Skeletal Muscle Phosphocreatine Depletion Depresses Myocellular Energy Status During Sepsis

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Objective: To determine the effects of phosphocreatine (PCr) depletion on myocellular energetics.

Design: Randomized controlled study.

Setting: University laboratory.

Materials: Thirty-eight adult male Wistar rats (110-121 g).

Methods: The poorly metabolized creatine analogue β-guanidinopropionic acid, (β-GPA, 2% of a gel diet) was fed to the rats for 14 days to replace (75%) endogenous PCr stores before cecal ligation and puncture (CLP). Rats were randomized to receive sham operation and gel diet (sham-gel group [n = 10]), sham operation and β-GPA diet (sham–β-GPA group [n = 9]), CLP and gel diet (CLP–gel group [n = 10]), and CLP and β-GPA diet (CLP–β-GPA group [n = 9]). On day 14, all animals underwent operation. Twenty-four hours later, in vivo phosphorus 31–labeled magnetic resonance spectroscopy (31P-MRS) of the gastrocnemius muscle was performed. Muscle samples were collected to determine enzyme activities of β-hydroxyacyl-CoA dehydrogenase, phosphofructokinase, citrate synthase, and the metabolites adenosine triphosphate (ATP), PCr, inorganic phosphate, and creatine. Free adenosine diphosphate levels, the phosphorylation potential, and free energy change of ATP hydrolysis were then calculated.

Results: All animals undergoing CLP but no controls had positive results of blood cultures. Although sham–β-GPA animals had altered bioenergetics, CLP–β-GPA rats experienced a greater deterioration of energy state compared with CLP-gel controls. Glycolytic and oxidative enzyme activities were not significantly different between groups and therefore could not explain the observed differences.

Conclusions: There is an overall decrease in energy availability during sepsis, which is worsened by PCr depletion. These changes support the contention that PCr plays an important role as an ATP buffer during systemic infection.

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Sepsis is the primary cause of morbidity and mortality in intensive care units. Skeletal muscle comprises 50% to 60% of the total body mass and thus is the largest organ affected by systemic infection. As such, it is quantitatively and metabolically one of the most important tissues in the body.

Skeletal muscle energy metabolism is rapidly altered during sepsis. Previous investigations have shown that changes in high-energy phosphate metabolism occur early in the course of infection. Prominent among these changes is an increase in the consumption of adenosine triphosphate (ATP) to provide fuel for sodium and potassium ATPase and other metabolic processes. Under normal conditions, myocytes obtain ATP from glycolysis, mitochondrial oxidative phosphorylation, and the breakdown of phosphocreatine (PCr). The rate of ATP in regeneration from PCr breakdown is very rapid, exceeding ATP use and ATP replenishment by oxidative phosphorylation and glycolysis. Within 24 hours of cecal ligation and puncture (CLP) in rodents, a 20% decrease in PCr stores is observed in the resting gastrocnemius muscle in association with a 20% increase in forward flux thought to compensate for an increase in ATP consumption induced by systemic infection. In the clinical setting, ATP and PCr levels are decreased in critically ill patients. Despite these observations, the role of PCr as an energy buffer during stress remains mainly unanswered and controversial.

The creatine (Cr) analog β-guanidinopropionic acid (β-GPA) can be used to deplete tissue Cr and PCr levels. It reduces uptake of Cr across cell mem-
branes by competitively inhibiting the Cr transporter. In addition, β-GPA and its phosphorylated counterpart P-β-GPA are poor substrates for creatine kinase (CK). The maximum velocity for β-GPA through the CK pathway is only 0.3% of that for Cr, whereas the maximum velocity for P-β-GPA is 0.1% of that for PCr.

In our study, β-GPA was administered to Wistar rats to replace myocellular Cr and PCr to study the roles of these compounds and the CK reaction in myocellular energy metabolism during sepsis. In vivo phosphorus 31–labeled magnetic resonance spectroscopy (31P-MRS) was used to measure tissue levels of adenine diphosphate (ADP), ATP, PCr, and inorganic phosphate (Pi). Our objective was to determine the effect of PCr depletion on skeletal muscle bioenergetics. Our data support the hypothesis that PCr plays an important role in cellular energy metabolism during systemic infection.
and 51%, and Cr concentrations were reduced by 26% analyses showed that PCr concentrations were reduced by PCr levels in the sham– and CLP–GPA peaks could be readily identified. At 15 days, is shown in the Figure. At 6 days of 10% (n = 1) in the CLP–operation died, whereas the 24-hour mortality rate was A typical31P-MRS spectra of resting gastrocnemius muscle produces data equivalent to those obtained using traditional methods.9

The absolute value of ΔGATP depends on an estimation of the standard change in free energy, which is dependent on pH, free magnesium2+, and temperature. Thus, a value of −36.27 kJ/mol was used as the standard change in free energy, which according to Mijan de la Torre et al11 closely approximates our KCK value and pH at 37°C. 

The phosphorylation potential (PP) was calculated according to the following formula:

$$PP = \frac{ATP}{ADP}$$

The free energy change of ATP hydrolysis (ΔGATP) was calculated using the following formula:

$$ΔG_{ATP} = ΔG^\circ + 2.58 \ln \left( \frac{ADP + Pi}{ATP} \right)$$

where ln indicates natural log.

Substituting the right side of the first equation for ADP yields the following:

$$ΔG_{ATP} = ΔG^\circ + 2.58 \ln \left( \frac{Cr}{PCr} \right) \times K_{CK}$$

The activity of each enzyme was expressed in millimoles per gww of muscle at 37°C. Muscle protein content was determined using a modified Lowry technique (Diagnost, Microprotein determination, Procedure 690, Sigma Chemical Co). All samples were stored at −80°C until the assays were performed.

In vitro assays

Immediately after 31P-MRS scanning on day 15, gastrocnemius muscle tissue from the scanned leg was rapidly freeze-clamped with liquid nitrogen-chilled metal tongs and immediately submerged in liquid nitrogen. From these tissues, the concentrations of ATP, PCr, Pi, and Cr were measured after perchloric acid extraction using standard enzymatic methods.14 The gastrocnemius muscle from the opposite limb was then frozen in liquid nitrogen without clamping for determination of β-hydroxyacyl-CoA dehydrogenase (BHACD, a measure of fatty acid oxidation), citrate synthase (CS, the rate-limiting enzyme of the citric acid cycle), and phosphofructokinase (PFK, the rate-limiting enzyme of glycolysis) enzyme activities. This tissue was homogenized in potassium phosphate buffer and 2-mercaptoethanol for the CS assay and in phosphate buffer for the PFK and BHACD assays. Enzyme activities were determined spectrophotometrically as follows: (1) CS activity was measured according to Bulletin C-3260 (Sigma Chemical Co); (2) BHACD activity was measured according to Bulletin H-3516 (Sigma Chemical Co); and (3) PFK activity was measured using the modified method of Shonk and Boxer.15 The activity of each substrate was expressed in millimoles per gww of muscle at 37°C. Muscle protein content was determined using a modified Lowry technique (Diagnost, Microprotein determination, Procedure 690, Sigma Chemical Co). All samples were stored at −80°C until the assays were performed.

Bacterial cultures

After gastrocnemius muscle tissue was harvested, a peritoneal fluid sample was cultured on blood agar plates (tryptic case soy agar with 5% sheep blood, Becton-Dickinson Microbiology Systems, Cockeysville, Md), and a right-ventricle blood sample was instilled into aerobic blood culture bottles (tryptic soy broth with sodium polyanetholesulfonate and carbon dioxide, Becton-Dickinson Microbiology Systems). Culture plates and bottles were incubated at 37°C, and results were noted after 24 and 48 hours.

Statistical analyses

Results are expressed as the mean ± SEM. Data were analyzed using 1- and 2-way analysis of variance (ANOVA) (STATISTICA for PCs, version 4, StatSoft Inc, Tulsa, Okla). Post hoc comparisons were performed using the Fisher least significant different test. P values of less than or equal to .05 were considered statistically significant. P values of .05 to .10 were considered significant trends.

Effect of β-GPA Administration

A typical31P-MRS spectra of resting gastrocnemius muscle is shown in the Figure. At 6 days of β-GPA feeding, the β-GPA peaks could be readily identified. At 15 days, β-GPA was the predominant phosphagen and had replaced 75% of PCr levels in the sham– and CLP–β-GPA groups. In vitro analyses showed that PCr concentrations were reduced by 56% and 51%, and Cr concentrations were reduced by 26% and 18%, in the sham– and CLP–β-GPA groups, respectively, compared with their gel-fed counterparts (Table 1).

Changes in ATP and Pi Levels, Enzyme Activities, and Protein Content

Two weeks of β-GPA feeding decreased gastrocnemius muscle ATP levels, whereas CLP had no effect. Levels of Pi were not changed by the administration of β-GPA. However, in gel-fed rats, CLP induced a statistically significant 26% increase in Pi level (Table 1).

Twenty-four hours after operation, CS activity was elevated in the CLP-gel group (56.9 ± 11 mmol/g of wet weight per minute), reaching statistical significance, compared with the sham-gel animals (35.8 ± 3.7 mmol/g of

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wet weight per minute). There was no significant β-GPA feeding effect on CS activity (sham–β-GPA group, 40.4 ± 4.7 mmol/g of wet weight per minute; CLP–β-GPA group, 46.5 ± 11 mmol/g of wet weight per minute), nor were BHACD and PFK activities different between groups. In addition, muscle protein contents did not vary significantly, with protein levels ranging from 205 ± 22 to 236 ± 17 mg/g of wet weight.

### IN VIVO 31P-MRS INDEXES

As anticipated, β-GPA feeding alone significantly altered myocellular bioenergetics, inducing a 61% reduction in PCr-ATP ratios in animals undergoing sham operation and CLP (Table 2). The CLP effects were less pronounced, with the PCr-ATP ratios declining by 8% in gel-fed rats and 10% in β-GPA–fed animals. Changes in PCr-Pi ratio followed a similar pattern whereby β-GPA feeding induced a 70% fall in this measure of thermodynamic capacity. In this instance, the PCr-Pi ratio was significantly lower in CLP-gel rats compared with sham-gel animals, whereas CLP–β-GPA rats were not significantly different from their counterparts undergoing sham operation. Although the magnitude of the CLP effect was similar between gel- and β-GPA–fed groups, CLP–β-GPA and sham–β-GPA groups were not significantly different from each other. Intracellular pH measurements remained within normal limits in all rats and were not significantly different between groups.

The β-GPA feeding resulted in significantly higher free ADP levels in animals undergoing sham operation and CLP (Table 3). There was no significant CLP effect in the gel-fed rats; however, in β-GPA–fed animals, CLP resulted in significantly elevated ADP levels. Moreover, this rise was higher than that of any other group. Feeding with β-GPA resulted in significantly lower PP values in animals undergoing sham operation and CLP. In gel-fed animals, CLP resulted in significant PP decline of 42%; in β-GPA–fed animals, a 47% drop that trended toward significance (P = .07). Analysis of the changes in ΔG_{ATP} revealed a similar pattern, with β-GPA feeding alone reducing ΔG_{ATP} in animals undergoing sham operation to a degree similar to that induced by CLP. The percentage decrease in this index relative to sham–β-GPA controls was equivalent to the fall evident when comparing CLP-gel animals with sham-gel rats. Values for PP and ΔG_{ATP} were lowest in the CLP–β-GPA group.

### COMMENT

Previous studies have shown that PCr breakdown is accelerated during early sepsis to maintain ATP levels, providing fuel for diverse cellular processes. We undertook this study to examine the effect of PCr depletion by β-GPA administration on myocellular energetics during sepsis.

Phosphocreatine does not seem to be essential for aerobic metabolism or for maintaining skeletal muscle ATP levels during muscle stimulation at low workloads. However, during severe metabolic stress such as occurs during sepsis, there is an additional requirement for ATP production that is not met by glycolysis or oxidative phosphorylation. High concentrations of Cr and PCr may be essential to maintain ATP levels during increased metabolic demand, such as occurs during running, thyrotoxicosis, and perhaps even cancer cachexia.

Weight loss is characteristic of β-GPA administration, as has been reported previously. In our study, after an initial decline, β-GPA–fed rats gained weight at a rate equivalent to that of gel-fed controls. At harvest, β-GPA–fed animals were slightly but significantly smaller, but their muscle protein levels were equivalent to those of gel-fed animals. Thus, the contribution of changes in nutritional status to the experimental results was likely insignificant.

In vivo 31P-MRS scans demonstrated that P-β-GPA became the dominant intracellular phosphagen, replacing more than 70% of normal PCr stores after 2 weeks of feeding. Greater replacement occurs with longer periods of β-GPA administration. The percentage of decrease in PCr measured in vitro was similar in magnitude to that observed in vivo, although the concentrations were lower. The PCr levels obtained using 31P-MRS are typically higher than those obtained using

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### Table 1. In Vitro Metabolite Concentrations*

<table>
<thead>
<tr>
<th>Metabolites, µmol/gww</th>
<th>Groups Undergoing Sham Operation</th>
<th>Groups Undergoing CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel-Fed (n = 10)</td>
<td>β-GPA–Fed (n = 9)</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>7.15 ± 0.15</td>
<td>4.67 ± 0.19†</td>
</tr>
<tr>
<td><strong>PCr</strong></td>
<td>25.6 ± 0.87</td>
<td>11.1 ± 1.9§</td>
</tr>
<tr>
<td><strong>Cr</strong></td>
<td>9.80 ± 0.76</td>
<td>7.22 ± 0.32†</td>
</tr>
<tr>
<td><strong>Pi</strong></td>
<td>5.47 ± 0.54</td>
<td>4.94 ± 0.31†</td>
</tr>
</tbody>
</table>

*CLP indicates cecal ligation and puncture; gww, gram of wet weight; β-GPA, β-guanidinopropionic acid; ATP, adenosine triphosphate; PCr, phosphocreatine; Cr, creatine; and Pi, inorganic phosphate. Groups are described in the “Study Protocol” subsection of the “Materials and Methods” section.

†P < .05 vs gel-fed animals.

‡P = .06 vs CLP-gel group, 2-way analysis of variance and post hoc least significant difference.

§P < .05 vs animals undergoing sham operation and CLP–β-GPA group.
Compared with controls, the ATP levels were greatly decreased in both β-GPA–fed and CLP-β-GPA animals. This accumulation likely reflects increased ATP consumption because skeletal muscle has a lower oxidative capacity for aerobic ATP production than other tissues such as the heart, brain, or kidney. An important role of PCr may be to maintain myocellular ADP levels in a range where they may help regulate mitochondrial respiration, glycogenolysis, and glycolysis.

Intracellular free ADP concentrations were greatly increased in the gastrocnemius muscle of septic rats fed β-GPA. This accumulation likely reflects increased ATP consumption induced by systemic infection in the presence of decreased ADP rephosphorylation secondary to PCr depletion and the inefficient phosphate donor capacity of P-β-GPA. Changes in intracellular free ADP mediate the balance between ATP oxidative production and consumption because skeletal muscle has a lower overall oxidative capacity for aerobic ATP production than other tissues such as the heart, brain, or kidney. An important role of PCr may be to maintain myocellular ADP levels in a range where they may help regulate mitochondrial respiration, glycogenolysis, and glycolysis.

### Table 2. High-Energy Phosphate Ratios, Intracellular pH, and β-GPA Replacement

<table>
<thead>
<tr>
<th></th>
<th>Groups Undergoing Sham Operation</th>
<th>Groups Undergoing CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel-Fed (n = 10)</td>
<td>β-GPA-Fed (n = 9)</td>
</tr>
<tr>
<td>PCR-ATP ratio</td>
<td>2.59 ± 0.05</td>
<td>1.02 ± 0.14†</td>
</tr>
<tr>
<td>PCr-Pi ratio</td>
<td>14.24 ± 1.00§</td>
<td>4.30 ± 0.64†</td>
</tr>
<tr>
<td>Intracellular pH</td>
<td>7.16 ± 0.02</td>
<td>7.17 ± 0.20</td>
</tr>
<tr>
<td>β-GPA replacement, %</td>
<td>...</td>
<td>75.00 ± 0.03</td>
</tr>
</tbody>
</table>

*Abbreviations are given in the first footnote to Table 1. Groups are described in the “Study Protocol” subsection of the “Materials and Methods” section. Ellipses indicate not applicable.

†P < .05 vs gel-fed animals.
‡P < .05 vs sham-gel group, 2-way analysis of variance and least significant difference.
§P < .05 vs β-GPA–fed and CLP-gel animals.
∥P < .05 vs gel-fed animals.
¶P < .05 vs sham-gel group, 2-way analysis of variance and least significant difference.

### Table 3. Phosphorus 31–Labeled Magnetic Resonance Spectroscopy–Derived Indexes

<table>
<thead>
<tr>
<th></th>
<th>Groups Undergoing Sham Operation</th>
<th>Groups Undergoing CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gel-Fed (n = 10)</td>
<td>β-GPA-Fed (n = 9)</td>
</tr>
<tr>
<td>Free ADP, µmol/gww</td>
<td>51.7 ± 4.3</td>
<td>91.4 ± 9.1†</td>
</tr>
<tr>
<td>Phosphorylation potential</td>
<td>112.9 ± 12.6§</td>
<td>49.2 ± 6.4</td>
</tr>
<tr>
<td>∆GATP, kJ/mol</td>
<td>−66.5 ± 0.3§</td>
<td>−64.4 ± 0.3</td>
</tr>
</tbody>
</table>

*ADP indicates adenosine diphosphate; ∆GATP, free energy change of ATP hydrolysis. Other abbreviations are given in the first footnote to Table 1. Groups are described in the “Study Protocol” subsection of the “Materials and Methods” section.

†P < .05 vs gel-fed animals.
‡P < .05 vs sham-gel group.
§P < .05 vs β-GPA–fed animals and animals undergoing CLP.
∥P < .05 vs animals undergoing sham operation and CLP-gel group.
¶P < .05 vs sham-gel group, 2-way analysis of variance and least significant difference.
#P < .06 vs sham-β-GPA group, 2-way analysis of variance and least significant difference.
PK activity and decreases in BHACD activity, these were associated with long-term β-GPA administration. In our investigation, these adaptive changes may not yet have occurred after 2 weeks of β-GPA feeding when there was only 70% PCr depletion, further highlighting the potential significance of the metabolic changes observed.

Due to the critical roles that Cr, PCr, and the enzyme CK are believed to play in energy metabolism, the effects of Cr supplementation on muscle function are being actively investigated. Oral Cr supplementation is known to increase muscle Cr and PCr content, as well as the rate of PCr resynthesis after muscle activity in normal human adults. Supplementation also appears to increase body mass and improve muscle strength and endurance by 10% to 20%. Although skeletal muscle Cr content in patients with heart failure is decreased metabolic demand and suggest that PCr plays an important role in the control of myocellular energy status during sepsis.

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