White Paper for Cyclocreatine

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Background

Creatine [N-(aminoiminomethyl)-N-methyl glycine or methylglycocyamine] is a nonessential, amino-acid-like dietary compound found in small quantities within the brain, liver, kidneys, and testes, while approximately 95% of creatine stores are found in skeletal muscle [1]. Creatine is naturally ingested through omnivorous diets, with the greatest natural quantity of creatine present in red meats, and to a lesser extent in fish. Moreover, creatine is endogenously synthesized from the amino acids arginine, glycine, and methionine [2]. Additionally, creatine can be orally ingested as a dietary supplement. The most commonly used forms of supplemental creatine are hydrates, salts, esters, and alcohols. Creatine is a vital molecule necessary for muscle contraction because energy is provided to the body from the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (Pi). Figure 1 illustrates that the phosphagen system provides a rapid re-synthesis of ATP from ADP with the use of phosphocreatine (PCr) through the reversible reaction of creatine kinase [2,3,4]. Of the 95% of creatine stored within skeletal muscle, approximately 40% is free creatine and approximately 60% is PCr [3]. The average 70 kg (155 pounds) person has a total creatine pool of 120-140 grams. Specifically, the range of creatine in skeletal muscle is 110-160 mmol/kg dry mass [1,2,5].
Some forms of creatine undergo phosphorylation at higher rates than others. As an example, most creatine salts, esters and alcohols are phosphorylated in cells at a rate of about 75%, leaving about 25% in its free form. Rats, chicks, and mice fed cyclocreatine [6], a synthetic analogue of creatine [7], accumulate large amounts of phosphorylated cyclocreatine (1-carboxymethyl-2-imino-3-phosphonoimidazolidine) in skeletal muscle. Heart muscle from rats and chicks fed cyclocreatine were shown to be less depleted in phosphorylated cyclocreatine and ATP than creatine [8], and have less delayed onset of rigor relative to control animals [9], thereby suggesting that cyclocreatine might aid heart muscle and other tissues in sustaining ATP levels during prolonged metabolic and/or ischemic stress. Interestingly, it has been shown in chick skeletal muscle that under favorable conditions 98% of intracellular cyclocreatine was phosphorylated compared to 70% for creatine. Furthermore, cyclocreatine was shown to delay the utilization of glycogen and
onset of rigor in ischemic skeletal muscle [10]. These results indicate the importance of sustaining intramuscular levels of PCr on sustaining intramuscular glycogen levels and bioenergetics PCr capacity, thereby prolonging skeletal muscle contractile capacity.

**Rationale for Creatine Supplementation in Sport and Exercise**

Creatine and PCr serve as both an intracellular buffer for ATP and an energy shuttle for the movement of high-energy phosphates from mitochondrial sites of production to cytoplasmic sites of utilization. The spontaneous loss of creatine and of PCr to creatinine requires that creatine be continuously replaced, which can only occur by a combination of diet and endogenous synthesis. The endogenous synthesis of creatine makes major demands on the metabolism of glycine, arginine, and methionine. Large doses of creatine supplements are widely taken, particularly by athletes, as an ergogenic means of improving muscular performance.

The major rationale of creatine supplementation relative to exercise and athletic performance is to maximally increase the intracellular pool of total creatine (creatine + PCr). This is based on the premise that the intracellular concentration of PCr plays a significant role during the immediate bio-energetic system, which is most active during exercise at high intensity, short duration, and repeated bouts of exercise. Through the depletion of intracellular PCr stores, the intracellular concentration of ATP is maintained and replenished. This occurs via a freely reversible reaction in which PCr phosphorylates ADP to replenish ATP stores, catalyzed via the enzyme creatine kinase. PCr levels within the muscle are almost 3 to 4 times more abundant than intramuscular ATP stores. While the concentration of PCr is more abundant than ATP, the rate in which ATP is utilized is likely to exceed the overall energy substrate regeneration necessary at activities of high intensity. However, the PCr supply is sufficient in providing a temporary ATP source until other bio-energetic systems reach maximal rates. Assuming the total creatine pool to be full, the level of intramuscular creatine is considered to be saturated at 160 mmol/kg dry mass; however, only 20% of users achieve this amount and another 20-30% do not respond to creatine supplementation at all [1]. Nevertheless, creatine supplementation can provide a way in which to help maintain an elevation in the intramuscular pool of total creatine.
Research Supporting the Effectiveness and Efficacy of Cyclocreatine

1. Ability of cyclocreatine to increase total skeletal muscle phosphorylated cyclocreatine:

Accumulation of analog of phosphocreatine in muscle of chicks fed 1-carboxymethyl-2-iminoimidazolidine (cyclocreatine).

Newly-hatched chicks were fed a commercial diet containing 1% cyclo-creatine and the breast muscle was shown to rapidly accumulate a derivative of cyclocreatine within a few days. Interestingly, during this time the concentration of total creatine declined during the accumulation of cyclocreatine. When chicks fed cyclocreatine for 6 days were restored to the control diet containing no cyclocreatine, it was found that cyclocreatine persisted in breast muscle, and the creatine level was restored to the normal value (Figure 2). Cyclocreatine was also taken up by rat muscle, heart, and brain. Specific to chick breast muscle, however, the data suggested the derivative to be N-phosphorylated cyclocreatine. Under favorable conditions, the derivative reacted in vitro with the specific reagents, creatine kinase and MgADP, to yield cyclocreatine. Essentially all of breast muscle cyclocreatine appeared to be in the form of phosphorylated cyclocreatine, which persisted in muscle long after cyclocreatine was removed from the diet. Long term conservation of phosphorylated cyclocreatine in muscle was aided by the fact that, unlike PCr, phosphorylated cyclocreatine was not continuously degraded to an inactive cyclic lactam. It was suggested that the maximal phosphorylated cyclocreatine (and PCr) attained in sarcoplasm would not only affect the phosphorylation potential of muscle cells, but also account for more than half of the normal inorganic cation concentration of muscle sarcoplasm, and hence play an important role in muscle function. Griffiths G. Walker J. Accumulation of analog of phosphocreatine in muscle of chicks fed 1-carboxymethyl-2-iminoimidazolidine (cyclocreatine). Journal of Biological Chemistry. 251(1):2049-54,1976.

The level of creatine kinase of Ehrlich ascites tumor cells grown intraperitoneally in mice is only 0.4% of mouse leg muscle, and these cells have no appreciable pool of PCr. However, Ehrlich cells grown for 10 days in mice fed 1% cyclocreatine increased PCr at a level exceeding that which accumulated in host mice. The ascites fluid from mice fed a 1% cyclocreatine diet contains 0.6 mM cyclocreatine; therefore, cells were incubated with cyclocreatine concentrations of 6 mM and 40 mM in order to determine if cells incubated with greater concentrations of cyclocreatine could increase the intracellular levels of phosphorylated cyclocreatine. It was observed that cells exposed to 40 mM cyclocreatine accumulated more phosphorylated cyclocreatine in one hour than did cells grown for 10 days in mice fed 1% cyclocreatine (Figure 3). These data suggest that under favorable conditions, approximately 98% of the intracellular cyclocreatine and 70% of creatine were phosphorylated to form PCr. The reason being is that differences between the phosphorylated products of cyclocreatine and creatine in amount accumulated, percentage phosphorylated, and rate of utilization are consequences of the fact that cyclocreatine has a Gibbs energy of hydrolysis 2 kcal/mol lower than that of PCr.
To demonstrate the formation and utilization of the phosphorylated levels of cyclocreatine and creatine co-existing in the same cells, cells harvested from control-fed mice were incubated with 80 mM of creatine and 30 mM cyclocreatine. After 1 hour, cells were washed and incubated in 2-deoxyglucose in order to deplete PCr and mimic an increase in energy demand. It was demonstrated that the pool of phosphorylated creatine was depleted at a rate 50-100 times faster than the phosphorylated cyclocreatine pool (Figure 4). Annesley T. Walker J. Formation and Utilization of Novel High Energy Phosphate Reservoirs in Ehrlich Ascites Tumor Cells. Journal of Biological Chemistry. 253(22):8120-25, 1978.

Figure 3. Creatine of a High-Energy Phosphate Reservoir with Cyclocreatine

Figure 4. Formation and Utilization of Phosphorylated Cyclocreatine and Creatine
2. Ability of cyclocreatine to protect bioenergetic and muscle function during ischemia:

Creatine and cyclocreatine effects on ischemic myocardium: 31P nuclear magnetic resonance evaluation of intact heart.

The purpose of this study was to investigate the effects of prior dietary supplementation with creatine or cyclocreatine on high energy phosphate metabolism of the ischemic rat myocardium. For 21 days rats were fed a diet containing either 1% Cr by weight, 1% cyclocreatine by weight, or control. At the end of the feeding period, rats were anesthetized, hearts harvested and perfused. 31P nuclear magnetic resonance (NMR) studies of myocardial bioenergetics were performed. After acquisition of pre-ischemic spectra, global ischemia was produced by clamping aortic inflow. Ischemia was maintained until adenosine triphosphate (ATP) was depleted, which occurred significantly quicker in control and creatine than in cyclocreatine (Figure 5). In addition, during ischemia the levels of phosphorylated creatine in the control and creatine condition were depleted significantly more than cyclocreatine (Figure 6). The time for return of mechanical function (heart rate x systolic pressure) after ischemia was similar for all three groups (CON = 28 +/- 28, CR = 34 +/- 22, and CY = 22 +/- 15 min), even though the cyclocreatine group was subjected to longer periods of ischemia (55 min vs. 35). These data indicate that cyclocreatine, but not creatine, pre-treatment provides myocardial protection either during and/or after ischemia and allows return of mechanical function after much longer episodes of ischemia than in control and creatine. One factor in the mechanism of protection may be the prolonged maintenance of phosphagen (Figure 6) due to the higher equilibrium concentration of phosphorylated cyclocreatine which in turn provides substrate for continued synthesis of ATP (Figure 5) during and after ischemia, thus defining cyclocreatine as a bioenergetic protective agent. Osbakken M, Ito K, Zhang D, Ponomarenko I, Ivanics T, Jahngen E, Cohn M. Creatine and cyclocreatine effects on ischemic myocardium: 31P nuclear magnetic resonance evaluation of intact heart. Cardiology, 80(3-4):184-95, 1992.
Enhanced ability of skeletal muscle containing cyclocreatine phosphate to sustain ATP levels during ischemia following beta-adrenergic stimulation.

In young chicks fed 1% cyclocreatine for 19 days, breast muscle accumulated a substantial amount of phosphorylated cyclocreatine. The levels of ATP were sustained at high values substantially longer in breast muscle of cyclocreatine-fed chicks, compared to control-fed chicks, during total ischemia initiated 2 hours after injection of both groups with the beta-adrenergic
agonist isoproterenol (5 mg/kg subcutaneous) to initiate glycogenolysis. Feeding chicks creatine resulted in a dramatic reduction in muscle glycogen levels, whereas cyclocreatine had much less of an effect of muscle glycogen utilization, thereby contributing to glycogen sparing (Figure 7).

Feeding chicks 5% creatine increased the total creatine pool in breast muscle and resulted in a slight ATP-sustaining action, while 1% cyclocreatine was effective in sustaining ATP during ischemia. Furthermore, the ATP sustaining activity in ischemic breast muscle was detected after 4 days, but not after only 2 days. Moreover, when chicks were fed cyclocreatine for 12 days and then returned to the control diet for 6 days, significant ATP-sustaining activity was still demonstrated (Figure 8). The phosphorylated creatine reserves in isoproterenol-stimulated breast muscles of all dietary groups were essentially exhausted within the first hour of ischemia. In contrast, breast muscle of chicks fed either 1% cyclocreatine underwent a maintenance of phosphorylated cyclocreatine for up to 2 hours of ischemia as the muscle was able to rely more on muscle glycogen. Although adaptive factors are also involved, it is suggested that a significant portion of the ATP-sustaining activity of dietary cyclocreatine in ischemic breast muscle can be attributed to the unique thermodynamic properties of the accumulated phosphorylated cyclocreatine. These properties enable phosphorylated cyclocreatine to continue to thermodynamically buffer the adenylate system and transport high energy phosphate in skeletal muscle at cytosolic pH values and phosphorylation potentials well below the range where the PCr system can function effectively. Cyclocreatine can help delay depletion of ATP levels in skeletal muscles during ischemia. Furthermore, cyclocreatine provides a protective role against ATP degradation and against irreversible cellular damage in skeletal and cardiac muscles during ischemic episodes. Turner D. Walker J. Enhanced ability of skeletal muscle containing cyclocreatine phosphate to sustain ATP levels during ischemia following beta-adrenergic stimulation. J Biol Chem. 262(14):6605-9, 1987.
Figure 7. Time Course of Glycogenolysis in Breast Muscle of Chicks Injected with Isoproterenol

<table>
<thead>
<tr>
<th>Time After Isoproterenol Injection (min)</th>
<th>Muscle Glycogen (μM/g)</th>
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<tr>
<td>0</td>
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<td>20</td>
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<tr>
<td>120</td>
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</tbody>
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- Control
- Cyclocreatin

Figure 8. Time Course of ATP Depletion During Ischemia in Breast Muscle of Chicks Injected with Isoproterenol 2 Hours Prior to Initiation of Ischemia

<table>
<thead>
<tr>
<th>Duration of Ischemia (min)</th>
<th>ATP (μM/g)</th>
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<tr>
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<td>5</td>
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Summary

Cyclocreatine has been shown to effectively increase the level of phosphorylated cyclocreatine in chick skeletal muscle compared to creatine’s ability to form PCr. As a result, 98% of intracellular cyclocreatine is phosphorylated compared to 70% for creatine. Furthermore, cyclocreatine reduces the amount of muscle glycogen utilized and ATP degraded when subjected to exercise-mimicking, bioenergetic conditions. Cyclocreatine also delays the onset of rigor in ischemic skeletal muscle. These results indicate the importance of sustaining intramuscular levels of PCr on sustaining intramuscular glycogen levels and bioenergetics PCr capacity, thereby prolonging skeletal muscle contractile capacity.

References

