Creatine supplementation and age influence muscle metabolism during exercise

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Creatine supplementation and age influence muscle metabolism during exercise. J. Appl. Physiol. 85(4): 1349–1356, 1998.—Young [n = 5, 30 ± 5 (SD) yr] and middle-aged (n = 4, 58 ± 4 yr) men and women performed single-leg knee-extension exercise inside a whole body magnetic resonance system. Two trials were performed 7 days apart and consisted of two 2-min bouts and a third bout continued to exhaustion, all separated by 3 min of recovery. 31P spectra were used to determine pH and relative concentrations of P, phosphocreatine (PCr), and β-ATP every 10 s. The subjects consumed 0.3 g·kg⁻¹·day⁻¹ of a placebo (trial 1) or creatine (trial 2) for 5 days before each trial. During the placebo trial, the middle-aged group had a lower resting PCr compared with the young group (35.0 ± 5.2 vs. 39.5 ± 5.1 mmol/kg, P < 0.05) and a lower mean initial PCr resynthesis rate (18.1 ± 3.5 vs. 23.2 ± 6.0 mmol·kg⁻¹·min⁻¹, P < 0.05). After creatine supplementation, resting PCr increased 15% (P < 0.05) in the young group and 30% (P < 0.05) in the middle-aged group to 45.7 ± 7.5 vs. 45.7 ± 5.5 mmol/kg, respectively. Mean initial PCr resynthesis rate also increased in the middle-aged group (P < 0.05) to a level not different from the young group (24.3 ± 3.8 vs. 24.2 ± 3.2 mmol·kg⁻¹·min⁻¹). Time to exhaustion was increased in both groups combined after creatine supplementation (118 ± 34 vs. 154 ± 70 s, P < 0.05). In conclusion, creatine supplementation has a greater effect on PCr availability and resynthesis rate in middle-aged compared with younger persons.

aging; creatine monohydrate; phosphocreatine; skeletal muscle; magnetic resonance spectroscopy

STUDIES HAVE REPORTED age-related reductions in skeletal muscle size, type II fiber diameter, mitochondrial enzyme activity, and high-energy phosphate metabolism, which are associated with the decline in skeletal muscle strength and endurance capacity that occurs with aging (5, 7, 8, 21, 22, 34). However, the underlying processes through which these changes occur are not well understood. Reduced levels of resting phosphocreatine (PCr) reported in the elderly may be in part responsible for these declines (22, 29). A decline in PCr availability has been shown to be a potential contributing factor to muscle fatigue during moderate- to high-intensity exercise (6, 16). In addition, resynthesis rates of PCr after exercise have been reported to decline with age by ~8% every 10 yr after 30 yr of age (24). It is generally believed that PCr resynthesis is regulated by creatine kinase bound to the outer membrane of mitochondria and that the initial rate of PCr recovery is proportional to the rate of mitochondrial oxygen consumption (18, 20, 21, 26, 31, 33, 34).

Dietary creatine supplementation (20–30 g/day for 4–6 days) has been reported to increase muscle creatine concentration by as much as 50% and enhance muscle performance during intermittent high-intensity exercise bouts (2–4, 10, 12, 13, 15). The performance-enhancing effect of creatine may result from increased muscle creatine availability that sustains the initially rapid rate of PCr resynthesis further into recovery and increases available PCr during later exercise bouts (3, 12, 15). No studies have investigated the effects of creatine in older persons who, because of intrinsic deficits in muscle energy metabolism, may benefit from creatine supplementation.

The purpose of this study was to determine the effects of creatine supplementation and age on muscle PCr metabolism and performance by using 31P-magnetic resonance spectroscopy (MRS). 31P-MRS provides frequent, serial, noninvasive measurements of intramuscular phosphorus compounds and greatly improves measurement resolution over muscle biopsy techniques (27), the most frequently used method of assessing the effects of creatine supplementation on muscle metabolism to date. We hypothesized that middle-aged persons would have lower resting muscle PCr concentrations and slower PCr resynthesis rates than younger adults with similar activity and dietary habits and that creatine supplementation would have greater effects on muscle PCr metabolism in middle-aged persons.

METHODS

Subjects. Middle-aged (>50 yr) and young (<40 yr) subjects were recruited for participation in the study. The younger group consisted of 4 men and 1 woman and the middle-aged group of 3 men and 1 woman. The physical characteristics of the subjects are presented in Table 1. All subjects were free from chronic diseases and on no regular medications, as determined by a medical history questionnaire. The study was approved by the appropriate institutional review boards, and all subjects gave their voluntary and informed consent before participation.

The level of habitual physical activity was not different between the young and middle-aged groups, as was indicated by the Harvard Alumni Questionnaire (30). In addition, because of a potential effect of diet on skeletal muscle creatine, the subjects were questioned on their normal dietary habits. There were no vegetarians in the study, and all the subjects reported consuming at least five servings of meat per week.

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Creatine supplementation. Two single-blind exercise trials were performed: a placebo trial followed by a creatine trial 7 days later. The trials were not randomized, as skeletal muscle creatine levels can remain elevated above basal levels for 4–5 wk after supplementation stops (17). Five days before each trial, the subjects began consuming 0.3 g·kg$^{-1}$·day$^{-1}$ of either a placebo (granulated sugar) or 0.3 g·kg$^{-1}$·day$^{-1}$ of creatine monohydrate (Phosphagen, Experimental and Applied Sciences, Pacific Grove, CA) combined with 0.3 g·kg$^{-1}$·day$^{-1}$ of a flavored powder drink mix. The mixture was dissolved in water and consumed four times per day.

Exercise. Both groups performed single-leg knee-extension exercise while lying supine inside a whole body 1.5-T magnetic resonance (MR) system (General Electric SIGNA, General Electric Medical Systems, Milwaukee, WI). The exercise apparatus provided concentric resistance via a lever arm and pulley system integrated with a flywheel and resistance strap (Fig. 1). An elastic cord returned the lever arm to the starting position after each knee extension. Knee extensions were performed from $\sim$110 to $\sim$145° of knee extension at 37 contractions/min set by an audible metronome. Power output during exercise was determined by measuring the tension and displacement applied to an in-line pulley and by estimating leg mass (36).

During the experimental trials, three single-leg exercise bouts were performed, separated by 3 min of recovery. Bouts 1 and 2 were 2 min each in duration, and bout 3 was continued to exhaustion. Exhaustion was defined as the time when the rate and/or range of motion could not be maintained by the subject after being given verbal encouragement by the investigators. Before the experimental trials, two to three exercise practice sessions were performed to familiarize the subjects with the experimental procedures and to determine the appropriate exercise intensity. The maximum flywheel resistance at which each subject was able to perform two 2-min exercise bouts was determined. To achieve exhaustion in 1–2 min, the resistance was increased slightly for bout 3. The resistance for each bout was constant during both the placebo and creatine trials. Both legs were tested in each experimental condition.

$^3$P-MRS. $^3$P spectra were collected during exercise through a $^1$H/$^3$P dual radio-frequency transmit/receive 11-cm surface coil (USAsia, Columbus, OH) placed over the quadriceps muscles. $^3$P data were acquired by using a hard-pulse 25.85-MHz excitation (pulse width 600 µs), repetition time = 1,000 ms, spectral width 2,000 Hz, and 1,024 sampled free induction decay (FID) points. Before exercise, a proton MR image was acquired axially by using the $^1$H/$^3$P surface coil to verify coil placement and muscle group participation. A linear gradient shim procedure was performed to reduce field inhomogeneity within the sensitive volume. Surface coil transmitter and receiver gains for $^3$P-MRS were set once to maximize the PCr signal acquired from the muscle and kept constant throughout the study. Ten FID signals were averaged, producing one spectrum every 10 s. Care was taken to ensure that exercise began and ended at the onset of an FID cycle. The MR system was calibrated by using known standards on each testing day.

FID processing consisted of apodization of 10-Hz line broadening, zero-filling to 4,096 points and Fourier transformation, followed by zero and first-order phasing. Relative concentrations of Pi, PCr, and $\beta$ATP were determined from spectral peak areas. PCr/$\beta$ATP$_{rest}$, Pi/$\beta$ATP$_{rest}$, and $\beta$ATP/$\beta$ATP$_{rest}$ ratios (where $\beta$ATP$_{rest}$ was the mean area of the six initial resting $\beta$-ATP peaks collected before exercise) were converted to millimoles per kilogram wet weight by assuming that the area of $\beta$-ATP$_{rest}$ was equivalent to 5.5 mmol/kg wet wt (33). The relative quantity of PCr hydrolysis during exercise was represented as the change in PCr from rest to the lowest PCr value attained during each exercise bout. Finally, pH was calculated by using the chemical shift between the Pi, and PCr frequency (33).

The rate of PCr resynthesis and a time constant (Tc) indicating half-time of recovery were determined from a monoexponential curve fit to the PCr recovery data (18, 20, 26, 34). The following equation was used: $y = a(1 - \exp(bx)) + c$, where $y$ represents the PCr value at any given time $x$, $a$ is the change in PCr during recovery, $b$ is the rate constant ($1/b = T_c$), and $c$ is the initial PCr value at the onset of recovery. The values for initial PCr resynthesis rate were determined from the slope of the initial 10 s of the monoexponential curve fit (20, 34). The initial PCr resynthesis rate and PCr Tc were used to describe PCr kinetics, not to predict muscle oxidative capacity as in previous studies (1, 20, 34).

Analysis. Analysis of variance tests were used to analyze PCr, Pi, ATP, pH, PCr hydrolysis, initial PCr resynthesis rate, Tc, power output, and time to exhaustion with treatment (creatine vs. placebo), age (young vs. middle-aged), leg (right

### Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>5</td>
<td>31 ± 5.2</td>
<td>174 ± 12.8</td>
<td>67.9 ± 11.7</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>4</td>
<td>58 ± 4.5</td>
<td>179 ± 3.11</td>
<td>83.3 ± 12.8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of subjects.

Fig. 1. Schematic diagram of the exercise apparatus.
vs. left), and exercise or recovery bouts as factors. A Newman-Keuls post hoc test was used to determine mean differences between and within factors. A paired t-test was used to determine mean differences in body weight between the placebo and creatine trials for the young and middle-aged groups. The initial PCr concentration, PCr hydrolysis, and time to exhaustion for exercise bout 3 were correlated by using a Pearson product moment correlation. The significance level was set at P < 0.05 for all tests, and results are presented as means ± SD.

RESULTS

Age. During the placebo trial, resting and recovery PCr was lower in the middle-aged group compared with the young group (rest 35.0 ± 5.2 vs. 39.5 ± 5.1 mmol/kg, P < 0.05) as illustrated in Fig. 2. PCr hydrolysis (ΔPCr, Table 2) during the exercise bouts tended to be lower in the middle-aged group (P = 0.06), whereas power output was not different between groups (Table 2). As indicated by the practice trials, the exercise resistance was increased in bout 3 to ensure that exhaustion occurred in 1–2 min (Table 2). One young subject was excluded from all analysis of PCr hydrolysis, initial PCr resynthesis rate, and time to exhaustion because of experimental difficulties during exercise in the placebo trial. The young group for these measures consisted of 3 men and 1 woman, 30 ± 5 yr old.

After exercise, the initial PCr resynthesis rate was slower in the middle-aged group compared with the young group (18.1 ± 3.5 vs. 23.2 ± 6.0 mmol·kg·min⁻¹, P < 0.05), as illustrated in Fig. 3. Figure 4 shows the monoexponential curve fit to the PCr data from the first recovery period for a representative young and middle-aged subject. The mean coefficient of determination (r²) for all the monoexponential curve fit data was 0.97 ± 0.03 (P < 0.01). The Tc obtained from the monoexponential equation was similar for the young and middle-aged groups (Fig. 3). In addition, Pi (Fig. 5) and pH (Table 3) were not different between the groups during rest, exercise, or recovery.

Creatine supplementation. After creatine supplementation, resting and recovery PCr was increased in the young group by 15% (rest 45.7 ± 7.5 mmol/kg, P < 0.05) and in the middle-aged group by 30% (rest 45.7 ± 5.5 mmol/kg, P < 0.05), eliminating the difference in resting PCr between the young and middle-aged groups (Fig. 2). PCr hydrolysis during exercise increased in the middle-aged group (P < 0.05) after creatine supplementation and tended to increase in the young group (P = 0.1, Table 2). There was a positive correlation between the initial PCr concentration and PCr hydrolysis for both groups during exercise bout 3 (r = 0.72, P < 0.05).

Power output remained constant across the placebo and creatine trials for both groups (Table 2). Creatine supplementation increased the mean time to exhaustion during exercise bout 3 by 30% in the young and middle-aged groups combined (118 ± 34 vs. 154 ± 70 s, P < 0.05, Table 2). There were no significant interactions or differences in time to exhaustion with regard to age. All the subjects were encouraged by the investigators during exercise and appeared to give a maximal effort during the time to exhaustion bout. Time to exhaustion was not significantly correlated with the initial PCr concentration (r = 0.26, P = 0.14) or PCr hydrolysis (r = 0.34, P = 0.06) during exercise bout 3.

![Fig. 2. Results of phosphocreatine (PCr; mmol/kg wet wt) vs. time (s) at rest and throughout exercise and recovery bouts for creatine and placebo trials. ○, Young subjects; ●, middle-aged subjects. Values are means ± SD.](image-url)
This study investigated the effects of age on muscle PCr metabolism during exercise by comparing young and middle-aged subjects (Table 1). In addition, the effects of oral creatine supplementation on muscle PCr metabolism and performance were examined. To collect performance and metabolic data simultaneously, knee-extension exercise was performed inside an MR system (Fig. 1). During the exercise, cadence and resistance were held constant, and time to exhaustion was used as a measure of quadriceps muscle endurance capacity. Noninvasive 31P-MRS was employed to measure quadriceps muscle PCr, P_i, ATP, and pH throughout exercise and recovery during a repeated-measures placebo and creatine exercise trial. We hypothesized that older persons would have lower resting PCr concentrations and slower initial PCr resynthesis rates than younger persons and that oral creatine supplementation would elicit greater improvements in muscle PCr metabolism in older persons.

Age. Our results confirm reports that older persons have a lower resting muscle PCr concentration compared with younger persons (22, 29) (Fig. 2). Resting PCr availability was 11% lower in the middle-aged group (P < 0.05), and the quantity of PCr hydrolyzed during exercise tended to be lower (P = 0.06, Table 2). An increase in the percentage of type I fibers within muscle has been reported to occur with age and may explain the reduced resting PCr concentration found in older subjects due to the characteristically lower PCr concentrations of type I fibers (7). However, it should be noted that not all studies investigating age-related changes in muscle metabolism report reductions in resting PCr and increases in the percentage of type I fibers (21, 22, 32). McCully et al. (22) reported resting

The initial PCr resynthesis rate increased in the middle-aged group (24.3 ± 3.8 mmol·kg⁻¹·min⁻¹, P < 0.05) to a level not different from the young group (24.2 ± 3.2 mmol·kg⁻¹·min⁻¹) after creatine supplementation (Fig. 3). In addition, the initial rapid rate of PCr resynthesis appeared to continue further into recovery, maintaining the elevated PCr concentrations in both groups over time (Fig. 4). Creatine supplementation did not significantly change the PCr resynthesis T_c in either group (Table 2, Fig. 3).

Resting P_i was unaffected by creatine supplementation in the young and middle-aged groups (Fig. 5). P_i production during exercise tended to increase following creatine supplementation (Fig. 5) and was similar to the changes observed in PCr hydrolysis (Table 2). The β-ATP (not shown) remained constant from rest throughout exercise and recovery, and there were no differences in β-ATP between groups or experimental trials. Additionally, pH declined during exercise (P < 0.05) and was not significantly different between groups or trials (Table 3).
PCr/Pi levels to be ~38% lower in middle-aged (66.8 ± 1.9 yr) and elderly (80.0 ± 5.1 yr) subjects compared with young (24.6 ± 4.7 yr) subjects. In a subsequent study, McCully et al. (21) found no difference in resting PCr/Pi values and no difference in fiber type distribution in older (66.0 ± 6.0 yr) vs. young (28.2 ± 6.8 yr) subjects. Given these findings, the effect of aging on resting PCr concentration and fiber type distribution requires further investigation.

The results of this study also agree with reports that older individuals have a reduced PCr resynthesis rate after exercise (8, 21–24) (Fig. 3). The initial PCr resynthesis rate represents the rate at which creatine kinase replenishes PCr immediately after exercise and may be affected by muscle creatine concentration (1, 12, 26, 33). The middle-aged subjects had a 22% slower initial PCr resynthesis rate, which may be associated with their reduced resting PCr concentrations (Fig. 2). As PCr is hydrolyzed to resynthesize ATP during exercise, the free creatine concentration in the muscle increases. During recovery, the elevated muscle creatine concentration drives the creatine kinase reaction toward the production of PCr (28). A low muscle PCr concentration is indicative of a low total creatine availability, which may limit creatine kinase resynthesis of PCr (3, 12, 15). Furthermore, an increase in muscle creatine availability elicited by oral creatine supplementation has been reported to prolong the initial rapid rate of muscle PCr resynthesis during recovery (12).

Muscle mitochondrial oxidative capacity and pH also affect PCr recovery and, therefore, may influence the initial PCr resynthesis rate and Tc measurements (1, 26, 33). In this study, there was no difference in pH between groups or trials (Table 3). Both the PCr recovery Tc and initial PCr resynthesis rate have been reported to be independent of pH and exercise intensity when the variation in pH is similar to that observed between the young and middle-aged groups in this
Muscle oxidative capacity has been found to decline with age and may have limited the initial PCr resynthesis rate in the middle-aged group by limiting the supply of ATP required by creatine kinase to resynthesize PCr after exercise (5, 7, 8, 21, 22, 34). However, given that the only change in the subjects' daily routine between trials was the ingestion of creatine, we assume that muscle oxidative capacity was unaffected during the 7 days between trials and that changes in PCr metabolism after creatine supplementation resulted from factors other than oxidative capacity.

Creatine supplementation. After oral creatine supplementation, resting PCr concentration increased in the young group (P < 0.05) and, to a greater extent, in the middle-aged group (P < 0.05), eliminating the difference in resting PCr between the young and middle-aged subjects (Fig. 2). The variation in response to creatine supplementation by the young and middle-aged groups is consistent with reports that persons having relatively low resting muscle PCr concentrations tend to have a larger increase in resting muscle PCr after creatine supplementation (12, 15).

In addition to the increase in resting PCr, there was an increase in PCr hydrolysis during exercise in the middle-aged group without a change in exercise power output (Table 2), suggesting that a greater proportion of ATP supplied to the working muscle fibers was derived from PCr after creatine supplementation. PCr appears to be the preferred energy source of skeletal muscle to replenish ATP during activity. Thus, as PCr availability increases, PCr hydrolysis during exercise increases, as indicated by the positive correlation between the initial PCr concentration and PCr hydrolysis during exercise bout 3 (r = 0.72, P < 0.05). Others have reported an increase in PCr use and a reduction in muscle lactate during exercise after creatine supplementation (3). The young group tended to use more PCr after creatine supplementation (P = 0.1).

The slower initial PCr resynthesis rate observed in the middle-aged subjects was increased (P < 0.05) to a rate similar to that of the young group after creatine supplementation (Fig. 3). These results support the concept presented previously that muscle creatine availability may affect creatine kinase activity during recovery and, consequently, affect PCr resynthesis rate (3, 12, 15). Similar increases in PCr resynthesis rate have been reported to occur after intense physical training (19), and similar differences exist when trained and untrained populations are compared (14, 25, 31). However, the increase in PCr resynthesis rate in these studies was attributed to improvements in mitochondrial oxidative capacity associated with training, which enable the mitochondria to supply ATP to the creatine kinase system at a faster rate. Assuming that muscle oxidative capacity was not affected by creatine supplementation in the present study, the increase in initial PCr resynthesis rate observed in the middle-aged group most likely resulted from the increase in muscle creatine availability. These results suggest that the availability of muscle creatine may significantly influence the initial PCr resynthesis rate after exercise in healthy middle-aged persons, independent of oxidative capacity and pH.

The monoexponential $T_c$ is a function of the quantity of PCr hydrolyzed and the PCr resynthesis rate (18, 20, 26, 34). Because of the combined increase in PCr hydrolysis and resynthesis rate (P < 0.05) in the middle-aged group after creatine supplementation, the $T_c$ remained relatively constant (Table 2). Similarly, the lack of a significant difference between the young and middle-aged subjects' mean $T_c$ during the placebo trial (Fig. 3) may be explained by the lower PCr hydrolysis (P = 0.06) and slower PCr resynthesis rate (P < 0.05) observed in the middle-aged group (Table 2). These results suggest that the $T_c$ alone may not adequately describe differences in muscle energy metabolism that may exist between experimental groups and conditions.

The initial rapid rate of PCr resynthesis in the young and middle-aged groups appears to proceed for a longer duration after creatine supplementation (Fig. 4). This maintains an elevated PCr level during recovery (Fig. 2), despite the increased PCr hydrolysis during exercise observed after creatine supplementation in the middle-aged group (Table 2). The prolonged high rate of PCr resynthesis observed in this study agrees with results reported by Greenhaff et al. (12) that show a sustained initial PCr resynthesis rate in subjects responding to

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### Table 3. Muscle pH results

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Bout 1</th>
<th>Recovery 1</th>
<th>Bout 2</th>
<th>Recovery 2</th>
<th>Bout 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>7.12 ± .04</td>
<td>6.66 ± .21</td>
<td>6.99 ± .13</td>
<td>6.70 ± .26</td>
<td>6.94 ± .26</td>
<td>6.56 ± .25</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>7.12 ± .04</td>
<td>6.58 ± .29</td>
<td>6.93 ± .28</td>
<td>6.65 ± .19</td>
<td>6.92 ± .18</td>
<td>6.48 ± .29</td>
</tr>
</tbody>
</table>

Values are means ± SD and represent the pH at end of exercise bouts 1 and 2 and at end of recovery periods 1 and 2 for young and middle-aged subjects. The pH values for bout 3 represent the pH at end of bout 3 or after completion of 2 min of exercise, whichever occurred first.
Creatine supplementation during the second minute of recovery from exercise. An increase in PCR availability and utilization during exercise and an improved PCR resynthesis capacity during recovery have been proposed as the means through which creatine supplementation improves muscle performance during intermittent exercise bouts (3, 12, 15).

Time to exhaustion in bout 3 of the exercise trial was increased after creatine supplementation in the young and middle-aged groups combined (Table 2), indicating that creatine supplementation improves resistance to fatigue. This is consistent with the results of several other creatine-performance studies involving high-intensity intermittent exercise (2–4, 10, 13). Fewer studies have reported that creatine supplementation did not significantly affect intermittent exercise performance (9, 11). A greater sample size is required to determine whether there is a difference in the magnitude of change in time to exhaustion between young and middle-aged persons. In control trials performed by two young subjects, there were no differences in exercise time to exhaustion, 99 ± 27 and 101 ± 37 s, respectively, or 31P-MRS measurements between trials, suggesting that time to exhaustion was a reliable measure of muscle performance.

Conclusion. It has been reported in previous studies (8, 21–24, 29) that resting PCR concentration and PCR resynthesis rate are reduced in elderly persons. We hypothesized that creatine supplementation would increase muscle PCR availability and resynthesis rate in middle-aged persons more so than in younger persons with similar activity and dietary habits. The results of this study indicated that middle-aged persons had greater improvements in muscle PCR availability, PCR hydrolysis during exercise, and initial PCR resynthesis rate after creatine supplementation, compared with younger persons, and that creatine supplementation improved muscle endurance capacity in both groups combined. Our results did not indicate that creatine supplementation improved muscle endurance to a greater magnitude in older vs. younger individuals.

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