ADP}]_{\text{end}}^{1/2}}\right)$$

the $V_{\text{PCR}}$ was calculated from the derivative of the next equation at time 0:

$$V_{\text{PCR}} = k \times \Delta[\text{PCr}]$$

in which $\Delta[\text{PCr}]$ represents the amount of PCr resynthesized during the recovery, and the rate constant $k = 1/e$ (40). The first-order PCr recovery rate constant ($k$) was determined from a fitting of the PCr time-dependent changes during the recovery period to a single exponential curve described by the equation:

$$Y(t) = Y_{\text{end}} + Y_{\text{res}} \left[1 - e^{-t/T_{\text{DP}}}\right]$$

where $Y_{\text{end}}$ is the level of [PCr] measured at the end of exercise, and $Y_{\text{res}}$ refers to the amount of PCr resynthesized during the recovery.

Model variables were determined with an iterative process by minimizing the sum of squared residuals between the fitted function and the observed values. Goodness of 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 ($r^2$) were calculated as follows (68):

$$r^2 = 1 - \frac{(SS_{\text{res}}/SS_{\text{tot}})}$$

where $SS_{\text{res}}$ is the sum of squares of the residuals from the fit, and $SS_{\text{tot}}$ is the sum of squares of the residuals from the mean.

ATP production from anaerobic glycolysis. Throughout the exercise period, glycogen breakdown to pyruvate and lactate, proton efflux, buffering capacity, protons produced by oxidative phosphorylation, and the consumption of protons by the CK reaction lead to changes in intramuscular pH (40). Assuming that the glycolytic production of 1 mol of $H^+$, when coupled to ATP hydrolysis, yields 1.5 mol of ATP, ATP production from anaerobic glycolysis (ATPgly) can be deduced from the total number of protons (P) produced throughout exercise (32):

$$\text{ATP}_{\text{gly}} = \frac{2.25 \times P}{30}$$
P = H_{\text{CK}}^+ + H_{\text{ox}}^+ + H_{\text{flux}}^+

H_{\text{CK}}^+ (in mM/min) was calculated from the time-dependent changes in [PCr] and from the stoichiometric coefficient (γ):

H_{\text{CK}}^+ = -\gamma \times ATP_{\text{CK}}

where γ is the proton stoichiometric coefficient of the coupled Lohmann reaction as described previously (48).

H_{\text{ox}}^+ (in mM/min) was calculated from the apparent buffering capacity β_{total} (in Sylkes, mmol acid added/unit change in pH) and from the rate of pH changes:

\[ H_{\text{ox}}^+ = -\beta_{total} \times dpH / dt \]

where

\[ \beta_{total} = \beta_{\text{bicarbonate}} - \beta_{\text{Pi}} + \beta_{\text{PME}} + \beta_{\text{PME}} \]

in which β was determined from the initial change in PCr (ΔPCr) and alkalization of pH (ΔpH) (14):

\[ \beta_x = \gamma \times (\Delta PCr / \Delta pHi) \]

β_{PME} were determined based on the dissociation constant of the buffer (K) according to the standard formula (16):

\[ \beta_x = (2.303 \times H^+ X / K \cdot X) / (K + H^+) \]

where X is either Pi or PME and K = 1.77 \times 10^{-7} for Pi and 6.3 \times 10^{-7} for PME.

In agreement with previous studies and assuming that muscle is a closed system during exercise (16, 42), \beta_{bicarbonate} was set to zero.

H_{\text{ox}}^+ (in mM/min) was calculated from the rate of pH changes:

\[ H_{\text{ox}}^+ = m \cdot ATP_{\text{ox}} \]

H_{\text{flux}}^+ (in mM/min) was calculated for each time point of exercise using the proportionality constant λ relating proton efflux rate to ΔpHi:

\[ H_{\text{flux}}^+ = -\lambda \Delta pHi \]

This proportionality constant λ (in mM-min^{-1}·pH unit^{-1}) was calculated during the recovery period:

\[ \lambda = -V_{\text{eff}} / \Delta pHi \]

During this period, PCr is regenerated throughout the CK reaction as the consequence of oxidative ATP production in mitochondria. Thus, H_{\text{flux}}^+ can be calculated from the rates of proton production from the CK reaction (H_{\text{CK}}^+, in mM/min) and mitochondrial ATP production (H_{\text{OX}}^+, in mM/min) on one side and the rate of pH changes on the other side. At this time, ATP production is exclusively aerobic, and lactate production is considered as negligible:

\[ V_{\text{eff}} = \beta_{total} \times dpH / dt + \gamma \times V_{\text{PCR}} + m \cdot ATP_{\text{ox}} \]

To improve precision, we use a modified version of this calculation (43) in which the total proton disappearance (i.e., ∫Edt) is estimated cumulatively from the start of recovery and then fitted to an exponential function to obtain the initial recovery rate E.

Total ATPase rate. The total ATPase rate (ATP_{ox}, mM/min) was calculated for each time point as:

\[ ATP_{\text{tot}} = ATP_{\text{ox}} + ATP_{\text{CK}} + ATP_{\text{gly}} \]

Energy cost of contraction (in mM/W) was calculated as the ratio between total ATP production (ATP_{ox} + ATP_{CK} + ATP_{gly}) and power output.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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