Parallel Increases in Phosphocreatine and Total Creatine in Human Vastus Lateralis Muscle During Creatine Supplementation

Jeffrey J. Brault, Theodore F. Towse, Jill M. Slade, and Ronald A. Meyer

Short-term creatine supplementation is reported to result in a decreased ratio of phosphocreatine (PCr) to total creatine (TCr) in human skeletal muscle at rest. Assuming equilibrium of the creatine kinase reaction, this decrease in PCr:TCr implies increased cytoplasmic ADP and decreased Gibbs free energy of ATP hydrolysis in muscle, which seems contrary to the reported ergogenic benefits of creatine supplementation. This study measured changes in PCr and TCr in vastus lateralis muscle of adult men (N = 6, 21–35 y old) during and 1 day after 5 d of creatine monohydrate supplementation (0.43 g·kg body weight$^{-1}$·d$^{-1}$) using noninvasive $^{31}$P and $^1$H magnetic-resonance spectroscopy (MRS). Plasma and red-blood-cell creatine increased by 10-fold and 2-fold, respectively, by the third day of supplementation. MRS-measured skeletal muscle PCr and TCr increased linearly and in parallel throughout the 5 d, and there was no significant difference in the percentage increase in muscle PCr (11.7% ± 2.3% after 5 d) vs. TCr (14.9% ± 4.1%) at any time point. The results indicate that creatine supplementation does not alter the PCr:TCr ratio, and hence the cytoplasmic Gibbs free energy of ATP hydrolysis, in human skeletal muscle at rest.

Key Words: creatine kinase, energetics, magnetic-resonance spectroscopy

Creatine monohydrate, referred to simply as creatine, is a popular dietary supplement for amateur and professional athletes for its reported ergogenic effects. Short-term oral creatine supplementation can increase skeletal-muscle total creatine (TCr = phosphocreatine [PCr] + creatine) by up to 30% and concomitantly improve exercise performance in various repetitive, burst-type tasks (2, 36). The beneficial exercise effects are presumably a result of increased intracellular PCr, which regenerates ATP during muscle contraction via the creatine kinase (CK) reaction (PCr + ADP + H$^+$ ↔ creatine + ATP). Because of the near-equilibrium nature of CK (21) and because of adequate phosphate availability (1) relative to creatine uptake rates (5), any increase in cellular TCr concentration might be expected to result in a proportional increase in PCr. In fact, the maintenance of muscle PCr:TCr
or PCr:creatine ratio has been shown repeatedly in studies of rodents fed excess creatine (3, 9, 16, 23, 27).

In contrast to rodent muscle, in human muscle PCr and creatine do not appear to increase proportionally with creatine supplementation. Instead, analyses of perchloric-acid extracts of muscle biopsies show that TCr content increases substantially more than PCr content in most (11, 12, 15, 22, 29, 38), though not all (10), human studies. For example, after 5–7 d of creatine-hydrate supplementation at 20 g/d, TCr measured by biopsy of vastus lateralis muscle increased by 17.0–20.1%, whereas PCr increased by only 7.5–9.2% (11, 14, 15, 22, 29), on average a greater than 2-fold difference. This decrease in the PCr:TCr ratio during creatine supplementation has been suggested to play a role in initiating the cellular adaptations reported in muscle after creatine supplementation (8, 26). On the other hand, it has been argued that if CK is at equilibrium, a decreased PCr:TCr ratio would require an increase in cytoplasmic ADP concentration and a decrease in cytoplasmic free energy of ATP hydrolysis (ΔG_{ATP}) (23). A decrease in the free energy available to perform muscle work seems inconsistent with the widely reported, although somewhat variable, ergogenic benefits of creatine (2, 19, 36).

The discrepancy between the “expected” and measured PCr:TCr ratios of creatine-supplemented human muscle could have at least 3 explanations. First, a sampling or freezing artifact that degrades PCr to creatine (4, 34) might be enhanced by creatine supplementation. Second, creatine supplementation might promote binding or compartmentation of creatine and, therefore, increase creatine independent of PCr. Third, creatine supplementation might alter the energetic balance in human muscle and, therefore, lead to a true fall in muscle cytoplasmic ΔG_{ATP}. The purpose of this study was to examine this third possibility by measuring changes in PCr and TCr in human vastus lateralis muscle during short-term creatine supplementation using noninvasive, high-resolution magnetic-resonance spectroscopy (MRS), which only detects soluble, metabolically active metabolites (25). The results show that, just as in rodent muscle, PCr and TCr increase to the same relative extent in human muscle during creatine supplementation.

Methods

Subjects
Six recreationally active male subjects participated in this study. They were 28 ± 6 y old (range 21–35) and 176 ± 5 cm tall and weighed 81 ± 9 kg. None of the subjects were vegetarians or had taken creatine supplements within the preceding year. All subjects provided informed, written consent before participation in the study, which was approved by the Michigan State University Committee on Research Involving Human Subjects.

Study Design
Subjects consumed powdered creatine monohydrate (GNC Pro Performance, 0.43 g·kg body weight^{-1}·d^{-1}) for 5 d. The creatine was provided as 5 individually packaged daily doses, and each daily dose was consumed in 4 equal aliquots, dissolved in the liquid of the subject’s choice, evenly spaced throughout the day. All subjects
reported completing all of the given doses with no adverse side effects. Subjects maintained their normal physical activity and dietary habits. \( ^1 \)H and \( ^31 \)P magnetic-resonance spectra were acquired from the left vastus lateralis muscle immediately before (day 0), during (days 1, 2, 3, and 5), and 1 d after (day 6) creatine ingestion. Testing for each subject was done at the same time each evening. Blood samples were collected immediately before the MRS sessions on days 0, 3, and 6.

**MRS Measurements**

Spectra were acquired on a 3T GE Excite system (GE Medical Systems, Milwaukee, WI) with subjects resting supine. \( ^31 \)P spectra (51.7 MHz, sweep width 2500 Hz, 2K complex data, TR 8 s, nominal 30° pulse at coil center, average of 40 scans) were acquired via an 8 × 5 cm elliptical transmit/receive surface coil placed on the belly of the vastus lateralis. Proton spectra (127.8 MHz, sweep width 5000 Hz, 2K data, TR 2 s, TE 35 ms, average of 128 scans) were acquired via a clinical flex coil from a 3 × 2 × 2 cm PRESS voxel (the 3-D equivalent of a pixel) centered under the phosphorous coil. After the coils were positioned during the first MRS session, their location on the leg and the position of the leg relative to the magnet’s landmark lights were recorded by pen markings on the subject’s skin, to ensure consistent positioning of the coil on the leg and positioning of the leg within the scanner for subsequent scans. Phosphorous spectra were zero-filled to 4K complex data and filtered with a 10-Hz exponential, and PCr and ATP peak areas were integrated between fixed PPM limits using custom-written software. Percentage changes in PCr and Pi were measured from the percentage change in the ratio of those peak areas to the mean of the 3 ATP peak areas. Proton spectra were zero-filled to 4K, filtered with a 2-Hz exponential, and resolution enhanced with a Gaussian function. Percentage changes in TCr were measured from the percentage change in the ratio of the TCr methyl peak area to the trimethylamine (TMA) peak area. Intracellular pH was estimated from the chemical shift of the inorganic phosphate peak relative to PCr (25).

**Blood Measurements**

Blood was drawn from the median cubital vein directly into 6-mL K\(_2\)-EDTA Vacutainer tubes (Becton Dickinson), mixed, and immediately put on ice. Tubes were centrifuged, and then the packed red blood cells (RBCs) and plasma were separated to different tubes. There was no visible hemolysis. Metabolites were extracted from the RBC and plasma fractions using 4.5% (vol/vol) cold perchloric acid and then neutralized with KOH and triethanolamine. Extracts were stored at –20 °C until analysis, which was within 1 wk. Creatine concentrations were determined using cation-exchange high-performance liquid chromatography (39).

**Statistics**

Blood plasma and red-cell creatine concentrations, Pi:ATP, and intracellular pH were compared across days by 1-way ANOVA, followed by Tukey’s honestly significant difference procedure. Relative changes in PCr:ATP versus TCr:TMA over time were compared by a repeated-measures 2-factor (metabolite vs. time)
general linear model. Statistics were calculated with SPSS software (SPSS Inc., Chicago, IL) at the \( P < 0.05 \) level of significance.

**Results**

Oral creatine supplementation resulted in significant increases in both plasma and RBC creatine content (Table 1). After 3 d of supplementation, plasma creatine increased 10-fold and RBC creatine increased more than 2-fold. Approximately 24 h after the final creatine dose, plasma creatine had returned to presupplementation level, whereas RBC creatine remained elevated. The increase and then decrease in plasma creatine confirms subject compliance in taking the supplements.

Consistent with elevated RBC creatine content, both PCr and TCr increased in skeletal muscle with oral creatine supplementation. Illustrative \(^3^P\) (for PCr) and \(^1^H\) (for TCr) spectra acquired from 1 subject over the entire 6-d protocol are shown in Figure 1. To better illustrate the changes in PCr and TCr after 5 d, downfield portions of the day 0 and day 5 spectra from the same individual as in Figure 1 are shown side by side (with a modest PPM offset) in Figure 2. PCr and TCr increased in all subjects by day 5, although the changes were somewhat less in other subjects (e.g., Figure 2, lower half).

Mean percentage changes in PCr:ATP and TCr:TMA for all subjects are shown in Figure 3. Both PCr:ATP and TCr:TMA increased nearly linearly and in parallel during the supplementation period (time effect, \( P = 0.03 \)). After 5 d, PCr had increased by 11.7% ± 2.3% (range 4–20%) and total creatine had increased 14.9% ± 4.1% (range 4–29%). There was no significant difference between the percentage changes in PCr and the percentage changes in TCr (metabolite effect, \( P = 0.84 \); time–metabolite interaction, \( P = 0.94 \)) and hence no evidence for a decreased ratio of PCr to TCr during the supplementation. Furthermore, creatine supplementation had no significant effect on Pi:ATP (0.52 ± 0.15, mean ± standard error, \( N = 6 \)) or on intracellular pH (7.05 ± 0.2).

**Discussion**

The major finding of this study is that MRS-measured PCr and TCr in human muscle increase in parallel during 5 d of oral creatine supplementation. The increase in muscle TCr concentration reported here is consistent in magnitude with findings of previous studies of creatine supplementation using MRS (20, 37) and chemical analysis of muscle biopsies (11, 12, 14, 15, 22, 29, 38). Furthermore, the increases

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Plasma and Red-Cell Creatine Concentrations (Mean ± Standard Error, ( N = 6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Plasma creatine (mmol/L)</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Red-cell creatine (mmol/L)</td>
<td>0.37 ± 0.03</td>
</tr>
</tbody>
</table>

*Significantly different than day 0, \( P < 0.05 \).
Figure 1 — Representative $^{31}$P (left) and $^1$H spectra from vastus lateralis muscle of a subject before (day 0), during (days 1–5), and after (day 6) creatine supplementation.
Figure 2 — Top: downfield portions of day 0 and day 5 $^{31}$P (left) and $^1$H (right) spectra from same subject as in Figure 1. Day 5 spectra are shifted to the right to illustrate the change in PCr and TCr peaks. Bottom: same regions of spectra from another subject in whom the changes in PCr and TCr were less than in the subject illustrated above.

Figure 3 — Mean (± standard error, $N = 6$) percentage changes in PCr:ATP and TCr:TMA ratios throughout the study period. On day 0 the PCr:ATP and TCr:TMA ratios were 4.15 ± 0.11 and 1.16 ± 0.07, respectively.
in plasma and RBC creatine also match those in studies in which blood was tested (14, 29). Therefore, this study replicated conditions that have previously resulted in a 2-fold larger increase in TCr than in PCr, as measured by biopsy of human vastus lateralis muscle (11, 12, 15, 22, 29, 38). Our results show no significant difference, however, in the percentage increase in MRS-measured, metabolically active TCr and PCr during short-term creatine supplementation.

The MRS measurements performed in this study yield only relative changes in TCr and PCr, not absolute concentrations. This is not a limitation, however, for estimating changes in cytoplasmic \( \Delta G_{\text{ATP}} \). Assuming equilibrium of the CK reaction, the cytoplasmic \( \Delta G_{\text{ATP}} \) depends on the creatine:PCr concentration ratio according to

\[
\Delta G_{\text{ATP}} = -RT \ln(K_c \times \text{Pi} \times \text{creatine/PCr})
\]

where \( K_c \) is a lumped constant that includes the equilibrium constants for ATP hydrolysis and the CK reaction, as well as the pH dependence of these reactions (see reference 13, equation 12). TCr is just the sum of PCr and free creatine, so

\[
\Delta G_{\text{ATP}} = -RT \ln[K_c \times \text{Pi} \times (\text{TCr} – \text{PCr})/\text{PCr}]
\]

Clearly, if TCr and PCr change by the same relative amount (e.g., by 10% or 1.1-fold) the creatine:PCr ratio does not change:

\[
[(1.1 \times \text{TCr}) – (1.1 \times \text{PCr})]/1.1 \times \text{PCr} = (\text{TCr} – \text{PCr})/\text{PCr} = \text{creatine/PCr}
\]

Thus, if Pi, pH, and TCr:PCr are all constant, as observed in this study during creatine supplementation, then \( \Delta G_{\text{ATP}} \) is also constant. We conclude that, just as in rodent muscle (23), oral creatine supplementation does not significantly alter the cytoplasmic energetic state in human muscle. Kan et al. (17) recently came to the same conclusion using a completely different spectroscopic approach based on ingestion of \(^{13}\text{C}\)-labeled creatine.

A potential limitation of the MRS measurements used in this study is that, to account for day-to-day variations in instrument tuning and spectral signal to noise, the PCr and TCr peak areas were normalized to ATP and TMA peak areas, respectively. Of course, this assumes that muscle ATP and TMA contents are not altered by short-term creatine supplementation. Previous biopsy studies have shown that ATP is not altered by creatine loading (11, 15, 38), and ATP is very commonly used as a constant reference peak in \(^{31}\text{P}\)-NMR studies (18). Therefore the normalization of PCr to ATP in this study seems justified. The TMA peak in skeletal muscle is primarily caused by carnitine and glycerophosphocholine (30). Total carnitine content is tightly regulated in human muscle, for example, only changing by 15% after 5 h of intravenous infusion of carnitine to a plasma concentration 10-fold above normal (35). Glycerophosphocholine and other choline-containing compounds can vary between muscles of different individuals (32), but there is no evidence that their concentrations change significantly from day to day in healthy subjects. Thus, although we cannot rule out the possibility that the TMA peak increased over 5 d to precisely the extent needed to keep the PCr:TCr ratio constant, this does seem unlikely.

A likely explanation for the discrepancy between our and Kan et al.’s (17) noninvasive results versus previous biopsy results is hydrolysis of PCr to creatine
Muscle Creatine During Creatine Supplementation

during the biopsy procedures (4, 34). Artifactual PCr hydrolysis can occur as a result of muscle damage during either collection (34) or freezing (24) of muscle biopsies. This artifactual hydrolysis might simply be enhanced in muscles with higher PCr content. In addition, creatine-supplemented muscles have been shown to increase in size within a few days (6, 41), and these swollen muscles might be more easily damaged during biopsy. Thus, it might be that creatine-supplemented muscle is more susceptible to a PCr hydrolysis artifact during biopsy.

A second possible explanation of the discrepancy between our noninvasive result and previous biopsy results is the presence of a bound-creatine pool that has no access to CK and is not seen by MRS. Because tightly bound metabolites are not visible in high-resolution spectra (25) but would be released in perchloric-acid muscle extracts, the PCr:creatine ratio would then appear lower in muscle extracts. The magnitude of the percentage increase in total creatine observed in this study, however, is not substantially lower than observed in the biopsy studies, so it appears unlikely the MRS is missing a large bound pool of creatine. Furthermore, any high-affinity binding sites for creatine would be expected to saturate early in the course of creatine supplementations, yet no rapid accumulation of creatine is observed by biopsy after only 1 d of supplementation (38).

It should be noted that both MRS and biopsy measurements of global creatine and PCr can be confounded by variations in creatine and PCr content in different compartments. For example, if there were a cellular or subcellular compartment with a lower PCr:TCr ratio, and if this compartment preferentially accumulated creatine during supplementation compared with other compartments with a higher PCr:TCr ratio, the globally measured PCr:TCr ratio would fall during creatine supplementation. Similarly, if blood and extracellular fluid, which contain negligible PCr content, were a substantial fraction of the sampled material, the increase in creatine would tend to exceed the increase in PCr during supplementation. Blood and extracellular fluid could not significantly contribute to these measurements, however, in healthy, intact muscle. Using liberal estimates of vastus lateralis extracellular space as 10% (31), RBC volume in whole muscle as 1%, muscle TCr content as 40 mM, and the plasma creatine contents as measured here, the RBCs plus extracellular creatine would only represent 0.2% of total creatine content.

A second finding of this study is the nearly linear TCr (and PCr) accumulation over 5 d of creatine supplementation. This finding in muscle is in stark contrast to plasma creatine concentration, which increases manyfold within hours (28). As demonstrated herein, plasma creatine increases 10-fold by our earliest measure, day 3, but muscle creatine has increased less than 10% at that point. This is consistent with findings that the accumulation of muscle creatine via the creatine transporter is near saturation at normal plasma creatine concentration (33). Although some have attempted to determine the time course of creatine accumulation by urine analysis (7, 14, 15), this is complicated by the fact that humans synthesize creatine in the liver, a process that might be down-regulated by creatine supplementation. Furthermore, creatine can be lost in the feces (40), and uptake occurs disproportionately quickly into other nonmuscle and nonneuronal tissues (16), both of which overestimate the initial creatine accumulation in skeletal muscle if using urine analysis. Although based on muscle-biopsy studies (14, 15), we would not expect muscle TCr or PCr to increase substantially more if we were to supplement for longer than 5 d. This could be examined in subsequent studies.
In summary, this study shows that MRS-measured, freely soluble PCr and TCr increase in parallel in human vastus lateralis muscle during short-term creatine supplementation and, thus, that creatine supplementation does not alter the energetic state in resting human muscle. The disproportionate increases in creatine and TCr compared with PCr reported in previous studies might be explained by artifactual hydrolysis of PCr during the biopsy procedures.

Acknowledgment

This study was supported by NIH AR043903.

References


