Creatine and Phosphocreatine: A Review of Their Use in Exercise and Sport

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Objective: Creatine and phosphocreatine (PCr) are important compounds in the normal energy metabolism of muscle. Recently, it has been shown that dietary creatine (5 to 20 g/day) can increase muscle creatine and PCr, with enhancement in anaerobic exercise performance after two weeks of administration caused by an increase in anaerobic capacity.

Data Sources: MEDLINE was searched from 1983 to 1996 using key word "creatine" along with "humans," "muscle," "exercise," and "transport." Also, APStracts, the American Physiology Society search engine for abstracts, was searched from 1994 to 1996.

Data Synthesis: Creatine is transported into the muscle cell by a specific transporter, resulting in increased intracellular creatine and PCr. The PCr is capable of acting as an energy buffer, protecting the adenosine triphosphate (ATP) concentration. Maintaining muscle nucleotides therefore enhances exercise performance and recovery. There have been reports that PCr protects the cells from ischemic damage and decreases the loss of nucleotides by stabilizing cell membranes. Indeed, intravenous PCr (2-4 g/day) has been administered to cyclists, resulting in a faster recovery time between training sessions.

Conclusions/Recommendations: It is becoming evident that oral creatine supplementation may yield certain benefits to enhance the athlete's performance during maximal anaerobic exercise and interval training.

Key Words: ATP, ADP, muscle, energy metabolism, creatine kinase, anaerobic exercise

Recently there has been a great deal of interest in the use of creatine and, to a lesser extent, phosphocreatine (PCr) in sports medicine and athletic training. Various published works claim exercise enhancement and specific benefits with the administration of creatine and PCr to athletes,[1–13] but their utility and possible side effects still need to be examined in greater detail. There are, however, various reports in the literature in which creatine administration is used for therapeutic purposes [14,15] or in which the absence or depletion of creatine is detrimental.[16] This review is an attempt to summarize the current knowledge regarding the application of creatine and/or PCr with a focus on sports medicine and muscle physiology.

The creatine/PCr system plays an essential role in the normal energy metabolism of muscle because it acts as a buffer for the adenosine triphosphate (ATP) concentration.[17,18] It can also prevent a rise in the adenosine diphosphate (ADP) concentration, which can slow cross-bridge cycling. In muscle, creatine is reversibly phosphorylated by the enzyme creatine kinase (CK) in the following reaction:

$$\text{ATP} + \text{creatine} \overset{\text{CK}}{<=======>} \text{PCr} + \text{ADP} + \text{H}^+. \quad (1)$$

The H^+ produced in this reaction also means that the CK reaction is capable of buffering the pH of exercising muscle. Though important, the pH buffering by CK will not be addressed in this paper. There are two isoenzymes of creatine kinase found in skeletal muscle and both are required for normal muscle function. There is a cytosolic form of creatine kinase and a mitochondrial form, and they function simultaneously to form a rapid interconversion of PCr and ATP, which maintains an equilibrium in the muscle. This reaction is readily reversible and may be used to buffer the ATP concentration by rephosphorylating ADP from the energy contained within the PCr molecule. The other significant effect of the reaction is to keep the ADP concentration relatively low in the muscle (which, if elevated, will slow cross-bridge cycling).[19] As much as 70% of the immediate high-energy stores contained within skeletal muscle are in the form of PCr.[20,21] The total creatine concentration (PCr + creatine) in striated muscle is about 30 μmol/g muscle, which represents 4 grams of creatine per kilogram of Type I muscle fibers. For this reason, creatine can be described as an extract of muscle solids. The cellular concentration of creatine is determined by specific transporters that transport creatine into the cell with sodium (Na^+) against its concentration gradient.[2,22–25] The concentration of PCr is determined by the equilibrium constant of the CK reaction.[19] Therefore, the concentration of PCr in muscle is dependent upon the ATP and total creatine concentrations.

CREATINE SYNTHESIS

Creatine is synthesized in the liver and kidney and released into the blood stream[26] to be actively taken up by the muscle cells via specific transport protein(s). The liver plays an important role in the control of creatine content in the body because it methylates guanidinoacetate to produce creatine[15] for release into the blood stream. This methylation reaction utilizes the amino acid methionine. Guanidinoacetate is produced from the amino acids glycine and arginine in the following reaction:

$$\text{glycine} + \text{arginine} ======> \text{guanidinoacetate} + \text{ornithine}. \quad (2)$$

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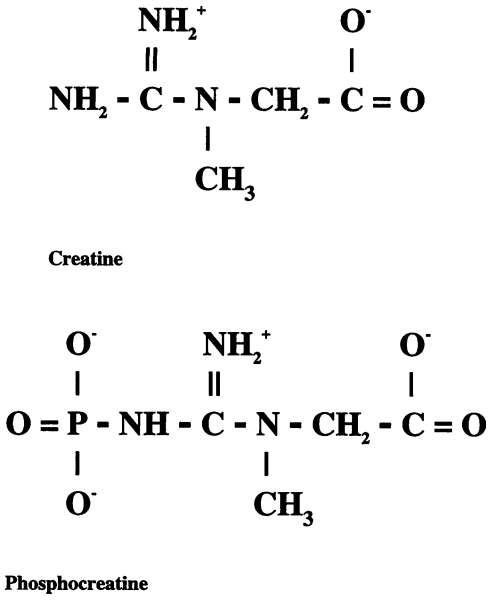

Creatine

Phosphocreatine

Fig 1. The chemical structures of the creatine molecule (top) and its phosphorylated counterpart phosphocreatine (bottom).

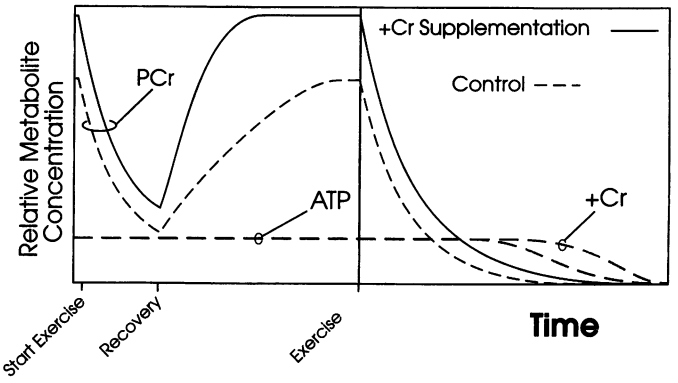

Fig 2. A schematic representation of the relative concentration changes that occur in muscle during two bouts of exercise. The PCr concentration at the start of the anaerobic exercise is greater in the muscle with creatine supplementation compared to control. The PCr concentration falls rapidly in both groups as the PCr molecule is used to buffer the ATP concentration. When exercise stops and the muscle is allowed to recover, the rate of PCr recovery is faster in the muscles with creatine supplementation (this is explained in Fig 3). Furthermore, when the anaerobic exercise is prolonged in the second bout of exercise, the ATP concentration is better maintained with creatine supplementation. The ATP buffering capacity of the muscle is enhanced due to the increased PCr concentration in the muscle. Note: the rates of concentration changes during exercise are dependent upon intensity of the exercise. For maximal anaerobic exercise, such as a sprint, the entire right hand box of this figure would take about 15 seconds.

The ornithine is used in the urea cycle and the guanidinoacetate is methylated to form creatine (Fig 1; creatine molecule). Therefore the synthesis of creatine requires three amino acids: glycine, arginine, and methionine. The net synthesis of creatine in the body is 1 to 2 g/day.

CREATINE SUPPLEMENTATION

Harris et al[21,27] have shown that feeding creatine at 2 to 5 g/day increases intracellular creatine and PCr in human muscle.[3] Greenhaff and colleagues have extended these observations using 5 to 20 g/day creatine.[9,28,29] They fed volunteers 5 g of creatine four times a day with a daily total of 20 g. This is considered the loading dose and is decreased to 2 to 5 g/day (maintenance dose) after 6 to 14 days, which is continued throughout the period of training. If athletes are given lower doses (2–3 g/day), without the loading dose, there is still increased muscle creatine but with a slower rate of accumulation. The current evidence is that the best loading of muscle creatine occurs during normal training and occurs concomitant with a low-fat, high-carbohydrate diet.[29]

In the intestine, creatine is absorbed *unchanged* from the lumen and passes directly into the blood stream. Following oral creatine administration serum creatine rises, and the amount of creatine taken into the cells increases, leading to higher intracellular PCr levels[1,2] (See Fig 2). This extra PCr enhances anaerobic output and muscle peak torque production[28] (as measured using a Cybex II isokinetic dynamometer; Lumex, Inc., Ronkonkoma, NY) in the working skeletal muscle. This was seen in athletes fed 24 g/day for 5 days, and the exercise protocol was five sets of quadriceps contractions, with 30 repetitions in each set. Part of the reason for the increase in muscle peak torque production was attributed to the accelerated rate of PCr resynthesis. Greenhaff et al[10] pointed out, however, that the dietary supplementation with creatine did not increase total muscle creatine in all athletes. It appears that about 70% of the people studied had an increase in total muscle creatine and exercise enhancement.[20] This was attributed to the fact that some of the athletes (30%) had creatine levels near the physiological maximum. Another study showed that increased PCr does not change the maximal force of contraction.[27] Increases in maximal force may occur if there is an anabolic action of creatine or PCr, but these observations have not been fully evaluated.[14,30]

Active uptake of creatine has been found in all muscle cells studied, including skeletal, heart, and smooth muscles.[19,25,31] Creatine has been shown to have an anabolic effect on muscle tissues[14,30] because it caused an increase in protein synthesis.[30] Elevation of PCr in the muscle cell is also capable of stimulating protein synthesis in the cell organelles. Interestingly, Bessman and Mohan[30] likened the PCr stimulation of protein synthesis to that of exercise or insulin-stimulated growth. Even in humans there was a 46% increase in the size of Type II muscle fibers in gyrate atrophy patients fed 1.5 g/day of creatine for one year.[14] Conversely, inhibition of normal creatine metabolism prevents normal muscle growth as well as decreases protein and lipid synthesis.[16,32] Oral administration of creatine monohydrate to men and women has been found to lower plasma total cholesterol (6%), triacylglycerols (22%), and very-low-density lipoprotein-C (23%) after 56 days of administration (5 g/day).[33]

CREATINE TRANSPORT

Creatine is actively taken up into the cell via a specific transport protein using the energy contained in the Na^+

gradient.[28,34,35] This transport protein has a high affinity for creatine and concentrates it within the cell.[25] Once in the cell, very little creatine is lost (3 percent per day).[26,35] The concentration of creatine where the transport rate is half of its maximum (K_m) is about 30 μmol/L, which is in the range of circulating plasma creatine concentration.[24,26,34] Therefore at physiological levels of creatine, the activity of the creatine transport protein(s) is operating at about half maximal. The result is that small increases in plasma creatine, which can occur with oral creatine supplementation, cause an increase in creatine transport with a concomitant increase in intracellular creatine concentration.

In isolated cell and tissue preparations, Joseph F. Clark et al (unpublished observations) and Joseph Odoom et al (unpublished observations) have found that incubation of isolated vascular smooth muscle or cultured striated muscle both actively concentrate creatine. Odoom increased total creatine in cultured muscle cells and clearly demonstrated that its uptake was Na^+ dependent and cyclic adenosine monophosphate (cAMP) mediated.[34] Similar experiments using isolated vascular smooth muscle showed a striking two- to fourfold increase in intracellular creatine concentration and increased PCr when tissue was incubated with 20 mmol/L creatine for 24 hours.[36] From these studies it is apparent that creatine is taken up by living tissue and that increasing intracellular creatine increases PCr and anaerobic capacity (Fig 2).

There appears to be an optimum or maximum total intracellular creatine concentration of about 160 nmol/kg dry mass where normal is about 120 nmol/kg dry mass.[21] This has been seen in human studies where all subjects approached the same relative creatine content in the muscle.[30] In subjects who had relatively low levels of muscle creatine, supplementation caused a striking increase in muscle creatine content. However, if the subjects started with elevated levels of creatine in their muscle, the increase with oral creatine administration was diminished. Subjects started with a range of creatine concentrations between 110 and 140 nmol/kg dry mass.[21] In experiments on isolated muscle tissue a similar maximum is seen, and the tissue tends to maintain that level (Claire Willott and Joseph F. Clark, unpublished observations). These data strongly indicate that there are definite limits to the benefits possible from creatine supplementation and that, once muscle creatine levels have plateaued, there will be no further increase.

CREATINE SUPPLEMENTATION AND WATER/URINE HOMEOSTASIS

During the early phase of oral creatine supplementation there is a decrease in water excretion with concomitant water retention.[29] The cause of the water retention in these athletes has not yet been determined, although it is likely that there is water associated with the creatine during cotransport with sodium ion.[34] This water retention may be related to the unpublished reports of muscle cramps and heat intolerance seen during creatine supplementation. From a metabolic perspective, these may be reasonable suggestions, and, therefore, anyone involved with creatine supplementation should strongly recommend that their athletes be properly hydrated and avoid strenuous exercise (of the double-session type) during the initial days of creatine supplementation. It has not yet been reported if there is a correlation between the loading dose regime and muscle cramps or heat intolerance, and, therefore, more research must be performed regarding the indications and contraindications of creatine supplementation and water homeostasis.

Creatine is converted spontaneously and nonenzymatically to creatinine in all tissues with PCr. The creatinine cannot be reconverted to creatine and is not used by the muscle so it must be excreted in the urine. When athletes were given 20 grams of creatine per day their urinary excretion of creatinine (as well as serum creatinine) was increased.[29] Urinary creatinine levels quickly return to normal as the creatine intake is decreased. There were no other alterations in urinary metabolism reported due to creatine supplementation.[33] The loss of free creatine in cultured skeletal muscle cells is less than 3% per day.[23,35] This is close to the amount of creatinine produced nonenzymatically by living human muscle [19,26,31]. The major way in which creatine is lost from the body is via the irreversible conversion to creatinine.[34,35] The excretion of creatinine in the urine is constant at about 2 g/day. Therefore, creatine efflux from the cell is considered to be negligible, and the advantages of creatine administration are not prone to being lost with exercise.[23,26]

CREATINE'S CONTROL OF OXIDATIVE METABOLISM

The rapid resynthesis of PCr seen with creatine supplementation is likely to be oxidative in origin[19,37,38] as the increased creatine stimulates oxidative phosphorylation (Fig 3).[6,18,19,38] This is due to mitochondrial creatine kinase (Mi-CK) bound to

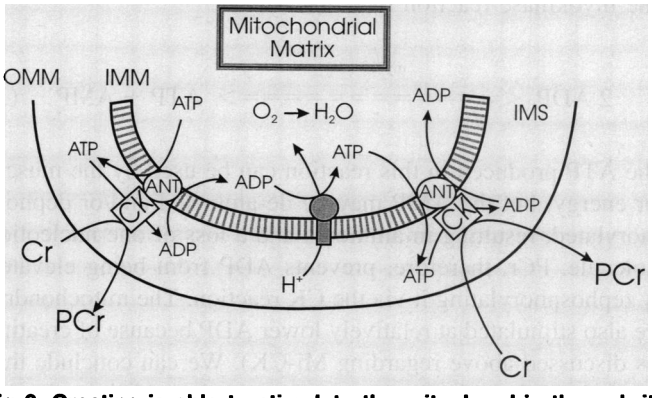

Fig 3. Creatine is able to stimulate the mitochondria through its action on mitochondrial creatine kinase (CK). The result is that ADP is produced "in" the mitochondria and the ADP enters the mitochondrial matrix to be resynthesized. The result is that creatine, when it arrives at the mitochondria from the muscle, gets phosphorylated to PCr and therefore has the same stimulatory effect as ADP because it causes the production of one ADP molecule. Creatine stimulates the mitochondria by amplifying the ADP signal at the mitochondria. ANT, adenine nucleotide translocase or the protein that transports ADP into the mitochondrial matrix and ATP out; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; IMS, intermembrane space; H^+, the protons used to synthesize ATP from ADP; $O_2 \rightarrow H_2O$, oxidative part of oxidative phosphorylation that is consuming oxygen.

the outer surface of the inner mitochondrial membrane.[19,38] Mi-CK is functionally coupled to oxidative phosphorylation and can therefore stimulate respiration because of enzymatic amplification of ADP at or near the ADP transport protein. The result is that instead of 30 μmol/L ADP needed for half maximal stimulation of oxidative phosphorylation, only 10 μmol/L is needed.[19,38] Therefore, creatine administration increases anaerobic capacity (as seen in Fig 2) and also increases aerobic recovery by stimulating the mitochondria and oxidative phosphorylation[16,19,38](Fig 3).

CREATINE AND MUSCLE BIOENERGETICS

Greenhaff et al[20,28] found that following oral creatine supplementation there was a greater peak torque production in the final few contractions in a series. These athletes were able to perform more anaerobic work after oral administration of creatine.[2,20] Oral creatine did not influence plasma lactate levels, but Greenhaff et al[28] did find that PCr was better able to maintain muscle ATP concentrations. Creatine supplementation produced a significant decrease in plasma ammonia during exercise. Plasma ammonia is generally due to a loss of muscle adenine nucleotide stores (originating from ATP). This lost ATP is slowly resynthesized, but the decrease in ammonia may reflect the increased ATP that is staying in the muscle. Therefore the augmented PCr in muscle protects the total nucleotide concentration.

The mechanism by which PCr decreases nucleotide loss is due to its ability to rapidly phosphorylate ADP and not due to effects on AMP deaminase or myokinase. Therefore, ADP levels are not elevated. Elevated ADP would have two detrimental consequences in muscle. One is to slow cross-bridge cycling, and the other is to activate the myokinase reaction. The myokinase reaction is:

$$2\ \mathrm{ADP} \xleftrightarrow{\text{myokinase}} \mathrm{ATP} + \mathrm{AMP}. \quad (3)$$

The ATP produced in this reaction can be used by the muscle for energy, but the AMP may be de-aminated and/or dephosphorylated, resulting in ammonia and a loss of one nucleotide molecule. PCr, therefore, prevents ADP from being elevated by rephosphorylating it via the CK reaction. The mitochondria are also stimulated at relatively lower ADP because of creatine (as discussed above regarding Mi-CK). We can conclude that increasing PCr with creatine supplementation aids in maintaining muscle phosphorylation potential and in this way also maintains maximal anaerobic performance.

After administration of creatine, and the concomitant increase in muscle PCr, there is an increase in the rate of PCr resynthesis.[10,20,28] To the athlete this is seen as a shorter recovery time after intense exercise (Fig 2). In athletes being tested with 30 quadriceps contractions and a 1-minute rest between sets, the creatine supplementation group (24 g/day) was able to develop greater peak torque production compared with placebo controls.[28] The rapid resynthesis of PCr is essential for repeated bouts of exercise, and, coupled with this information, it is important to realize that creatine is not lost from the cell[23,35] (discussed above).

PHOSPHOCREATINE

Because creatine and phosphocreatine are in rapid exchange via the reversible transphosphorylation reaction catalyzed by creatine kinase, they must both be discussed in a paper such as this. As discussed above, PCr is required for normal cellular metabolism, and increased PCr concentrations can enhance anaerobic exercise. In the cell, PCr is used in no other pathways known because it is not involved in any other reactions and is acted upon only by CK. PCr does, however, have another use that does not involve enzymes and is due to its amphipathic (or ionic) nature.[9,38–40] The ionic characteristics of PCr enable it to bind to the polar phospholipid heads of membranes[39](Fig 1). This binding to the phospholipid heads effectively stabilizes the membrane phospholipid bilayer by decreasing fluidity.[39] The more fluid-like a membrane, the more leaky it is. Stabilizing the membrane prevents some of the damage caused by transient ischemia and hypoxia (which can occur during exercise) and thus prevents loss of essential cellular substances (such as nucleotides).[37,39]

Membrane stabilization by PCr has been shown in striated muscle using electron spin resonance and functional studies on blood cells, as well as cardiac and skeletal muscle.[37,39,40] PCr has been given to patients by intravenous (IV) injection as part of the treatment for ischemia and myocardial infarction.[40] In cardiac muscle it improves the energetic status (phosphorylation potential) and decreases electrical anomalies that sometimes lead to lethal arrhythmias. It is even used as a cardioplegic cardioprotective agent during heart surgery.[37,38] There is also a preserving effect upon the intracellular concentration of soluble enzymes and nucleotides, which may be due to less cytoplasmic leakage.[39]

Other trials have reported favorable actions of PCr on skeletal muscle function and performance. PCr augmentation in the muscle improves maximum anaerobic power,[24] increasing the ability of the muscle to produce maximum burst output compared to placebo.[33] During conditions of maximal exercise such as "cronoscalata" (timed uphill cycling), enhanced performance has been shown in amateur cyclists given intravenous PCr (2 g twice a day).[12] In three controlled studies using an isokinetic dynamometer, PCr increased the rate of recovery after intense exercise.[1,11,] Shorter rests were needed between competitions in these athletes as well as increased muscle fiber recruitment in athletes given 0.5 g/day PCr.[1] Another study was also performed on patients with hypotonotrophy (resulting from knee pathology) and demonstrated promising therapeutic effects for this pathology by preserving muscle mass.[11,41] The authors, however, did not speculate on the application to healthy individuals. Notwithstanding, these studies all support exercise enhancement by IV administration of PCr.

Intravenous PCr (2g/day) has been given to athletes during strenuous endurance training (Valdure A. Saks, unpublished observations). The athletes given PCr were able to train longer and reported less muscular discomfort. They also had a striking reduction in morning muscle stiffness. It was postulated that

the decreased stiffness and myalgia seen was due to less muscle damage occurring during training. The decreased muscle damage may be an effect of the membrane stabilization of PCr and/or possible improvement in the metabolism of the tissue.[7,11] This result is consistent with the observation of cardioprotection in which PCr stabilizes membranes and possibly protects the muscle from damage during strenuous aerobic exercise.

One drawback of PCr administration is that it must be given intramuscularly or intravenously because it is readily broken down in the intestinal tract. After intramuscular or intravenous administration its pharmacodynamic effects last for 2 to 5 hours, as demonstrated by the increased concentrations of ATP in human red blood cells[42] and rat cardiac cells,[43] as well as the observations of antiarrhythmic effects.[44] Nonetheless, IV PCr has beneficial effects that creatine alone does not have. Most of these effects are attributed to the membrane-stabilizing action, but, when PCr is hydrolyzed, it forms inorganic phosphate (Pi) and creatine. As discussed earlier, creatine is readily and actively taken into the cells and used to supplement the energy metabolism of muscle. Therefore, PCr has the same benefits as oral creatine administration in addition to the membrane effects.

PHOSPHOCREATINE ENERGY SHUTTLE

PCr is intimately involved in the energy metabolism of muscle.[17,34,45] It is synthesized at sites of energy production via localized creatine kinases such as Mi-CK and MM-CK (the muscle form of creatine kinase). Mi-CK is found in the mitochondria and synthesizes PCr in a reaction that is functionally coupled to oxidative metabolism (Figs 3 and 4).[19,38] Creatine kinase has been described as an integral protein of the contractile apparatus[19] and is capable of regenerating ATP for contraction. Rephosphorylation of ATP in this way produces creatine that diffuses back to the mitochondria to stimulate respiration further and gets rephosphorylated back to PCr. This cycling of PCr to creatine and back is referred to as the phosphocreatine shuttle mechanism[19,30,37,38] and is essential in normal function of muscle, especially during periods of high activity such as exercise (Figs 2 and 4). Therefore, PCr is an important metabolite in the normal function of the muscle because it can buffer the ATP concentration (Fig 2), prevent elevation of ADP, and function in the muscle's energy shuttle. These roles may indeed be enhanced by supplementation, as discussed above, and thus may partially explain some of the benefits of supplementing creatine and/or PCr. Much more information on the phosphocreatine shuttle and its relative importance in cellular function can be obtained in the review of Wallimann et al.[19]

CONCLUSION

This paper has discussed some of the actions of creatine and PCr on muscle metabolism and performance. Twenty grams per day of creatine can be added to the athlete's diet for 1 to 2 weeks and reduced to 5 g/day for the remainder of the sports season. The result is that this creatine supplementation increases muscle creatine and muscle PCr for the duration of the sports season. Because of the apparent maximum for muscle creatine, higher doses have negligible additional effects. The increased creatine and PCr are able to enhance anaerobic capacity as well as stimulate protein synthesis. Along with buffering ATP, PCr has the ability to stabilize membranes and protect cells from damage. When PCr is degraded via CK, ADP is converted to ATP and there is an improvement in the muscle bioenergetics. The drawback of PCr applications in the sports medicine field is that it must be administered intravenously or intramuscularly, whereas creatine can be taken orally.

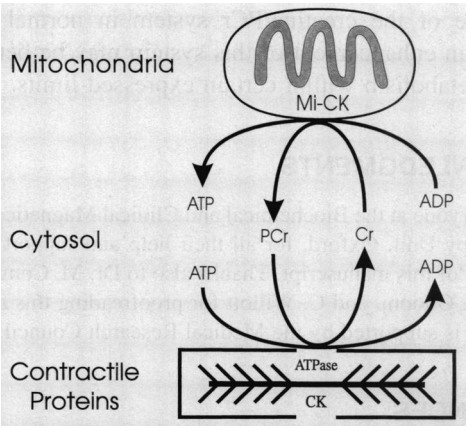

Fig 4. Schematic representation of the energy metabolites being shuttled between the contractile proteins and the mitochondria in muscle. During exercise and recovery there is a shuttling of energy-rich molecules (such as ATP and PCr) from the mitochondria to the contractile proteins. The contractile proteins utilize the energy within these molecules to perform work and produce creatine and ADP. (Note: ATP is absolutely required for muscle work, but PCr is used to maintain ATP.) In the cell the creatine resulting from this cycle is rephosphorylated to PCr in the mitochondria. Interestingly creatine has the ability to stimulate mitochondria and therefore helps accelerate or amplify the communication between the mitochondria and the contractile proteins. Therefore, ADP and creatine can signal the mitochondria to increase oxidative ATP production. ATPase, myosin ATPase used to perform muscle work; CK, creatine kinase at or near the contractile proteins used to rephosphorylate ADP to ATP; Mi-CK, creatine kinase specifically found in the mitochondria and used to resynthesize PCr.

There are several unexplored areas of research and investigation regarding creatine and PCr supplementation and administration. Controlled double-blind studies examining water/urine homeostasis and their relationship to muscle cramps, stiffness, and heat intolerance have yet to be reported. Differential benefits to athletes in the same sport have not been examined and there appear to be gender differences that have not been explained.[20,29,33] Also, the importance of creatine supplementation in athletes with low or no meat intake (vegetarians) needs to be examined.

The use of any dietary supplement is highly variable and always has limits. This review has discussed some of the apparent strengths and weaknesses of these two closely related compounds. What role they may play in the fields of applied physiology or exercise physiology remains to be determined. One may speculate, however, that because of the central

importance of the creatine/PCr system in normal muscular function, an enhancement of this system may be beneficial to muscle metabolism within certain expressed limits.

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REFERENCES

1. Agnese L, Tarello M, Grazioli I. Efficacia della creatina fosfato nel trattamento della ipotonotrofia da non uso della muscolatura scheletrica. *Ort e Traum Oggi.* 1992;12:107–113.
2. Balsom PD, Ekblom B, Soderlund K, Sjodin B, Hultman E. Creatine supplementation and dynamic high-intensity intermittent exercise. *Scand J Med Sci Sports.* 1993;3:143–149.
3. Birch R, Novel D, Greenhaff PL. The influence of dietary creatine supplementation on performance during repeated bouts of maximal isokinetic cycling in man. *Eur J Appl Physiol.* 1994;69:268–270.
4. Casey A, Constantin-Teodosiu D, Howell S, Hultman E, Greenhaff PL. Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *Am J Physiol.* 1996;271:E31–E37.
5. Casey A, Constantin-Teodosiu D, Howell S, Hultman E, Greenhaff PL. Metabolic response of type I and II muscle fibers during repeated bouts of maximal exercise in humans. *Am J Physiol.* 1996;271:E38–E43.
6. Clark JF. Uses of creatine phosphate and creatine supplementation for the athlete. In: Conway MA, Clark JF, eds. *Creatine and Creatine Phosphate: Scientific and Clinical Perspectives.* London: Academic Press; 1996:217–225.
7. Dal Monte A, Leonardi LM, Figura F, Cappozzo A, Marchetti M, De Sanctis AM, Nofroni I. Effetti dell'apporto esogeno di fosfocreatina sulla potenza muscolare umana. *Gazz. Med. Ital.* 1976;135:2–11.
8. Gelfgan EB, Dalili IG, Shakhtakhtinskaya FN, Yagisarova NM, Shirinova EA. The effect of phosphocreatine on the tolerance of physical exercise in patients with ischemic heart disease. In: Saks VA, Bobkov YG, Strumia E, eds. *Creatine Phosphate: Biochemistry, Pharmacology, and Clinical Efficiency.* Torino Italy: Edizioni Minerva Medica; 1987:270.
9. Green AL, Hultman E, Macdonald IA, Sewell DA, Greenhaff PL. Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in man. *Am J Physiol.* 1996;271:E821–E826.
10. Greenhaff PL, Bodin K, Soderlund K, Hultman E. Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. *Am J Physiol.* 1994;266:E725–E730.
11. Pirola V, Pisani L, Teruzzi P. Valutazione del recupero del trofismo muscolare in pazienti anziani con frattura di femore trattati con creatina fosfato e fisokinesiterapia. *Clin. Ter.* 1991;139:115–119.
12. Tegazzin V, Rossi M, Schiavon M, Schiavon R, Bettini V, Aragno R, Bitozzi A. Indagine sulla performance di ciclisti trattati e non trattati con fosfocreatina. *Biol Med.* 1991;13:121–135.
13. Walker JB. Bioenergetic engineering with guanidino compounds: loading tissues with extended-range synthetic thermodynamic buffers. In: De Deyn PP, Marescau B, Stalon V, Qureshi IA, eds. *Guanidino Compounds in Biology and Medicine.* London: John Libbey and Co.; 1992:187–194.
14. Sipila I, Rapola J, Simell O, Vannas A. Supplementary creatine as a treatment for gyrate atrophy of the choroid and retina. *N. Engl. J. Med.* 1981;304:867–870.
15. Stockler S, Hanefeld F, Frahm J. Creatine replacement therapy in guanidinoacetate methyltransferase deficiency: a novel inborn error of metabolism. *Lancet.* 1996;348:789–790.
16. Sanford L, Tikunov B, Henson D, Lamanca J, Sweeney HL. Creatine depletion elicits structural, biochemical, and physiological adaptations in the rat costal diaphragm. *Am J Physiol.* 1996;40:C1480–C1486.
17. Conway MA, Ouwerkerk R, Rajagopalan B, Radda GK. Creatine phosphate: in vivo human cardiac metabolism studied by magnetic resonance spectroscopy. In: Conway MA, Clark JF, eds. *Creatine and Creatine Phosphate: Scientific and Clinical Perspectives.* London: Academic Press; 1996:127–160.
18. Trump ME, Heigenhauser GJF, Putman CT, Spriet LL. Importance of muscle phosphocreatine during intermittent maximal cycling. *J Appl Physiol.* 1996;80:1574–1580.
19. Wallimann T, Wyss M, Brdczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation structure and function of creatine kinase isozymes in tissue with high and fluctuating energy demands: the phosphocreatine circuit for cellular energy homeostasis. *Biochem J.* 1992;1102:21–40.
20. Greenhaff PL, Bodin K, Harris RC, Hultman E, Jones DA, McIntyre DB, Soderlund K, Turner DL. The influence of oral creatine supplementation on muscle phosphocreatine resynthesis following intense contraction in man. *J Physiol.* 1993;467:75P.
21. Harris RC, Soderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci.* 1992;83:367–374.
22. Balsom PD, Harridge SDR, Soderlund K, Sjodin B, Ekblom B. Creatine supplementation per se does not enhance endurance performance. *Acta Physiol Scand.* 1993;149:521–523.
23. Bloch K, Schoenheimer R, Rittenberg D. Rate of formation and disappearance of body creatine in normal animals. *J Biol Chem.* 1941;138:155–166.
24. Earnest CP, Snell PG, Rodriguez R, Almada AL, Mitchell TL. The effect of creatine monohydrate ingestion on anaerobic power indices, muscular strength, and body composition. *Acta Physiol Scand.* 1995;153:207–209.
25. Moller A, Hamprecht B. Creatine transport in cultured cells of rat and mouse brain. *J Neurochem.* 1989;52:544–550.
26. Walker JB. Creatine: biosynthesis, regulation, and function. In: Meister A, ed. *Advances in Enzymology.* Vol 50. New York: John Wiley and Sons; 1979:177–242.
27. Harris RC, Viru M, Greenhaff PL, Hultman E. The effect of oral creatine supplementation on running performance during maximal short term exercise in man. *J Physiol.* 1993;467:74P.
28. Greenhaff PL, Casey A, Short AH, Harris R, Soderlund K, Hultman E. Influence of oral creatine supplementation of muscle torque during repeated bouts of maximal voluntary exercise in man. *Clin. Sci.* 1993;84:565–571.
29. Hultman E, Soderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in man. *J Appl Physiol.* 1996;81:232–237.
30. Bessman SP, Mohan C. Phosphocreatine, exercise, protein synthesis, and insulin. In: De Deyn PP, Marescau B, Stalon V, Qureshi IA, eds. *Guanidino Compounds in Biology and Medicine.* London: John Libbey and Co.; 1992:181–186.
31. Fitch CD, Shields RP. Creatine metabolism in skeletal muscle. I. Creatine movement across muscle membranes. *J Biol Chem.* 1966;241:3611–3614.
32. Carpenter CL, Mohan C, Bessman SP. Inhibition of protein and lipid synthesis in muscle by 2:4-dinitrofluorobenzene, an inhibitor of creatine phosphokinase. *Biochem Biophys Res Comm.* 1983;111:884–889.
33. Earnest CP, Almada AL, Mitchell TL. High performance capillary electrophoresis-pure creatine monohydrate reduces blood lipids in men and women. *Clin. Sci.* 1996;91:113–118.
34. Clark JF, Odoom J, Tracey I, Dunn J, Boehm EA, Paternostro G, Radda GK. Experimental observations of creatine and creatine phosphate metabolism. In: Conway MA, Clark JF, eds. *Creatine and Creatine Phosphate: Scientific and Clinical Perspectives.* London: Academic Press; 1996:33–50.
35. Rose WC. The metabolism of creatine and creatinine. *Ann Rev Biochem.* 1933;2:187–206.
36. Scott DP, Davidheiser S, Coburn RF. Effects of elevation of phosphocreatine on force and metabolism in rabbit aorta. *Am J Physiol.* 1987;253:H461–H465.
37. Saks VA, Javadov SA, Pozin E, Preobrazhensky AN. Biochemical basis of the protective action of phosphocreatine on the ischemic myocardium. In: Saks VA, Bobkov YG, Strumia E, eds. *Creatine Phosphate: Biochemistry,*

Pharmacology, and Clinical Efficiency. Torino Italy: Edizioni Minerva Medica; 1987:270–273.
38. Saks VA, Kuznetsov AV, Kupriyanov VV, Miceli MV, Jacobus WE. Creatine kinase of rat heart mitochondria. *J Biol Chem.* 1985;891:138–144.
39. Saks VA, Strumia E. Phosphocreatine: molecular and cellular aspects of the mechanism of cardioprotective action. *Curr Ther Res.* 1993;53:565–598.
40. Sharov VG, Saks VA, Kupriyanov VV, Lakomkin VL, Kapelko VI, Steinschneider AY, Javadov SA. Protection of ischemic myocardium by exogenous phosphocreatine I. Morphologic and phosphorus 31-Nuclear magnetic resonance studies. *J Thorac Cardiovasc Surg.* 1987;94:749–761.
41. Satolli F, Marchesi G. Creatine phosphate in rehabilitation of patients with muscles hypotonotrophy of the lower extremity. *Curr Ther Res.* 1989;46: 67–73.
42. Piacenza G, Strumia E, Lorenzi E, Borgoglio R. Creatine phosphate effects on red blood cells. In: Saks VA, Bobkov YG, Strumia E, eds. *Creatine Phosphate: Biochemistry, Pharmacology, and Clinical Efficiency*. Torino Italy: Edizioni Minerva Medica; 1987:177–184.
43. Down WH, Chasseaud LF, Ballard SA. The effect of intravenously administered phosphocreatine on ATP and phosphocreatine concentrations in the cardiac muscle of the rat. *Arzneim Forsh.* 1983;33:552–554.
44. Fagbemi O, Kane KA, Parratt JR. Creatine phosphate suppresses ventricular arrhythmias resulting from coronary artery ligation. *J Cardiovasc Pharmacol.* 1982;4:53–58.
45. Conway MA, Rajagopalan B, Radda GK. Skeletal muscle metabolism in heart failure. In: Conway MA, Clark JF, eds. *Creatine and Creatine Phosphate: Scientific and Clinical Perspectives*. London: Academic Press; 1996:161–184.