Creatine reduces human muscle PCr and pH decrements and $P_i$ accumulation during low-intensity exercise

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Rico-Sanz, J esús. Creatine reduces human muscle PCr and pH decrements and $P_i$ accumulation during low-intensity exercise. J Appl Physiol 88: 1181–1191, 2000.—The purpose of this study was to examine with 31P-magnetic resonance spectroscopy energy metabolism during repeated plantar flexion isometric exercise (Ex-1–Ex-4) at 32 ± 1 and 79 ± 4% of maximal voluntary contraction (MVC) before and during a creatine (Cr) feeding period of 5 g/day for 11 days. Eight trained male subjects participated in the study. ATP was unchanged with Cr supplementation at rest and during exercise at both intensities. Resting muscle phosphocreatine (PCr) increased ($P < 0.05$) from 18.3 ± 0.9 (before) to 19.6 ± 1.0 mmol/kg wet wt after 9 days. At 79% MVC, PCr used, $P_i$ accumulated, and pH at the end of Ex-1–Ex-4 were similar after 4 and 11 days of Cr supplementation. In contrast, PCr utilization and $P_i$ accumulation were lower and pH was higher for exercise at 32% MVC with Cr supplementation, suggesting aerobic resynthesis of PCr was more rapid during exercise. These results suggest that elevating muscle Cr enhances oxidative phosphorylation during mild isometric exercise, where it is expected that oxygen delivery matches demands and predominantly slow-twitch motor units are recruited.

nuclear magnetic resonance; oxidative phosphorylation; skeletal muscle; phosphocreatine; inorganic phosphate

RECENT STUDIES (4, 19, 21, 22, 43) have demonstrated that human muscles retain ~10–35% of the 20–30 g of creatine (Cr) per day supplemented to the habitual diet for 3–6 days, and a recent report showed that 14 days of Cr feeding (3 g/day) raised the total Cr storage in human muscle as much as ingesting doses of 20 g/day for 6 days (22). The enlarged intramuscular total Cr storage after Cr supplementation has been associated with increments in the work performed in one and repeated bouts of intense muscle contractions (3, 4, 9, 10), although no alteration of performance has been observed in some cases (11, 33, 43). The improved muscle performance during repeated exercise after Cr supplementation was suggested to be due to a higher phosphocreatine (PCr) concentration and to an accelerated rate of PCr resynthesis during the recovery periods (4, 10, 19), and there is recent evidence that muscle mass might increase during Cr feeding, which might also play a role in the improvements in muscle work (42, 45). However, a role for Cr as a controller of oxidative phosphorylation, enabling higher rates of oxidative metabolism during muscle contraction, has been basically ignored.

If Cr enhances muscle oxidative phosphorylation during recovery from ischemic exercise, as suggested by the results of Greenhaff et al. (19), enhancement of PCr resynthesis should also occur during the exercise periods after Cr supplementation unless by some unexplained mechanism this physiological event can only occur during recovery from muscular contraction. One important condition for the enhancement of PCr resynthesis during exercise is that there is no limitation of oxygen supply, because the resynthesis of PCr in human muscle appears to occur solely in the presence of oxygen (36). Also, control of oxidative phosphorylation in slow-twitch (ST) and fast-twitch (FT) fibers might be different (25, 31, 32). Recent reports have shown that ADP is restricted in the outer mitochondrial membrane in cardiac myocytes and ST skeletal muscle fibers (37) and that Cr addition enhanced aerobic phosphorylation only in these fibers (26, 46). Therefore, after Cr supplementation, enhancement of PCr resynthesis during aerobic exercise should result in a lesser fall in muscle PCr. These differences should be detected in human muscle during protocols of different recruitment pattern and oxygen availability.

In the present study, the effect of a relatively low Cr dosage (5 g/day) on muscle metabolism was examined with 31P-magnetic resonance spectroscopy (31P-MRS) at rest, during four bouts of isometric plantar flexion at light [32% maximal voluntary contraction (MVC)] and high (79% MVC) intensities, and during recovery after each bout. The 79% MVC protocol was expected to have more restriction of oxygen supply and to recruit relatively fewer ST fibers than would the other because of the higher intensity of the isometric contraction. It was hypothesized that aerobic phosphorylation during the exercise periods would be enhanced after Cr supplementation during light but not intense exercise. The results support this hypothesis, as evidenced by a smaller net utilization of PCr during exercise, and suggest that Cr...
supplementation enhanced aerobic phosphorylation for light exercise where oxygen delivery could match demand in ST fibers being recruited.

METHODS

Subjects. Eight trained male subjects ranging in age from 23 to 30 yr participated in this study. Subjects were engaged in endurance training at least three times a week, and one of them competed in elite handball. The study was approved by the Ethics Committee of the University of Copenhagen. Informed consent was obtained from all subjects after they received a detailed explanation of the procedures and the risks and discomforts of the experiment.

Experimental design. In a preliminary visit to introduce the subjects to the experimental protocols and setup, three maximal voluntary isometric plantar flexion contractions (MVC) of 3-s duration separated by 2 min were performed. The highest value of the three trials was taken as the MVC. After being accustomed to the equipment set up (5), subjects came to the laboratory on several occasions to complete four exercise bouts (Ex-1–Ex-4) of 3-min 20-s duration separated by 4 min of rest. Subjects warmed up for 5 min at 10% MVC followed by 10 min of rest before performing the repeated exercise bouts. The intensity of the exercise was adjusted accordingly in subsequent visits until the target intensity was identified. The aim was for the subjects to endure four exercise bouts, developing the same tension in each bout and reaching near exhaustion by the end of the fourth bout so that they were unable to complete a fifth bout. Similar procedures were followed for the higher intensity protocol, which consisted of four exercise bouts of 40-s duration separated by 2 min of rest. These procedures required at least two to three visits for each protocol.

Once the intensities were identified for each subject, two additional visits before the Cr supplementation period (Bas) were scheduled for each protocol to test the reproducibility of measurements and to rule out a training effect. The two protocols were performed alternatively on separate days with 1–3 days in between and with 4–6 days between the two tests for each protocol. The average load for the high-intensity protocol was 79 ± 4% MVC and that for the low-intensity protocol was 32 ± 1% MVC. During the Cr supplementation period, the 79% MVC protocol was performed on days 4 (Cr-4) and 11 (Cr-11), and the 32% MVC protocol was performed on day 9 (Cr-9). During the exercise bouts, force was recorded by a calibrated strain gauge. Subjects kept force at target level by watching the strain-gauge readout and by continuous feedback from the experimenter. The same duration and tension for each exercise bout were applied, thus keeping the exercise bout as near as possible.

The unsaturated areas for all metabolites were converted to concentrations by assuming average integrated ATP signal for all subjects during the baseline period to correspond to a resting concentration of 5.5 mmol/kg wet wt. Intracellular pH during the exercise periods was calculated from the chemical shift difference of the P<sub>i</sub> peak with respect to the PCr peak (2). The parameters for the time course of PCr resynthesis were determined by fitting the points to a monoeponential growth curve.

Statistics. Values are expressed as means ± SE. Differences between results of the two protocols during the first and second visits of the Bas and between before and after Cr for each protocol were analyzed by repeated-measures analysis of variance. A post hoc Scheffé's F-test was used to analyze any significant differences. Differences were considered significant at the 5% probability level. Because no significant differences between the rates of PCr breakdown and recovery, P<sub>i</sub> accumulation, and pH changes were found between the two baseline visits for either of the protocols, the average of all the values for all parameters for these two visits was taken for comparison with the values during the supplementation period.

RESULTS

Resting muscle metabolite concentrations. Muscle ATP and P<sub>i</sub> remained unchanged during the supplementation period (Table 1). Average resting muscle PCr during baseline increased (P < 0.05 and P < 0.01, respectively) after the 9th and 11th days. Assuming a PCr-to-total Cr (TCr) ratio of 0.65 and 0.61 before and after supplementation, the ratio for PCr at day 9 was 0.9 ± 0.1 and 0.9 ± 0.1, respectively.

Table 1. Effect of oral creatine feeding of 5 g/day on resting muscle ATP, PCr, and P<sub>i</sub> during baseline period and after 4, 9, and 11 days of Cr supplementation

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>PCr</th>
<th>P&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bas</td>
<td>5.5 ± 0.2</td>
<td>18.3 ± 0.9</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Cr-4</td>
<td>5.2 ± 0.3</td>
<td>18.6 ± 0.7</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Cr-9</td>
<td>5.8 ± 0.4</td>
<td>19.6 ± 1.0*</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Cr-11</td>
<td>5.0 ± 0.2</td>
<td>19.5 ± 0.7†</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE given in mmol/kg wet wt. PCr, phosphocreatine; Bas, baseline period; Cr-4, Cr-9, and Cr-11: 4, 9, and 11 days of creatine supplementation, respectively. *Significantly different from Bas, P < 0.05. †Significantly different from Bas, P < 0.01.
after the supplementation period, respectively (values obtained from 84 subjects of Refs. 3, 9, 14, 24; assuming 77% muscle water), the free Cr was calculated to be 9.9 ± 0.5 mmol/wet wt at Bas and 12.5 ± 0.6 and 12.4 ± 0.6 mmol/kg wet wt after Cr-9 and Cr-11, respectively. The calculated increase in TCr amounted to 14% of the resting value.

Muscle metabolism. The ATP changes during exercise were not significantly altered by Cr supplementation in either of the protocols. The dynamic changes of PCr during the exercise periods during the 79% MVC protocol are shown in Fig. 1. The net PCr utilization during Ex-1–Ex-4 (11.9 ± 0.8, 13.9 ± 1.1, 14.9 ± 1.3, and 15.9 ± 1.3 mmol/kg wet wt, respectively) did not change after Cr-4 and Cr-11. However, during the 32% MVC protocol, the net PCr breakdown during Ex-1 and Ex-2 (4.6 ± 0.5 and 7.7 ± 0.8 mmol/kg wet wt, respectively) was significantly lower (P < 0.01) after Cr-9 (3.5 ± 0.6 and 5.7 ± 0.8 mmol/kg wet wt, respectively; Fig. 2). Also, during Ex-3, the net muscle PCr utilized was lower between 50 s and the end of exercise after Cr-9, although the differences were only significant at 72.5 (P < 0.05), 92.5 (P < 0.05), and 172.5 s (P < 0.05) into the exercise. During Ex-4, PCr used was also significantly lower at 92.5 (P < 0.05) and 112.5 s (P < 0.05). After this time, the net PCr utilized was not significantly lower after Cr-9 compared with Bas. During the 79% protocol, the half time of PCr resynthesis was lengthened (P < 0.05) after Cr-11 compared with Bas during recovery from Ex-1 and Ex-4, whereas it was lengthened (P < 0.05) during recovery from Ex-2 after Cr supplementation during the 32% MVC protocol (Tables 2 and 3).

The change in muscle P_i accumulation at the end of the exercise bouts during the 79% MVC protocol (Ex-1, 4.6 ± 0.8; Ex-2, 6.1 ± 1.0; Ex-3, 6.0 ± 0.9; and Ex-4, 6.7 ± 1.0 mmol/kg wet wt) was not significantly altered (Fig. 3). On the other hand, muscle P_i accumulation during the 32% MVC protocol before Cr supplementation of 3.9 ± 0.2 (Ex-1), 5.3 ± 0.7 (Ex-2), 8.8 ± 1.3 (Ex-3), and 11.1 ± 1.3 mmol/kg wet wt (Ex-4) was significantly lower (P < 0.01) at the end of Ex-1, Ex-2, and Ex-3 and tended to be lower (P = 0.06) at the end of Ex-4 after Cr supplementation. The muscle P_i accumulation was significantly lower during a large part of all the exercise periods in this protocol (Fig. 4).

The resting muscle pH did not change during the supplementation period (mean 7.05 ± 0.01; range 7.04–7.06). The pH changes during the four exercise periods at 79% MVC and the end-exercise pH in Bas of 7.03 ± 0.03, 6.99 ± 0.03, 6.98 ± 0.02, and 6.97 ± 0.03 for Ex-1, Ex-2, Ex-3, and Ex-4, respectively, were similar to those observed after Cr-4 and Cr-11 (Fig. 5). The time course of muscle pH during the 32% MVC protocol is shown in Fig. 6. The muscle pH was higher after Cr supplementation at several time points during the exercise periods. Muscle pH values at the end of Ex-1 (6.97 ± 0.03), Ex-2 (6.96 ± 0.02), Ex-3 (6.88 ± 0.03), and Ex-4 (6.76 ± 0.03) were higher after Cr supplementation, with the differences reaching statistical significance (P < 0.05, P < 0.01, and P < 0.05, respectively) with the exception of Ex-2 (P = 0.09).

For further inspection of the kinetic changes of PCr utilization and resynthesis and of P_i and H^+ accumulation, data for each subject were averaged for the four exercise and recovery bouts. Because the responses for Cr-4 were not different from those of Bas, only the data for Bas and Cr-11 during the 79% MVC protocol are

Fig. 1. Muscle phosphocreatine (PCr) during 4 exercise (Ex) periods of 40 s at intensity of 79 ± 4% maximal voluntary contraction (MVC) separated by 2 min of rest before (Bas) and after 4 (Cr-4) and 11 (Cr-11) days of creatine supplementation (5 g/day). Values are means ± SE.
shown for clarity. The average PCr utilization for Ex-1–Ex-4 did not change after Cr-11 compared with Bas (Fig. 7A). However, the half time of PCr resynthesis was lengthened \((p < 0.01)\) after Cr-11 \((18.4 \pm 1.1 \text{ vs. } 22.3 \pm 1.4 \text{ s})\). The analysis revealed that the amount of PCr resynthesized was not significantly altered up until \(30 \text{ s}\) of recovery \((8.8 \pm 0.7 \text{ vs. } 7.8 \pm 0.8 \text{ mmol/kg wet wt in Bas and Cr-11, respectively})\). However, the amount of PCr resynthesized from \(30 \text{ to } 120 \text{ s}\) was larger \((p < 0.05)\) after Cr-11 \((4.0 \pm 0.4 \text{ vs. } 4.6 \pm 0.4 \text{ mmol/kg wet wt})\). The average rate of Pi accumulation for Ex-1–Ex-4 up to \(120 \text{ s}\) of contraction was \(0.127 \pm 0.021 \text{ in Bas and } 0.183 \pm 0.030 \text{ mmol·kg wet wt}^{-1} \cdot \text{s}^{-1}\) after Cr-11, and during the last \(20 \text{ s}\) of contraction it was \(0.171 \pm 0.028 \text{ and } 0.204 \pm 0.034 \text{ mmol·kg wet wt}^{-1} \cdot \text{s}^{-1}\) in Bas and Cr-11, respectively. Pi was not significantly changed (Fig. 9A).

Averaging the values for Ex-1–Ex-4 during the 32% MVC protocol revealed that the PCr degradation from 0 to \(120 \text{ s}\) was lower \((p < 0.05)\) after Cr supplementation \((5.2 \pm 0.7 \text{ vs. } 4.0 \pm 0.5 \text{ mmol/kg wet wt})\). The net degradation from \(120 \text{ s}\) to the end of exercise was not different \((4.4 \pm 0.5 \text{ vs. } 4.1 \pm 0.5 \text{ mmol/kg wet wt}; \text{Fig. 7B})\). The average half time of PCr resynthesis did not change significantly after Cr supplementation \((18.4 \pm 0.9 \text{ vs. } 17.4 \pm 1.1 \text{ s in Bas and Cr-9, respectively})\). The average rate of Pi accumulation for Ex-1–Ex-4 up to \(32\%\) MVC was also lower \((p < 0.01)\) after Cr-9 \((0.016 \pm 0.006 \text{ mmol·kg wet wt}^{-1} \cdot \text{s}^{-1}; \text{Fig. 8B})\). The rate of Pi accumulation from \(120 \text{ s}\) to the end of exercise tended to be lower \((p = 0.06)\) after Cr-9 \((0.048 \pm 0.006 \text{ vs. } 0.033 \pm 0.006 \text{ mmol·kg wet wt}^{-1} \cdot \text{s}^{-1})\). The rates of H+ buffered \((0–50 \text{ s})\) and accumulation \((50–120 \text{ s})\) to \(120 \text{ s}\) did not change after Cr-9, but the rate of H+ accumulation from \(120 \text{ s}\) until the end of contraction was significantly lower \((p < 0.01)\) after Cr-9 \((0.41 \pm 0.08 \text{ vs. } 0.22 \pm 0.09 \text{ nM/s}; \text{Fig. 9B})\).

**DISCUSSION**

The main aims of this study were to investigate the effects of Cr supplementation at a daily dose of 5 g on resting muscle metabolites and muscle energy metabolism during repeated exercise of different intensities. It was hypothesized that an effect of Cr on aerobic metabolism during moderate-intensity exercise would be observed. However, the results did not support this hypothesis. There were no significant changes in muscle PCr, ATP, or Pi levels in response to Cr supplementation. The average rate of PCr resynthesis and degradation did not change significantly after Cr supplementation. The rate of Pi accumulation during moderate-intensity exercise was not different between Bas and Cr-9. The rate of H+ accumulation was also not different between Bas and Cr-9.

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**Table 2. Half time of PCr resynthesis during recovery periods after 4 isometric plantar flexion exercise bouts at an intensity of 79% MVC at Bas and after Cr-4 and Cr-11**

<table>
<thead>
<tr>
<th></th>
<th>Bas</th>
<th>Cr-4</th>
<th>Cr-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rec-1</td>
<td>20.4±1.7</td>
<td>17.5±1.3</td>
<td>26.6±2.3*</td>
</tr>
<tr>
<td>Rec-2</td>
<td>18.3±0.8</td>
<td>20.0±1.8</td>
<td>20.2±1.3</td>
</tr>
<tr>
<td>Rec-3</td>
<td>19.1±1.7</td>
<td>16.9±1.8</td>
<td>20.9±1.5</td>
</tr>
<tr>
<td>Rec-4</td>
<td>17.1±1.4</td>
<td>16.3±1.1</td>
<td>23.3±2.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE given in s. Rec, recovery period; MVC, maximal voluntary contraction. *Significantly different from Bas, P < 0.05.

**Table 3. Half time of PCr resynthesis during recovery periods after 4 isometric plantar flexion exercise bouts at an intensity of 32% MVC at Bas and after Cr-9**

<table>
<thead>
<tr>
<th></th>
<th>Bas</th>
<th>Cr-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rec-1</td>
<td>16.8±0.9</td>
<td>15.9±1.9</td>
</tr>
<tr>
<td>Rec-2</td>
<td>13.2±1.3</td>
<td>17.0±1.6*</td>
</tr>
<tr>
<td>Rec-3</td>
<td>19.4±1.0</td>
<td>16.1±1.6</td>
</tr>
<tr>
<td>Rec-4</td>
<td>21.2±1.4</td>
<td>17.5±1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE given in s. *Significantly different from Bas, P < 0.05.
Lactate would occur during the exercise periods for light but not for heavy isometric exercise. The results support this hypothesis, as evidenced by the lower PCR breakdown, P_i accumulation, and pH drop in the lower intensity protocol after Cr feeding.

During the Cr supplementation period, the ATP values remained unchanged, which is in agreement with the results of other studies (4, 10, 13, 19, 21, 22, 43). Despite the comparatively low daily dose of Cr given in the present study, intramuscular PCR increased 1.3 mmol/kg wet wt after 9 days of Cr feeding. It is also very likely that in this study the muscle free Cr increased more than PCR did, as has been demonstrated in all the recent studies (4, 10, 13, 19, 21, 22, 43). Calculations showed that, after Cr supplementation, ~2.5 mmol/kg wet wt of free Cr were deposited in the muscle examined. Assuming 30 kg muscle mass and the same uptake in all muscles as occurred in the

Fig. 3. Muscle P_i during 4 Ex periods of 40 s at intensity of 79 ± 4% MVC separated by 2 min of rest at Bas and after Cr-4 and Cr-11. Values are means ± SE. * Significant difference in net P_i accumulation by that point in time between Bas and Cr-4, P < 0.05.

Fig. 4. Muscle P_i during 4 Ex periods of 200 s at intensity of 32 ± 1% MVC separated by 4 min of rest at Bas and after Cr-9. Significant difference in the net P_i accumulation by that point in time during exercise between Bas and Cr-9: #P < 0.01; *P < 0.05.
gastrocnemius, ∼16 g of Cr would be retained by the 11th day, which corresponded to 29% of the dose given. Studies have shown that 10–35% of the Cr supplement is retained for doses ranging from 3 to 30 g during periods of up to 44 days (4, 10, 13, 19, 21, 22, 35, 43). It is clear from these experiments that Cr entry into and retention by muscle can be accomplished by different regimens, but a large part of the Cr supplement is lost. Human muscle cells take up Cr from blood by a Na\(^{+}\)-dependent and Na\(^{+}\)-independent specific transport process (27). The Na\(^{+}\)-dependent transport is downregulated by physiological plasma Cr (27), which might be a limitation when very high doses are given for a prolonged period. Insulin (23), exercise (21), vitamin E (15), and high-carbohydrate feeding have been shown to have a positive influence on the uptake of Cr in muscle (16, 17). The subjects’ diet and activity were not recorded in the present experiment, but they were requested to keep the same activity and food intake patterns during the course of the study. Nevertheless, the significant gain in muscle PCr of ∼7% indicates the supplementation was successful.

The effect of Cr supplementation on muscle energy metabolism was investigated noninvasively during two
protocols of repeated isometric contraction: one at high intensity (79% of MVC) and another at low intensity (32% of MVC). During high-intensity isometric contraction, a large number of fibers (both ST and FT) must be recruited, and above 50–70% of MVC, a large reduction in blood flow during exercise is expected. For instance, during contraction of the quadriceps at 50% MVC, lower blood flow and muscle oxygen uptake were observed compared with contraction at 25% MVC (14). Furthermore, endurance time of the quadriceps muscle during contraction at 67% of MVC was the same with or without circulatory occlusion (12), indicating that above ~67% MVC local ischemia occurs. Because PCr resynthesis is dependent on adequate blood flow and oxygen delivery (36), Cr supplementation would not be expected to enhance PCr resynthesis during exercise at 79% MVC where such a limitation is likely to occur. The findings at this high intensity are consistent with this hypothesis. That is, the PCr decrease, P_i accumulation, and pH change during the four exercise bouts at Bas were not different from those after Cr-4 and Cr-11. In contrast, the 32% MVC protocol recruits a fewer num-

Fig. 7. Average muscle PCr of 4 Ex and recovery bouts at the intensities of 79 ± 4% (A) and 32 ± 1% of MVC (B) at Bas and after Cr-11 (A) and Cr-9 (B). Values are means ± SE. *Significant (P < 0.05) increase of half time of PCr resynthesis in 79%MVC protocol (A) and decrease in net PCr breakdown until 120 s in 32%MVC protocol (B) after Cr supplementation.

Fig. 8. Average muscle P_i of 4 four Ex and recovery bouts at intensities of 79 ± 4% (A) and 32 ± 1% of MVC (B) at Bas and after Cr-11 (A) and Cr-9 (B). Values are means ± SE. # Significant decrease of rate of P_i accumulation until 120 s in 32%MVC protocol after Cr supplementation, P < 0.01.
ber of fibers, causing significantly less restriction of blood flow and muscle oxygenation (14). Supplementation, in this case, is expected to enhance the resynthesis of PCr, thereby reducing the PCr loss, P\(_i\) accumulation, and pH fall during light exercise as found in the present study.

It has been shown that the rate of PCr degradation of rat and human muscle follows a monoexponential decay during submaximal isotonic exercise of different intensities (28, 29). In the present study, PCr degradation during isometric exercise at 32\% MVC was more complex. Up to \(-120\) s of exercise, the PCr decline followed a monoexponential pattern, but from then on its decay was linear. Furthermore, the data showed a progressive tendency for the monoexponential decay to be linear with the number of bouts. Cr supplementation tended to attenuate this pattern. The explanation for this complex response currently is unknown. A possibility is a progressive change in the type and number of muscle fibers recruited during light isometric exercise as fatigue develops. At low exercise intensities, there is an orderly recruitment of ST, FTa, and FTb fibers as time progresses to fatigue (47). The recruitment of a larger number of FT fibers with time and in repeated bouts could have produced a progressive limitation in blood flow and oxygen delivery to working muscle, thereby changing PCr kinetics from monoexponential to a linear dynamic. Experiments on isolated mitochondria have shown that PCr decreases linearly when mitochondrial respiration exhausts the oxygen available and oxidative phosphorylation becomes oxygen limited (20). Alterations in H\(^+\) per se do not appear to be involved. The finding that Cr supplementation affected the net PCr utilization and P\(_i\) accumulation but did not affect pH during the monoexponential decay period excludes any effect of H\(^+\) on the kinetics of PCr during this time. Furthermore, H\(^+\) accumulation was lower from 120 to 200 s after Cr supplementation, suggesting that the rate of H\(^+\) production through anaerobic glycolysis was lower, but the PCr degradation rate was not changed by supplementation during this later period of low-intensity exercise. In other studies, Balsom et al. (4) found a lower muscle lactate accumulation at the end of repeated 6-s bouts of supramaximal cycling, whereas Febbraio et al. (13) found no change in glycogen degradation or lactate accumulation after five 1-min bouts after Cr supplementation. These controversial findings would need to be clarified in future research. Thus it is proposed that, whereas the oxygen delivery was not impeded and a larger relative proportion of ST fibers was recruited, oxidative phosphorylation during the exercise periods was likely enhanced after Cr supplementation.

Contrary to the present suggestion of an increment in oxidative phosphorylation during the exercise periods, Balsom et al. (4) proposed that the lower net PCr breakdown at the end of repeated bouts of maximal exercise was due to higher resynthesis of PCr during the recovery periods as demonstrated by Greenhaff et al. (19) during recovery from ischemic exercise. However, other results obtained in Greenhaff’s laboratory with \(^{31}\)P-MRS and biochemical analysis have shown that the amount of PCr resynthesized during recovery from ischemic and nonischemic exercise in tibialis and vastus lateralis, respectively, did not change after Cr supplementation (10, 18), whereas no explanation for the discrepant results has been given (10). Also, Balsom et al. (4) measured PCr from muscle biopsies obtained only before the first bout and after the fifth bout, and the recovery periods between exercise bouts were 30 s, whereas the results of Greenhaff et al. (19) showed no change in the rate of PCr resynthesis during the first minute of recovery. A recent study showed that the amount of PCr resynthesized during 2 min of recovery...
HUMAN MUSCLE ENERGETICS DURING EXERCISE AFTER Cr FEEDING

The mechanism by which oxidative phosphorylation in human muscle might have been increased after Cr supplementation might not differ from that of animal muscle. The enhancement of respiration by Cr in muscle was first observed by Thunberg and supported by findings of Katz (in Ref. 8) and was strengthened by the observations of Belitser and Tsybakova (6). The phenomenon was proposed to occur via CK in the mitochondrial intermembrane space (Mit-CK) and a Cr-PCr shuttle, which, by some mechanism yet undiscovered, carried Cr to mitochondria and provided PCr to myofibrils, sarclemma, and sarcoplasmic reticulum where the energy is needed during muscle contraction (7, 8, 38). Later investigations on skeletal muscle cell cultures and cardiomyocytes also confirmed that additional Cr increased the rate of oxidative phosphorylation and highlighted the role of the CK system in the transport of intracellular energy from mitochondria to myofibrils and other sites of energy utilization (8, 37, 38, 41). In cardiomyocytes, the higher respiration rates and higher rates of PCr production observed after Cr addition were suggested to be caused by an amplification of the sensitivity of respiration to ADP (K_m: 300 µM before Cr vs. 36 µM after Cr) (40) because of the coupling of Mit-CK, adenine nucleotide translocase, and oxidative phosphorylation (37). In skeletal muscle, differences in regulation of oxygen consumption in FT and ST muscle were first suggested by Meyer et al. (31) and Kushmerick et al. (25). Meyer et al. (31) proposed that perhaps the mitochondria in the two fiber types were not the same. It was later reported that ADP was restricted in the outer mitochondrial membrane in cardiac myocytes and ST skeletal muscle fibers (37) and that this restriction could be the explanation for the controlling role of Cr on oxidative phosphorylation in these fibers and not in FT fibers (26, 46). Meyer and Foley (32) have also reported differences in the regulation of oxygen consumption in ST and FT fibers. Moreover, the Mit-CK of the human gastrocnemius muscle has been shown to qualitatively and kinetically resemble that of heart muscle (1, 40). The M-CK activity is also higher in human trained vs. untrained muscle (1) and higher in ST compared with FT muscle (48). Therefore, there is a higher likelihood to observe an effect of Cr on oxidative phosphorylation during exercise demanding larger relative involvement of ST fibers in highly active humans. The present results show that this was likely the case. In contrast, after assuming that the increase in TCr concentration after Cr supplementation was larger in FT fibers, Casey et al. (10) proposed increased mitochondrial ATP production in this fiber type (10). However, their own data do not support larger increases in TCr concentration in FT fibers after Cr supplementation (Table 2 in Ref. 10). Also, results from animal muscle showed that depletion of up to 90% of TCr had no effect on aerobic metabolism in FT fibers (30), and it was suggested that in FT fibers the CK system acts as a simple buffer of adenine nucleotide levels (29, 30). Furthermore, in support of the contention that the effect of Cr on oxidative metabolism is confined to the ST fibers are the results of a
recent report that showed a correlation between the percentage of ST fibers and the enhancement of oxidative phosphorylation after Cr was added to human skinned fibers obtained after exercise (44). An additional or synergistic possibility is the proposed regulation of PCR/Cr on AMP-activated protein kinase (AMPK) as the control mechanism of CK in muscle (34). According to this novel mechanism, during the 32% MVC protocol a larger decrease in PCR/Cr during exercise after Cr feeding would have inhibited PCR breakdown and enhanced oxidation of fatty acids by the dual control of AMPK inhibiting MM CK isofrom and acetyl-CoA carboxylase, the latter eventually causing a drop in malonyl-CoA relieving the inhibition of carnitine palmitoyltransferase I. The possible ischemic conditions during the exercise periods of the 79% MVC protocol would have prevented oxidative phosphorylation from taking place. However, because PCR/Cr was lower after Cr feeding, particularly during the latter phase of recovery, this might have enhanced the oxidation of fatty acids and thereby increase the rate of PCR resynthesis. Possible differences in the control of AMPK in ST and FT fibers by PCR/Cr remain to be discovered, which might add to explain further the role of Cr in human muscle energy metabolism.

In conclusion, the results of this study demonstrate that adding a daily dose of 5 g Cr to the habitual diet increases muscle PCR in 9 days. Net muscle PCR utilization, P, accumulation, and pH drop were reduced during repeated bouts of relative low-intensity exercise leading to exhaustion after Cr supplementation, indicating that muscle oxidative phosphorylation during contraction is increased by Cr supplementation. This did not occur at high-intensity exercise. This difference is likely the result of better matching of oxygen delivery with demand and greater recruitment of ST fibers.

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