PAPER

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 (^{31}P-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.[1] 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.[2] 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.[3] In the clinical setting, ATP and PCr levels are decreased in critically ill patients.[4] 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-

MATERIALS AND METHODS

ANIMAL PREPARATION

The animals were cared for in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals of the Institute for Laboratory Animal Resources, the National Research Council, and the protocol was approved by the Animal Care Committee of the Harvard Medical School, Boston, Mass. Virus-free male Wistar rats were purchased at a weight of 110 to 121 g (Harlan Sprague Dawley, Inc, Indianapolis, Ind) and allowed to acclimatize for at least 3 days. They were housed at a constant temperature of 22°C, with 12-hour periods of light and dark exposure. During acclimatization standard, rat chow and tap water were allowed ad libitum.

STUDY PROTOCOL

Rats were randomized to 1 of the following 4 study groups: (1) sham operation and gel diet (sham-gel group [n = 10]); (2) sham operation and 2% β-GPA added to the gel diet (sham–β-GPA group [n = 9]); (3) CLP and gel diet (CLP-gel group [n = 10]); and (4) CLP and 2% β-GPA added to the gel diet (CLP–β-GPA group [n = 9]) and individually housed in metabolic cages. Daily food intake and body weights were recorded. On day 6, ^{31}P-MRS of the gastrocnemius muscle was performed on all animals to verify the replacement of PCr by β-GPA. On day 14, all animals underwent CLP or sham operation. Twenty-four hours later (day 15), ^{31}P-MRS scanning was repeated, and blood and muscle samples were harvested.

DIET PREPARATION AND ADMINISTRATION

A 2% β-GPA gel diet was prepared by combining 27.5 g of agar and 472.5 g of gel diet (Basal gel diet, F0927, Bioserve, Frenchtown, NJ) in 1 L of boiled distilled water. The liquid gel preparation was then cooled, and 10 g of β-GPA (Sigma Chemical Co, St Louis, Mo) was added. This last step was taken as a precaution against β-GPA high-temperature inactivation. The control group received the same gel diet without β-GPA. The gel diets were stored at –20°C until use. Animals were provided adequate calories (approximately 60 g/d per rat) for weight gain, and water was provided ad libitum.

CECAL LIGATION AND PUNCTURE

On day 14, animals underwent anesthetization using intraperitoneal pentobarbital sodium, 50 mg/kg, followed by sham operation or CLP. The CLP procedure was performed as described by Wichterman et al.[8] Briefly, the abdomen was shaved and scrubbed with povidone-iodine solution, and the peritoneal cavity was entered through a 3-cm lower midline abdominal incision. Peritonitis and sepsis were induced by nonobstructively ligating the cecum 1.9 cm from its proximal extent and puncturing it twice with a sterile 18-gauge needle along the antimesenteric border. Six milliliters of isotonic sodium chloride solution per 100 g of body weight was then instilled into the peritoneal cavity. The abdominal wall and skin incisions were closed using 3-0 silk and polyglactin 910 (Vicryl) sutures, respectively. In rats undergoing sham operation, the peritoneal cavity was entered; the cecum was exteriorized, manipulated, and returned to the peritoneal cavity; and the animals were resuscitated using the same procedure. After operation, all animals were housed individually and allowed free access to water but not to food, and 24-hour mortality rates were recorded.

^{31}P-MRS PROCEDURE AND INDEXES

On days 6 and 15, each rat was anesthetized with intraperitoneal pentobarbital sodium, 50 mg/kg, and placed in an aluminum probe machine-tooled to fit an 8.45-T magnet (79 mm, Nicolet 360, Nicolet Instruments, Madison, Wis). Within the probe, the gastrocnemius muscle was securely positioned over a 1.4-cm 2-turn surface coil. The magnetic field was then homogenized by maximizing the sodium signal at 95.3 MHz using a shim supply (Oxford Instruments, Oxford, England). Line widths of less than 95 MHz were routinely obtained. The coil was then tuned to 145.75 MHz to acquire the phosphorus spectra using a sweep width of ±8000 MHz, quadrature detection, and 4000 data points per scan. Sixty-four scans were signal averaged after 15-microsecond broad band pulses with 3-second interpulse delays. The summed scans were multiplied by an exponential function corresponding to 5-Hz line broadening before Fourier analysis to enhance visualization of the β-GPA peaks. The peak areas of Pi, PCr, β-GPA, and β-ATP (**Figure**) were measured from curve-fitted spectra (Nuclear Magnetic Reso-

Continued on next page

branes by competitively inhibiting the Cr transporter.[5,6] 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.[7]

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 (^{31}P-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.

RESULTS

BODY WEIGHT CHANGES AND BACTERIAL CULTURES

Although the β-GPA–fed animals initially lost weight, the rate at which they gained weight after 4 days was equivalent to that of animals fed the control diet. At the end of the 15-day study, the mean body weight of the β-GPA–fed rats was lower than that of controls (238 ± 4.3 vs 220 ± 6.1 g; $P<.05$ by 1-way ANOVA, LSD). All animals undergoing CLP, but not those undergoing sham opera-

nance 1, Schnectady, NY) and used to calculate PCr-ATP (a measure of energy reserves) and PCr-Pi (a measure of thermodynamic capacity) ratios. Intracellular pH (pH_i) was measured in vivo from the chemical shift of Pi relative to PCr. This method accurately and noninvasively measures hydrogen ion (H^+) concentration and produces data equivalent to those obtained using traditional methods.[9]

Free ADP concentrations were calculated from the CK equilibrium, using an assumed equilibrium constant, K_{CK}, of $1.66 \times 10^9 \cdot M^{-1}$, the PCr-ATP ratio, the pH_i derived from the ^{31}P-MRS scans, and the Cr concentration determined spectrophotometrically according to the following formula:

$$ADP = (ATP \times Cr)/(PCr \times H^+ \times K_{CK})$$

The ADP concentration was converted to micromoles using a value of 0.65 g of unbound cell water per gram of wet weight (gww) for blood-perfused skeletal muscle.[10] The contribution of β-GPA and phosphorylated β-GPA to calculated ADP levels and K_{CK} was assumed to be negligible.

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

$$PP = ATP/(ADP \times Pi)$$

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

$$\Delta G_{ATP} = -\Delta G° + 2.58 \ln (ADP \times Pi)/ATP,$$

where ln indicates natural log.

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

$$\Delta G_{ATP} = -\Delta G° + 2.58 \ln (Cr \times Pi)/(PCr \times H^+) \times K_{CK}$$

The absolute value of ΔG_{ATP} depends on an estimation of the standard change in free energy, which is dependent on pH, free magnesium^{2+}, 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 al[11] closely approximates our K_{CK} value and pH at 37°C.

IN VITRO ASSAYS

Immediately after ^{31}P-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.[12] 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.[13] 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 (Diagnostics, Microprotein determination, Procedure 690, Sigma Chemical Co).[14] 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 (trypticase 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.

tion had positive results of peritoneal and blood cultures at 24 and 48 hours. No rats undergoing sham operation died, whereas the 24-hour mortality rate was 10% (n = 1) in the CLP–β-GPA group and 0% in the CLP-gel group.

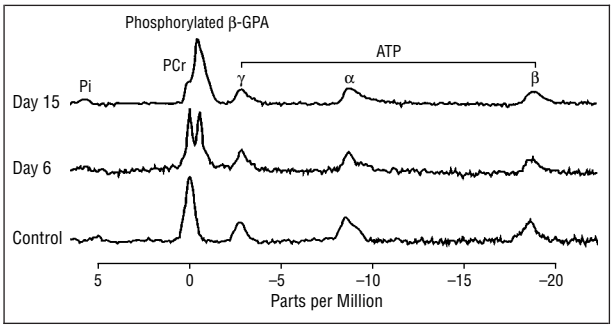
EFFECT OF β-GPA ADMINISTRATION

A typical ^{31}P-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



Results of in vivo phosphorus 31–labeled magnetic resonance spectroscopy of gastrocnemius muscle illustrating the following peaks: inorganic phosphate (Pi); phosphocreatine (PCr); and γ-, α-, and β-adenosine triphosphate (ATP) at baseline and after 6 and 15 days of β-guanidinopropionic acid (β-GPA) feeding. Note the new β-GPA peak downfield from the PCr peak (day 6), which replaces 75% of the PCr peak (day 15) in the upper spectra.

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 ^{31}P-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.

Table 1. In Vitro Metabolite Concentrations*

Metabolites, μmol/gww	Groups Undergoing Sham Operation		Groups Undergoing CLP	
	Gel-Fed (n = 10)	β-GPA–Fed (n = 9)	Gel-Fed (n = 10)	β-GPA–Fed (n = 9)
ATP	7.15 ± 0.15	4.67 ± 0.19†	7.37 ± 0.35	5.10 ± 0.25†
PCr	25.6 ± 0.87	11.1 ± 1.9†	25.23 ± 1.2	12.3 ± 2.5†
Cr	9.80 ± 0.78	7.22 ± 0.32†	10.43 ± 0.93	8.47 ± 0.69‡
Pi	5.47 ± 0.54	4.94 ± 0.31	6.89 ± 0.62§	5.42 ± 0.36

**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.

COMMENT

Previous studies have shown that PCr breakdown is accelerated during early sepsis to maintain ATP levels, providing fuel for diverse cellular processes.[3] 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.[15] 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,[6] thyrotoxicosis,[16] and perhaps even cancer cachexia.[17]

Weight loss is characteristic of β-GPA administration, as has been reported previously.[18,19] 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 ^{31}P-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 ^{31}P-MRS are typically higher than those obtained using

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

	Groups Undergoing Sham Operation		Groups Undergoing CLP	
	Gel-Fed (n = 10)	β-GPA–Fed (n = 9)	Gel-Fed (n = 10)	β-GPA–Fed (n = 7)
PCr-ATP ratio	2.59 ± 0.05	1.02 ± 0.14†	2.38 ± 0.04‡	0.92 ± 0.12†
PCr-Pi ratio	14.24 ± 1.00§	4.30 ± 0.64†	9.58 ± 0.49	2.98 ± 0.51†
Intracellular pH	7.18 ± 0.02	7.17 ± 0.20	7.19 ± 0.01	7.17 ± 0.01
β-GPA replacement, %‖	. . .	75.00 ± 0.03	. . .	75 ± 0.04

*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 = .07 vs sham-gel group, 2-way analysis of variance and least significant difference.
§P<.05 vs β-GPA–fed and CLP-gel animals.
‖Analyzed using 1-way analysis of variance.

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

	Groups Undergoing Sham Operation		Groups Undergoing CLP	
	Gel-Fed (n = 10)	β-GPA–Fed (n = 9)	Gel-Fed (n = 8)	β-GPA–Fed (n = 7)
Free ADP, μmol/gww	51.7 ± 4.3	91.4 ± 9.1†	63.6 ± 5.3	135.3 ± 20.0†‡
Phosphorylation potential	112.9 ± 12.6§	49.2 ± 6.4	66.2 ± 6.0	26.2 ± 3.7‖¶
ΔG_{ATP}, kJ/mol	−66.5 ± 0.3§	−64.4 ± 0.3	−65.2 ± 0.3#	−62.7 ± 0.4‖

*ADP indicates adenosine diphosphate; ΔG_{ATP}, 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–β-GPA group.
§P<.05 vs β-GPA–fed animals and animals undergoing CLP.
‖P<.05 vs animals undergoing sham operation and CLP-gel group.
¶P = .07 vs sham–β-GPA 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.

enzymatic methods.[20] In previous reports, PCr concentrations typically decreased by 90%, whereas Cr levels were 50% lower in β-GPA–fed rats compared with chow-fed controls.[15,16,18] Observed changes in PCr and Cr levels in our study were similar proportionally but not as profound, likely due to differences in the duration of β-GPA feeding.

Creatine concentrations in CLP–β-GPA rats were relatively higher than those measured in sham-gel controls. This is likely due to the accelerated PCr consumption that occurs with systemic infection[3] in the presence of inhibited rephosphorylation. The ATP levels were similarly decreased in both β-GPA–fed groups, as has been reported previously.

Changes in PCr-ATP ratios induced by CLP were not as large as those previously obtained in our laboratory[3] and reported in the literature,[8] and the effect of CLP on these ratios was not modified by the administration of β-GPA. Although all rats subjected to CLP had positive results of blood and peritoneal cultures, mortality rates in this group were lower than those reported in the literature. We therefore suspect that the lethality of the model was altered by the administration of the gel diet and β-GPA. Cultures obtained from all 4 groups of rats had fewer colonies than those plated from chow-fed animals (data not shown). Moreover, preliminary analyses of quantitative stool culture data suggest that the number and pathogenicity of bacteria are reduced in animals fed gel diets or β-GPA compared with animals that receive standard laboratory rat chow.

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.[2] 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.[21]

Septic animals with low levels of PCr also had lower PP and ΔG_{ATP}. These data suggest that cellular energy status is impaired in sepsis, and this impairment is exacerbated by PCr depletion. Cell energy status is a critical index of cellular function and regulates a variety of metabolic processes, including the distribution of water across cellular membranes, pH_i, and energy production.

Except for an increase in CS activity in the CLP-gel group compared with the sham-gel group, no other changes in enzyme activity were observed. Accordingly, skeletal muscle ATP production via anaerobic glycolysis, the citric acid cycle, and β-oxidation of fatty acids was not increased as a consequence of PCr depletion in rats undergoing sham operation or with sepsis. Although other investigators have reported increases in

PFK activity and decreases in BHACD activity, these were associated with long-term β-GPA administration.[18,22] 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,[23] as well as the rate of PCr resynthesis after muscle activity in normal human adults.[24] Supplementation also appears to increase body mass and improve muscle exercise performance[25,26] and may be important for diseased tissues. For example, despite apparently normal skeletal muscle Cr content in patients with heart failure, 1 week of Cr supplementation increased muscle strength and endurance by 10% to 20%.[25] Although these data are suggestive, key questions to be answered include whether Cr supplementation can improve or support other physiologic processes besides contraction and whether Cr supplementation is beneficial during systemic infection or other times when metabolic demands are increased.

We showed that muscle bioenergetics are disproportionately altered in septic animals depleted of PCr compared with controls. The findings are consistent with the hypothesis that PCr stores are used to buffer ATP levels and modulate free ADP levels during periods of increased metabolic demand and suggest that PCr plays an important role in the control of myocellular energy status during sepsis.

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