

# Clinical Pharmacology of the Dietary Supplement Creatine Monohydrate

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**Abstract**—Creatine is a dietary supplement purported to improve exercise performance and increase fat-free mass. Recent research on creatine has demonstrated positive therapeutic results in various clinical applications. The purpose of this review is to focus on the clinical pharmacology and therapeutic application of creatine supplementation. Creatine is a naturally occurring compound obtained in humans from endogenous production and consumption through the diet. When supplemented with exogenous creatine, intramuscular and cerebral stores of creatine and its phosphorylated form, phosphocreatine, become elevated. The increase of these stores can offer therapeutic benefits by preventing ATP depletion, stimulating protein synthesis or reducing protein degradation,

and stabilizing biological membranes. Evidence from the exercise literature has shown athletes benefit from supplementation by increasing muscular force and power, reducing fatigue in repeated bout activities, and increasing muscle mass. These benefits have been applied to disease models of Huntington's, Parkinson's, Duchenne muscular dystrophy, and applied clinically in patients with gyrate atrophy, various neuromuscular disorders, McArdle's disease, and congestive heart failure. This review covers the basics of creatine synthesis and transport, proposed mechanisms of action, pharmacokinetics of exogenous creatine administration, creatine use in disease models, side effects associated with use, and issues on product quality.

## I. Introduction

In 1994, the Food and Drug Administration passed the Dietary Supplement Health Education Act. This act defines a dietary supplement as

1. a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin, mineral, amino acid, herb or other botanical; or
2. a dietary substance for use to supplement the diet by increasing the total dietary intake; or
3. a concentrate, metabolite, constituent, extract, or combination of any ingredient described previously.

In addition, the act states that these products do not represent a conventional food or a sole item of a meal or the diet. Over the past 10 to 15 years, the field of dietary supplements has grown from \$3.3 billion business in 1990 to an estimated \$14 billion in the year 2000 (Zeisel, 1999). About \$200 million of this industry is spent on creatine monohydrate (Schnirring, 1998).

In the 1990s, creatine ( $\text{Cr}^3$ ) supplementation became a popular ergogenic aid to increase exercise performance. The benefits of Cr supplementation on exercise performance have been extended as a possible therapeutic agent in the treatment of disease conditions. Previous reviews have focused primarily on the improvements in exercise performance seen in human subjects ingesting Cr (Balsom et al., 1994; Mujika and Padilla, 1997; Volek and Kraemer, 1997; Juhn and Tarnopolsky, 1998a; Demant and Rhodes, 1999; Graham and Hatton, 1999; Jacobs, 1999; Kraemer and Volek, 1999; Benzi, 2000).

<sup>3</sup> Abbreviations: Cr, creatine; PCr, phosphocreatine; tCr, total creatine; AGAT, arginine:glycine amidino-transferase; GAMT, *S*-adenosylmethionine:guanidinoacetate *N*-methyltransferase; CreaT, creatine transporter; DM, dry muscle; IGF-1, insulin-like growth factor-1; GFR, glomerular filtration rate; AUC, area under the curve; GA, gyrate atrophy; MELAS, myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; 3-NP, 3-nitropropionic acid; ADC, apparent diffusion coefficient;  $T_{\max}$ , time of maximal concentration;  $C_{\max}$ , concentration at  $T_{\max}$ ; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine;  $\text{MPP}^+$ , 1-methyl-4-phenylpyridinium.

The purpose of this review is to focus on the clinical applications of Cr supplementation through the understanding of the physiological role of Cr, the benefits of Cr supplementation under healthy and diseased conditions, and on the limited information on the pharmacokinetics of exogenous Cr.

## II. Creatine Synthesis and Transport

Comprehending the synthesis and transport has become an important basis for the understanding of certain diseases of Cr metabolism (e.g., gyrate atrophy) and the effect of supplementation on regulation of these processes. Wyss and Kaddurah-Daouk (2000) and Walker (1979) have previously reviewed the systemic metabolism of Cr.

### A. Synthesis

Creatine ( $\alpha$ -methyl guanidino-acetic acid) is distributed throughout the body with 95% of Cr found in skeletal muscle (Walker, 1979). The remaining 5% of the creatine pool is located in the brain, liver, kidney, and testes (Walker, 1979). Cr is obtained through the diet ( $\sim 1$  g/day for an omnivorous diet) and synthesized in the liver, kidney, and pancreas ( $\sim 1$  g/day). The majority of synthesis in humans occurs in the liver and kidney (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). The dietary intake and endogenous production of Cr matches the spontaneous degradation of phosphocreatine (PCr) and Cr to creatinine at a rate of 2.6% and 1.1% per day, respectively (Walker, 1979). Therefore, creatinine production from Cr and PCr sums to 2 g/day or 0.017/day of total body Cr (Cr + PCr) based on a 70-kg human and a total Cr (tCr) pool of 120 g (Walker, 1979). Once creatinine is formed it enters circulation by diffusion and is eliminated from the body through glomerular filtration. Supplementation of Cr has been shown to reduce endogenous production in humans; however, normal rates return upon termination of supplementation (Walker, 1979). Circulating levels of creatinine also increase with supplementation (Kamber et al., 1999; Schedel et al., 1999; Volek et al., 2000).

Cr is derived from glycine and arginine by the formation of guanidinoacetate and ornithine in a reaction catalyzed by arginine:glycine amidino-transferase (AGAT) (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). It is theorized that guanidinoacetate is formed in the kidney and transferred via the blood to the liver (Wyss and Kaddurah-Daouk, 2000). In the liver, the methyl group from methionine, found as *S*-adenosylmethionine, is donated to guanidinoacetate by *S*-adenosylmethionine:guanidinoacetate *N*-methyltransferase (GAMT) (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). The rate-limiting step in Cr synthesis is the formation of guanidinoacetate by AGAT (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). Cr is capable of feedback inhibition of AGAT possibly by inhibiting steps before translation of AGAT mRNA (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). Other factors that have been shown to regulate Cr synthesis include thyroid hormone, growth hormone, testosterone, ornithine, and dietary deficiencies (e.g., fasting, vitamin E) (Walker, 1979; Wyss and Kaddurah-Daouk, 2000). Figure 1 is a simplistic representation of Cr synthesis and degradation.

### B. Transporters

In the body, there is little Cr found at the site of production, and therefore Cr must be transported from areas of synthesis to areas of storage and utilization. Typically, organs that contain the highest levels of AGAT and/or GAMT have the lowest levels of creatine kinase, the enzyme responsible for the phosphorylation of Cr to PCr (Walker, 1979). Since Cr is only produced in certain organs and utilized in others, it must enter the blood to reach other tissue systems such as skeletal muscle. The cellular uptake of Cr by organs is critical due to the potential down-regulation of these systems with chronic exposure to Cr (Guerrero-Ontiveros and Wallimann, 1998).

Once in the blood, Cr is transported into tissues against a concentration gradient through a sodium- and chloride-dependent transporter (CreaT). CreaT is similar to the transporters for dopamine, guanidino  $\gamma$ -aminobutyric acid, and taurine (Guerrero-Ontiveros and Wallimann, 1998). The location of expression of these transporters matches that of creatine kinase expression because the mRNA for CreaT has been found in kidney, heart, skeletal muscle, brain, testis, and colon, but not in the liver, pancreas, and intestine (Guimbal and Kilimann, 1993; Nash et al., 1994; Sora et al., 1994). The  $K_m$  for CreaT ranges from 20 to 160  $\mu\text{M}$  depending on species and location of transporter (i.e., red blood cell, macrophage, muscle fiber type) (Ku and Passow, 1980; Loike et al., 1986; Moller and Hamprecht, 1989; Guimbal and Kilimann, 1993; Schloss et al., 1994; Sora et al., 1994; Willott et al., 1999). Blood levels of Cr vary between species with rat > mouse > rabbit > human (Marescau et al., 1986). Table 1 summarizes the blood levels and  $K_m$  of Cr transporters in various species.

The content of tCr is dependent on the skeletal muscle fiber type. Type 2 fibers have higher levels of Cr and PCr (Meyer et al., 1985; Kushmerick et al., 1992; Casey et al., 1996). Rodent Type 2a and 2b fibers contain  $\sim 32$  mM PCr and 7 mM Cr and the EDL, a Type 2 fiber-rich muscle, has a higher  $K_m$  (160  $\mu\text{M}$ ) and higher  $V_{\text{max}}$  (100  $\text{nmol h}^{-1}$  g wet weight) compared with the Type 1 fiber-rich soleus. Type 1 fibers in rodents have  $\sim 16$  mM PCr and 7 mM Cr and the Type 1 fiber-rich soleus has a  $K_m = 73$   $\mu\text{M}$  and a  $V_{\text{max}} = 77$   $\text{nmol h}^{-1}$  g wet weight (Kushmerick et al., 1992; Willott et al., 1999). Therefore, Cr uptake is muscle fiber-type dependent. In humans, intramuscular levels of Cr have been found to be  $\sim 125$   $\text{mmol kg}^{-1}$  dry muscle (DM) with  $\sim 60\%$  of tCr in the form of PCr (Harris et al., 1992; Balsom et al., 1995; Casey et al., 1996; Hultman et al., 1996). For example, Hultman et al. found tCr levels in humans of 123  $\text{mmol kg}^{-1}$  DM of which 80.36  $\text{mmol kg}^{-1}$  DM was PCr ( $\sim 65\%$ ) and 43.01  $\text{mmol kg}^{-1}$  DM was Cr ( $\sim 35\%$ ). In general, human muscle tCr levels can range from 110 to 160  $\text{mmol kg}^{-1}$  DM (Harris et al., 1974).

Catecholamines, insulin-like growth factor 1 (IGF-1), insulin, and exercise can influence the net uptake of Cr into skeletal muscle. Odoom et al. (1996) used a G8 mouse skeletal muscle cell line to study the effects of  $\alpha$ - and  $\beta$ -agonists, IGF-1, and insulin on Cr uptake. Thyroid hormone ( $T_3$ ) increased tCr content up to 3-fold relative to controls, and IGF-1 increased tCr content by 40 to 60% relative to controls. Insulin at 3 nM stimulated tCr accumulation by 2.3-fold relative to control. Other studies have shown that both insulin and carbohydrate increase tCr accumulation in both humans and rodents (Haugland and Chang, 1975; Green et al., 1996a,b; Steenge et al., 1998, 2000). In the G8 cell line, the nonspecific  $\beta$ -agonist isoproterenol increased tCr content 40 to 60%, which is similar to that of the nonspecific  $\alpha$ , $\beta$ -agonist norepinephrine. The  $\alpha_1$ -agonist methoxamine decreased tCr content by 30% whereas the  $\beta_2$ -agonist clenbuterol increased tCr content by 30%. The  $\beta$ -antagonists (i.e., atenolol, butoxamine, and propranolol) caused a slight reduction ( $<10\%$ ) in tCr content.

Exercise has also shown stimulatory effects on Cr uptake (Harris et al., 1992; Robinson et al., 1999). Harris supplemented human subjects with Cr ( $4 \times 5$  g for 3–5 days) followed by one-legged cycle ergometry (Harris et al., 1992). The tCr in the exercised leg increased from 118.1  $\text{mmol kg}^{-1}$  DM to 162.2  $\text{mmol kg}^{-1}$  DM ( $\sim 37\%$  increase) with 103.1  $\text{mmol kg}^{-1}$  DM as PCr. The control leg increased from 118.1  $\text{mmol kg}^{-1}$  DM to 148.5  $\text{mmol kg}^{-1}$  DM ( $\sim 25\%$  increase) with 93.8  $\text{mmol kg}^{-1}$  DM of PCr. It was hypothesized that increased uptake resulted from enhanced blood flow, but changes in transport kinetics were not ruled out. It is possible the exercise may increase the translocation of CreaT to the muscle membrane similar to effects seen between exercise and GLUT-4 translocation (Thorell et al., 1999).

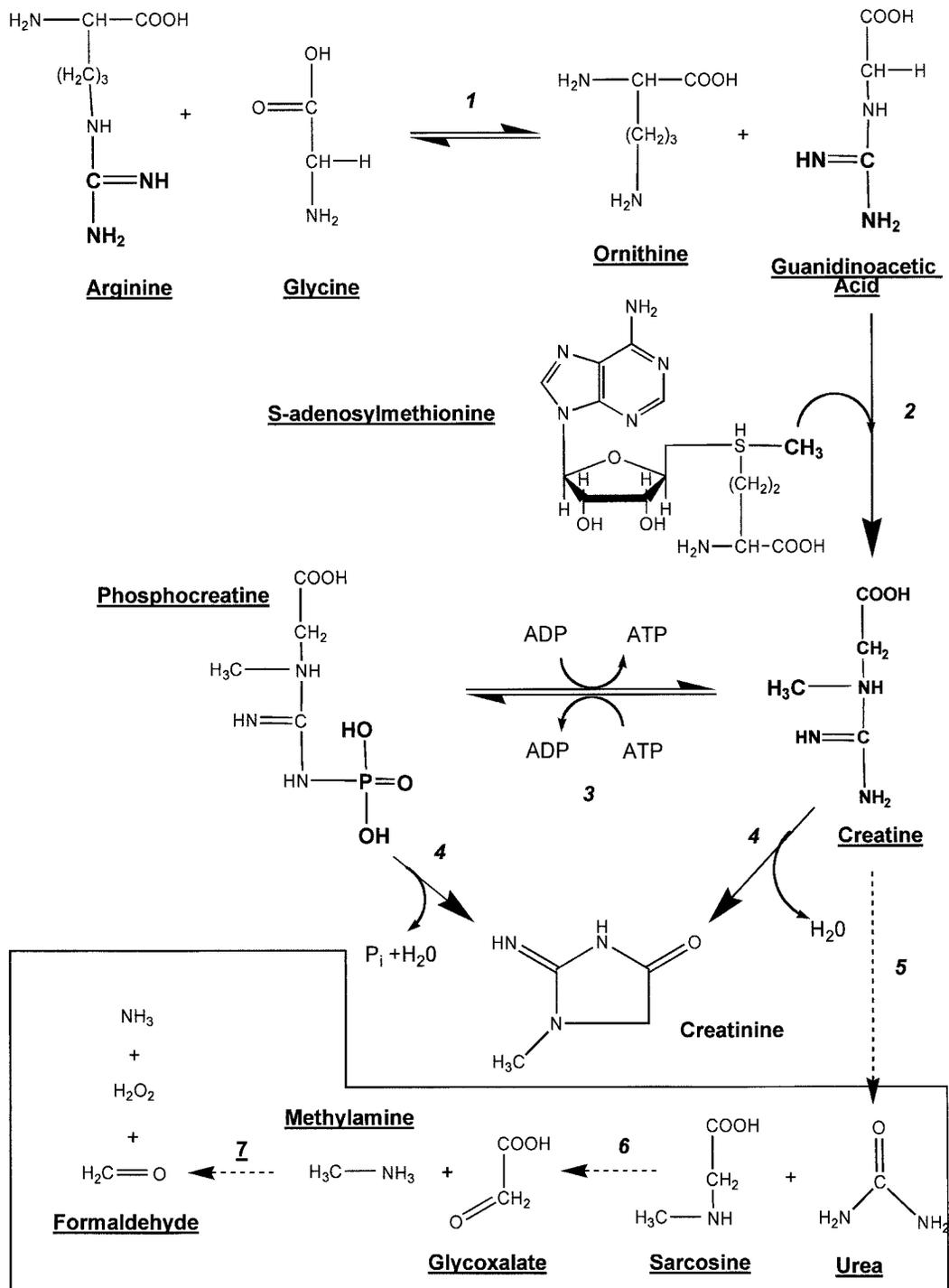


FIG. 1. Pathway of creatine metabolism. Catalyzed by AGAT (1), catalyzed by GAMT (2), catalyzed by creatine kinase (CK) (3), spontaneous (4), catalyzed by creatine amidohydrolase (5), catalyzed by glycine oxidase (6), and catalyzed by semicarbazide-sensitive amine oxidase (SSAO) (7). Dotted pathway indicates recently hypothesized toxic formation of formaldehyde by Yu and Deng (2000).

### III. Mechanisms of Action

Cr exerts various effects upon entering the muscle. It is these effects that elicit improvements in exercise performance and may be responsible for the improvements of muscle function and energy metabolism seen under certain disease conditions. Several mechanisms have been proposed to explain the increased exercise performance seen after acute and chronic Cr intake.

#### A. Energy Metabolism

Adenosine triphosphate (ATP) concentrations maintain physiological processes and protect tissue from hypoxia-induced damage. Cr is involved in ATP production through its involvement in PCr energy system. This system can serve as a temporal and spatial energy buffer as well as a pH buffer. As a spatial energy buffer, Cr and PCr are involved in the shuttling of ATP from the inner

TABLE 1  
 Values for blood levels of creatine and creatine transporter  $K_M$  across species

Species	Blood Cr	$K_M$
	$\mu M$	$\mu M$
Bovine		187 <sup>a</sup> (Dodd et al., 1999)
Dog	30,000 (Lowe et al., 1998), 50–100 (Harris and Lowe, 1995)	
Human	50–100 (Harris et al., 1992; Marescau et al., 1986)	15, <sup>a</sup> 20, <sup>f</sup> 30 <sup>c</sup> (Ku and Passow, 1980; Loike et al., 1986; Sora et al., 1994)
Mouse	200 (Marescau et al., 1986)	45, <sup>d</sup> 110 <sup>e</sup> (Moller and Hamprecht, 1989; Odoom et al., 1996)
Rabbit	150 (Marescau et al., 1986)	35 <sup>a</sup> (Guimbal and Kilimann, 1993)
Rat	500–600 (Horn et al., 1998; Marescau et al., 1986)	22, <sup>a</sup> 46, <sup>a</sup> 73, <sup>b</sup> 160, <sup>b</sup> 500, <sup>b</sup> 40–60, <sup>e</sup> 45 (Fitch and Shields, 1966; Fitch et al., 1968; Moller and Hamprecht, 1989; Schloss et al., 1994; Willott et al., 1999)
	140 (Fitch and Shields, 1966)	

<sup>a</sup> Cloned transporter.

<sup>b</sup> Intact muscle.

<sup>c</sup> White blood cell.

<sup>d</sup> Astroglia.

<sup>e</sup> Cell culture (L6 or G8).

<sup>f</sup> Red blood cell.

mitochondria into the cytosol (Meyer et al., 1984; Bessman and Carpenter, 1985). In the reversible reaction catalyzed by creatine kinase, Cr and ATP form PCr and adenosine diphosphate (ADP) (Fig. 2). It is this reaction that can serve as both a temporal energy buffer and pH buffer. The formation of the polar PCr “locks” Cr in the muscle and maintains the retention of Cr because the charge prevents partitioning through biological membranes (Greenhaff, 1997) (Fig. 2). At times during low pH (viz., during exercise when lactic acid accumulates), the reaction will favor the generation of ATP. Conversely, during recovery periods (e.g., periods of rest between exercise sets) where ATP is being generated aerobically, the reaction will proceed toward the right and increase PCr levels. This energy and pH buffer is

one mechanism by which Cr works to increase exercise performance.

Finally, Cr is also involved in regulating glycolysis. When humans and animals are depleted of tissue Cr, they adapt by increasing oxidative enzymes such as mitochondrial creatine kinase (O’Gorman et al., 1996), succinate dehydrogenase (Ren et al., 1993; O’Gorman et al., 1996), citrate synthase (Ren et al., 1993), and GLUT-4 glucose transporters (Ren et al., 1993). All of these proteins are involved in aerobic metabolism and can offset the lack of anaerobic energy supplied by the PCr system. Little information is available on whether enzyme activities are affected by increasing intracellular Cr stores. One study by Brannon et al. (1997) found citrate synthase activity increased in the soleus but not the plantaris in rodents supplemented with 3.3 mg of Cr per gram of diet. PCr and inorganic phosphate may also regulate energy processes by inhibiting the enzymes glycogen phosphorylase  $\alpha$ , phosphofructokinase, pyruvate kinase, and lactate dehydrogenase (Wyss and Kaddurah-Daouk, 2000). However, the control of PCr on these enzymes has come under debate since the PCr used in these studies contained impurities like inorganic pyrophosphate (Wyss and Kaddurah-Daouk, 2000).

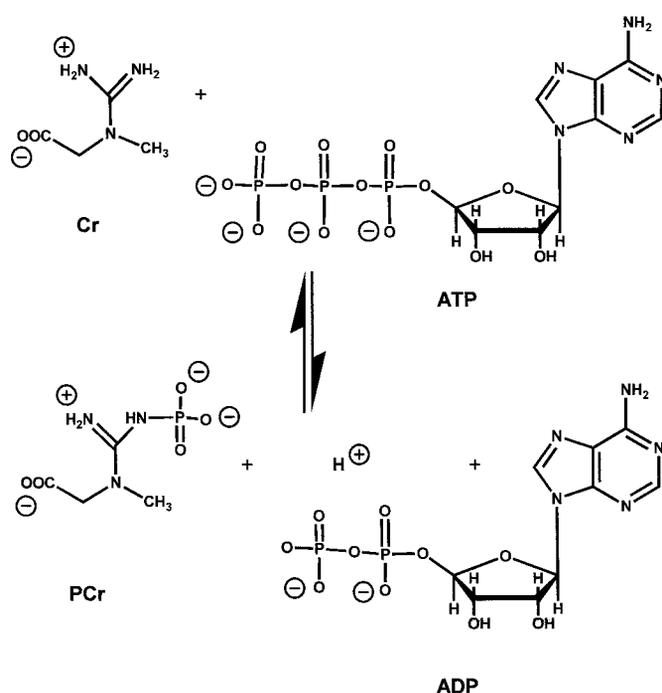


FIG. 2. Phosphorylation of Cr by ATP to form PCr and ADP. Large negative charges on phosphocreatine prevent diffusion across biological membranes thus locking phosphocreatine in the muscle cell.

### B. Protein Synthesis

One beneficial effect of Cr supplementation in young, healthy males is enhanced muscle fiber size and increased lean body mass. Typically, Cr loading of 20 g/day for 4 to 28 days in humans increases total body mass from 1 to 2 kg (Balsom et al., 1993; Greenhaff et al., 1994; Earnest et al., 1995; Green et al., 1996a; Vandenberghe et al., 1997; Kreider et al., 1998; Maganaris and Maughan, 1998; McNaughton et al., 1998; Snow et al., 1998) with increases coming from fat-free mass (Vandenberghe et al., 1997; Kreider et al., 1998; Volek et al., 1999; Becque et al., 2000; Mihic et al., 2000). Volek et al. (1999) found after 12 weeks of resistance training in men, Cr supplementation increased muscle fiber diameter in both Type 1 and Type 2 muscle fibers by 35%

(Fig. 3). Resistance-trained subjects not supplemented with Cr had fiber-type increases of 6 to 15%. Subjects both trained and supplemented had fat-free mass increases of 1.5 kg after 1 week and 4.3 kg after 12 weeks compared with the trained-only group that had a fat-free mass increase of 2.1 kg after 12 weeks. Sipila et al. (1981) found a 42% increase in Type 2 muscle fibers after 1 year of supplementation of 1.5 g/day in patients with gyrate atrophy without resistance training.

The increases in muscle mass may result from increased protein synthesis or reduced protein catabolism. Studies using cell culture by Ingwall and colleagues (Ingwall et al., 1972, 1974, 1975; Ingwall, 1976; Ingwall and Wildenthal, 1976) support the theory that exogenous Cr can increase protein synthesis both in vitro and in vivo. It was hypothesized by the authors that Cr, an end-product of contraction, may serve as a stimulus of protein synthesis and muscle hypertrophy. They found the rate of myosin and actin synthesis in chick embryo myoblasts increased in the presence of Cr, but the degradation rate of the muscle proteins remained unchanged. However, using a similar model to Ingwall, Fry and Morales (1980) did not find an effect of Cr on protein synthesis in cell culture. Recently, Tarnopolsky's group (Parise et al., 2000) reported measuring protein synthesis using whole body leucine kinetics and mixed muscle fractional protein synthetic rates during Cr supplementation in humans. They found no increase in protein synthesis, but a possible decrease in protein catabolism. The results from cell culture and the human study offer conflicting results as far as the role of Cr and regulation of protein metabolism. The equivocal results from cell culture may be the result of small changes in culture conditions or the method by which protein synthetic rates were determined. Future research should focus on humans especially with respect to changes in myosin and actin metabolism in Type 2 muscle fibers.

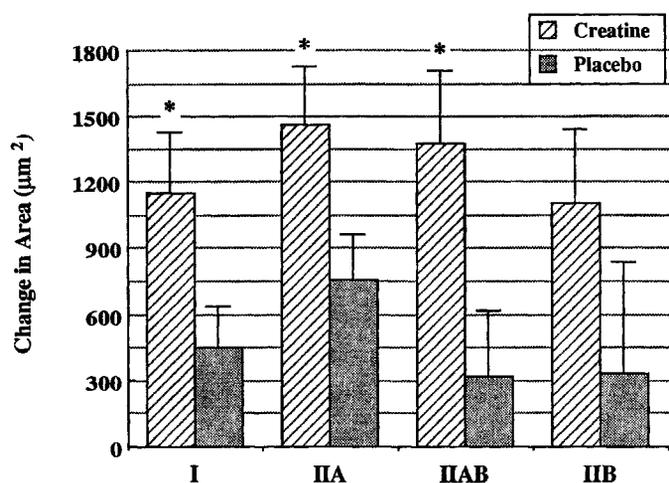


FIG. 3. Effect of weight-training and creatine supplementation on changes in cross-sectional area for different muscle fiber types. Human subjects were supplemented with  $5 \times 5$  g/day for 7 days and maintenance was 5 g/day for 11 weeks. Reprinted with permission from Volek et al. (1999).

The regulation of protein metabolism by an osmotic agent like Cr is supported by studies investigating the effect of cell swelling on protein synthesis. When Cr accumulates in cells, water drag occurs and increases cell hydration. Hyperhydration can act as an anabolic signal stimulating protein synthesis (Haussinger et al., 1994) or the hypo-osmolality can act as a protein-sparing signal and reduce protein degradation (Berneis et al., 1999). This theory of Cr-induced hydration affecting protein synthesis is still under debate because it has not been directly investigated.

Another mechanism by which Cr may increase muscle mass is Cr may be involved in satellite cell activity (Dangott et al., 2000). Dangott and colleagues examined the effect of Cr on compensatory hypertrophy in the rodent. There was no difference between supplemented and unsupplemented groups with regard to muscle mass and fiber diameter for muscles that underwent compensatory hypertrophy. The combination of Cr and increased functional loading did increase satellite cell mitotic activity.

### C. Membrane Stabilization

Cr can potentially prevent tissue damage by two possible mechanisms. The first mechanism involves stabilization of cellular membranes and the second involves maintenance of ATP. Cr, more specifically PCr, may stabilize membranes due to the zwitterion nature of PCr with negatively charged phosphate and positively charged guanidino groups. PCr binds to the phospholipid head groups and thus decreases membrane fluidity and decreases loss of cytoplasmic contents such as intracellular enzymes (e.g., creatine kinase). Sharov et al. (1987) administered PCr to attenuate ischemic damage to cardiomyocytes of rabbit. They found that PCr decreased the elevation in inulin diffusible space seen in untreated cardiomyocytes indicating maintenance of membrane integrity and reduced necrotic zone size (Fig. 4).

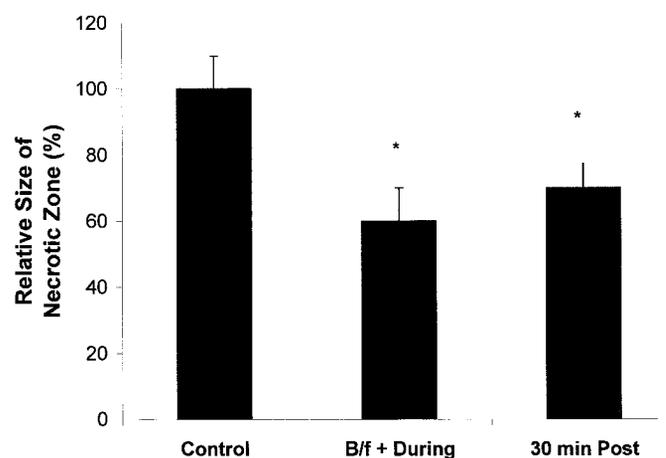


FIG. 4. Effect of phosphocreatine on myocardial infarct size. Necrotic zones were determined after coronary artery ligation of rabbit hearts. Phosphocreatine was administered either before and during (B/f + during) ligation (bolus  $20 \text{ mg kg}^{-1}$ , i.v. infusion of  $20 \text{ mg mg}^{-1}$  for 2 h) or 30 min after (30 min post) ligation in the same doses as before. Reprinted with permission from Sharov et al. (1987).

Recently studies have examined whether Cr supplementation would reduce exercise-induced muscle damage. No difference was found in the indirect indicators of muscle damage in a double-blind placebo study in males between the Cr supplement groups and unsupplemented control (Rawson et al., 2001). However, oxidative damage markers were not measured, and it may be possible that Cr attenuated oxidative stress by maintaining mitochondrial energy homeostasis.

The second mechanism of protection relates to ATP production. In cases of transient ischemia, the ability to generate ATP through oxidative pathways is reduced resulting in cell damage. Since Cr supplementation increases PCr, there is a higher reserve of ATP, thus providing the energy until eupoxic conditions are re-established.

#### IV. Pharmacokinetics

Research on Cr has predominately focused on the pharmacological properties of Cr; there have been few studies investigating the pharmacokinetics of Cr. Although some studies have shown plasma Cr versus time relationship (Fitch and Sinton, 1964; Harris et al., 1992; Green et al., 1996b; Schedel et al., 1999; Steenge et al., 1998, 2000; Vanakoski et al., 1998), the majority of studies have not reported any estimated or calculated pharmacokinetic parameters (i.e., volume of distribution, clearance, bioavailability, mean residence time, absorption rate, and half-life). If Cr is ever to be used clinically, then the pharmacokinetic profile is needed to establish optimal dosing. Figure 5 is the authors' proposed physiological model for Cr pharmacokinetics based on current literature.

##### A. Dosing

Currently, manufacturer's instructions and athletes' use of Cr follows a dosing regimen of a "loading" phase of 20 g/day ( $4 \times 5$  g) for 5 days and a maintenance dose of 3 to 5 g/day. Investigators have found that intramuscular tCr levels increase from 17 to >20% with a dosing regimen of 20 to 30 g for 2 or more days (Harris et al., 1992; Greenhaff et al., 1994; Balsom et al., 1995; Febbraio et al., 1995; Gordon et al., 1995; Hultman et al., 1996). It has also been reported that up to 20% of this increase is due to PCr (Harris et al., 1992; Gordon et al., 1995; Casey et al., 1996; Hultman et al., 1996; Vandenberghe et al., 1997, 1999). However, there does appear to be an upper limit of intramuscular tCr content at  $\sim 160$  mmol kg<sup>-1</sup> of DM (Harris et al., 1992; Casey et al., 1996). Similar intramuscular PCr levels from this dosing regiment can be accomplished by taking 3 g/day over 30 days (Hultman et al., 1996). After  $\sim 2$  days of loading, maximal accumulation of intramuscular Cr occurs and therefore amounts of >20 g/day are unnecessary (Terjung et al., 2000). The maximal accumulation of intramuscular tCr in humans is reflected in the progressive increase in urinary Cr with continuous Cr ingestion

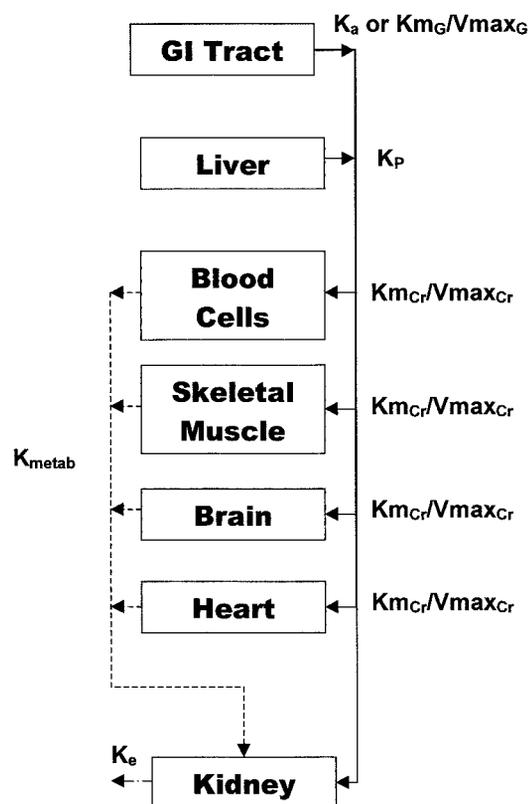


FIG. 5. Schematic of physiological model for creatine pharmacokinetics. Creatine enters the system through diet (first order rate constant  $K_a$  or Michaelis-Menten  $K_{mG}$  and  $V_{maxG}$ ) or zero-order hepatic production ( $K_p$ ).  $K_{mCr}$  and  $V_{maxCr}$  represent Michaelis-Menten kinetics for saturable uptake of creatine by the creatine transporter.  $K_{METAB}$  is the first order rate constant for the conversion of creatine to creatinine.  $K_e$  is elimination rate constant for creatinine. Solid lines indicate the creatine pathway and dotted lines indicate the creatinine pathway. Values for rate constants:  $K_a$  or  $K_{mG}/V_{maxG}$ , none available;  $K_p = 1$  g/day;  $K_{METAB} = 0.017$  days;  $K_{mCr}/V_{maxCr}$ , see Table 1; and  $K_e =$  GFR.

(Harris et al., 1992; Vandenberghe et al., 1997; Bermon et al., 1998; Maganaris and Maughan, 1998). Cr levels in humans can remain elevated for up to 1 month post-supplementation (Febbraio et al., 1995; Hultman et al., 1996).

Clinical studies have used different dosing regimens than those previously mentioned in the exercise literature. Table 2 describes some dosing regimens used in the literature in human subjects for exercise and treatment of disease. These differences in dosing amount and duration need to be addressed to better understand the regulation of endogenous synthesis of Cr and regulation of transporters.

##### B. Absorption and Distribution

Cr is administered orally either as a solution or solid dosage form. Oral absorption of Cr is determined by physicochemical properties of the molecule as well as splanchnic blood flow. Drugs and nutrients can pass through the gastrointestinal tract epithelia into the blood by diffusion, active transport, facilitated transport, or through paracellular pathways. Because Cr is structurally similar to basic amino acids (e.g., arginine,

TABLE 2  
Selected dosing amounts and duration for creatine supplementation

Study	Dosage	Duration	Application
Andrews et al. (1998)	4 × 5 g/day (20 g/day)	5 days	Congestive heart failure
Dechent et al. (1999)	4 × 5 g/day	4 weeks	Increase brain Cr
Hagenfeldt et al. (1994)	2 × 5 g/day (10 g/day)	2 weeks	MELAS
	2 × 2 g/day maintenance		
Heinänen et al. (1999)	1.5–2 g/day	8–15 years	Gyrate atrophy
Hultman et al. (1996)	3 g/day	>30 days	Exercise performance
Recreational use	4 × 5 g/day (20 g/day, 0.3 g kg <sup>-1</sup> )	4–6 days	Exercise performance
	3–5 g/day maintenance	Varies	
Tarnopolsky et al. (1997)	2 × 5 g/day (10 g/day)	2 weeks	Mitochondrial cytopathies
	2 × 2 g/day (4 g/day) maintenance	1 week	
Tarnopolsky et al. (1999)	10 g/day	5 days	Neuromuscular Disease
	5 g/day maintenance	5–7 days	
Vannas-Sulonen et al. (1985)	3 × 0.5 g/day (1.5 g/day) (adults)	5 years	Gyrate atrophy
	3 × 0.25 g/day (0.75 g/day) (children)		
Volek et al. (1999)	5 × 5 g/day (25 g/day)	7 days	Exercise performance
	5 g/day maintenance	11 weeks	
Vorgerd et al. (2000)	150 mg kg <sup>-1</sup> (~10 g/day)	1 week	Myophosphorylase deficiency
	60 mg kg <sup>-1</sup> (~3 g/day) maintenance	4 weeks	
Walter et al. (2000)	10 g/day (adults)	8 weeks	Various muscular dystrophies
	5 g/day (children)		
Willer et al. (2000)	20 g/day	5 days	Rheumatoid arthritis
	2 g/day maintenance	16 days	

lysine), Cr may enter systemic circulation through the amino acid transporter, peptide transporters, or specialized transporters (i.e., taurine).

Cr may also enter systemic circulation through the paracellular pathway. Creatinine has a molecular weight of 113, a net positive charge at intestinal pH, and a partition coefficient of  $-1.8$ , which allows it to move paracellularly through Caco-2 monolayers and diffuse through biological membranes (Karlsson et al., 1999). Cr has a molecular weight of 131, a net positive charge, and an estimated partition coefficient of  $-2.7$  and therefore should also cross through via the paracellular pathway. However, in a preliminary investigation, Cr was found to have very poor movement through the Caco-2 monolayer (Dash et al., 1999). This lack of movement could be caused by a lack of amino acid transporters specific for Cr or may indicate a lack of importance of paracellular transport in Cr absorption.

Oral administration of low doses of Cr in humans (1–10 g) show a time of maximal plasma concentration ( $T_{\max}$ ) of  $<2$  h (Harris et al., 1992; Green et al., 1996b; Schedel et al., 1999). At doses above 10 g,  $T_{\max}$  increases to  $>3$  h (Schedel et al., 1999). Once in the vasculature, Cr distributes into red blood cells, white blood cells, skeletal muscle, brain, cardiac muscle, spermatozoa, and the retina (Wyss and Kaddurah-Daouk, 2000). Because of low aqueous solubility ( $\sim 13$  mg ml<sup>-1</sup> water) and a low partition coefficient, the apparent volume of distribution should probably not exceed total body water. Protein and tissue binding also determine the volume of distribution; however, there currently is no data on the extent of protein binding.

### C. Clearance

Cr can be eliminated from the blood via two parallel pathways. The first pathway is a saturable uptake into

various organs and cells. The second pathway is renal elimination. As mentioned earlier, insulin, catecholamines, exercise, and IGF-1 can affect Cr uptake by the Na<sup>+</sup>-Cl<sup>-</sup>-dependent transporter. Therefore, clearance of Cr from the blood is dependent on intramuscular tCr levels, hormone levels, muscle mass, and kidney function [glomerular filtration rate (GFR)]. Pitts (1934) found that Cr is excreted at rates equivalent to that of xylose in humans, indicating renal elimination of Cr may be equivalent to GFR. However, Sims and Seldin (1949) found that Cr is reabsorbed in the kidney, which may explain the lack of Cr found in urine under healthy, unsupplemented conditions. This finding supports evidence that CreaT is found in the kidney and may serve to reabsorb Cr from the urine (Wyss and Kaddurah-Daouk, 2000).

### D. Pharmacokinetic Studies

To date, much of the work on Cr has focused on the pharmacological effects rather than on characterizing the pharmacokinetics. Of the studies that examined the behavior of Cr in blood, none have truly characterized the pharmacokinetics except for  $C_{\max}$  and  $T_{\max}$  thus leaving a gap in the research. Despite the lack of pharmacokinetic interpretation, these studies can serve as a basis for future work on Cr pharmacokinetics.

To truly understand the pharmacokinetics of Cr, data are needed after an intravenous bolus dose. Although some studies have administered Cr as an intravenous infusion in humans (Crim et al., 1976) there is only one available intravenous bolus study from Fitch and Sinton (1964). Small amounts of <sup>14</sup>C-Cr (2–60  $\mu$ Ci or 0.1–3 mg) were given as an intravenous bolus to five patients with various muscular disorders and followed over time. The half-life of <sup>14</sup>C-Cr in plasma was calculated to be 20 to 70 min. It appears the Cr follows a one-compartment body model. However, two of the five patients exhibited a

slight distribution phase of less than 40 min. Unfortunately, there is insufficient data at early time points to fully understand the profile after intravenous bolus administration. Clinically, the two patients that had a distribution phase were two of the oldest patients in the study (43 and 77 years of age) and also had two of the heavier body weights (63 and 100 kg). It is unknown how age or body weight would influence Cr pharmacokinetics.

Harris et al. (1992) investigated blood concentrations over time after oral administration of Cr monohydrate in young and middle-aged humans (ages 28–62 years). After a single 5-g dose, plasma Cr reached a mean  $C_{\max}$  of approximately  $100 \text{ mg l}^{-1}$  at a  $T_{\max}$  of 1 h. In another human study, Green et al. (1996b) investigated the effect of carbohydrate ingestion on plasma Cr levels at day 1 and day 3 of a 2-day, 20 g/day regimen. Following a 5-g dose on day 1, plasma Cr reached a  $C_{\max}$  of  $170 \text{ mg l}^{-1}$  at a  $T_{\max}$  of 50 min. When 5 g of Cr was ingested with 500 ml of an 18.5% w/v glucose simple sugar solution, the  $C_{\max}$  for plasma Cr was  $80 \text{ mg l}^{-1}$  and the  $T_{\max}$  was 90 min. The addition of carbohydrate during administration on day 1 caused over a 3-fold reduction in the AUC of plasma Cr. This reduction has been attributed to enhanced removal of Cr from blood caused by the stimulatory effect of insulin on Cr uptake by skeletal muscle. On day 3 after a 5-g dose, plasma Cr had a  $C_{\max}$  of  $234 \text{ mg l}^{-1}$  at a  $T_{\max}$  of 50 min, a nonsignificant 37% increase in  $C_{\max}$ . Interestingly, Green found a nonsignificant ~7% difference in AUC between day 1 and day 3 in the Cr without carbohydrate group. This lack of difference was probably caused by incomplete elimination of Cr from the blood on day 3. On day 1, plasma Cr reached near baseline by 270 min; however, at day 3, plasma Cr was 7 times higher than baseline at 270 min. These data suggest reduced volume of distribution after 2 days of 20 g/day Cr administration. Steenge et al. (1998) also tested the effects of insulin on plasma Cr in humans. In their study, 100 mM Cr was administered as an enteral infusion at  $2.5 \text{ ml min}^{-1}$  with an intravenous insulin infusion at varying rates. Peak Cr levels were reached 1 to 1.5 h after start of infusion. A decrease of 20% in plasma Cr AUC was shown to be dependent on insulin infusion rate.

Based on the work of Odoom et al. (1996) on the stimulatory effects of  $\beta$ -agonists on Cr uptake, Vanakoski et al. (1998) investigated the pharmacokinetics of Cr with and without caffeine ingestion. Following 3 days of  $3 \times 100 \text{ mg kg}^{-1}$  (~15 g/day) Cr ingestion, a single dose of  $100 \text{ mg kg}^{-1}$  (6–7 g) was administered for pharmacokinetic analysis. Cr had a  $C_{\max}$  of  $160 \text{ mg l}^{-1}$  at a  $T_{\max}$  of 92 min and a terminal half-life of 172 min. The concomitant administration of caffeine had no statistically significant effect on Cr pharmacokinetics. Because the pharmacokinetics were calculated after 3 days of loading, this profile may be more indicative of steady-state rather than single-dose pharmacokinetics. Additionally,

this was a double-blind, placebo-controlled crossover design study with 1 week washout between treatments. This would further conflict the pharmacokinetic data because elevated muscle tCr levels can last up to 28 days, and as such, accumulation could confound results by changing volume of distribution.

Recently, Schedel et al. (1999) administered increasing doses and measured plasma Cr over time. They found larger doses lead to longer absorption times, as a single 20-g dose demonstrated an absorption phase even after 4 h. Dr. E. S. Rawson (personal communication) recently compared blood levels of Cr after a 5-g dose in young healthy males and elderly healthy males. They found no difference in pharmacokinetic parameters between groups but found that intramuscular PCr levels in elderly males did not increase with supplementation. The lack of an increase in intramuscular PCr levels seen in this study supports this group's work with supplementation in the elderly in that exercise performance in the elderly does not increase with Cr supplementation.

It is very difficult to compare/contrast studies of Cr pharmacokinetics due to differences in the study design (dose, single versus after multiple doses or infusion), Cr products, and method of analysis (photometric, enzyme, high performance liquid chromatography). It is difficult to determine whether Cr pharmacokinetics is dose-dependent; however, the data by Schedel et al. (1999) indicate this possibility. The dose dependence can be caused by transporter-based uptake into muscle or transporter-based uptake from the gastrointestinal tract. As mentioned earlier, the reported studies are incomplete in the pharmacokinetic analysis, and further research is needed to establish standard pharmacokinetic parameters.

## V. Therapeutic Usage

Although the majority of studies on Cr have been on exercise performance in healthy subjects, recent evidence indicates Cr may be useful in the treatment of certain diseases. Patients with diseases that result in atrophy or muscle fatigue secondary to impaired energy production may benefit from Cr supplementation. The true mechanisms by which Cr can be effective in these diseases are unclear but the theorized mechanisms of increased energy in the form of PCr, increased muscle accretion, and stabilization of membranes may be influential as discussed previously.

Research has recently focused on the clinical application of Cr in rodents and humans, and therefore there is a limited amount of information available on the relationship between the rodent studies and human studies. Although studies involving rodents offer credence in the therapeutic use of Cr, the results may not fully explain the usefulness in humans. Rodents typically have a higher blood Cr level than humans (Marescau et al., 1986) and do not respond to supplementation in the

same manner that humans respond. For example, rats fed a 3% Cr diet for 40 days showed little increase in skeletal muscle tCr levels with large increases in tCr in liver and kidney (Horn et al., 1998). Therefore, the distribution processes in the rodent may differ from humans and may cause some differences in Cr application.

### A. Exercise Performance

The initial studies on Cr supplementation in the 1990s in humans focused on exercise performance, which served as a basis for subsequent clinical research and applications. As mentioned earlier, supplementation increases intramuscular tCr content. The increase in Cr in young healthy males has been shown to enhance anaerobic exercise performance by increasing power output (Earnest et al., 1995), muscular strength and work (Casey et al., 1996; Vandenberghe et al., 1997; Volek et al., 1999), and muscle fiber size (Volek et al., 1999). Studies have also been performed on young healthy females, middle-aged males (30–60 years of age), and the elderly (>60 years of age). Both females (Vandenberghe et al., 1997) and middle-aged males (Smith et al., 1998) benefited from Cr supplementation, but the elderly did not show an exercise performance enhancement (Bermont et al., 1998; Rawson et al., 1999; Rawson and Clarkson, 2000). The lack of an effect in the elderly may be explained by changes in transporter density associated with aging and decreased Cr uptake.

The American College of Sports Medicine recently had a roundtable discussion on the physiological and health effects of Cr supplementation (Terjung et al., 2000). Performance has been enhanced in swimming, all-out cycling, sprinting, repeated jumping, and resistance training (Juhn and Tarnopolsky, 1998a). The greatest improvements in performance have been found in series, high-power output exercises and the latter exercise bouts of a series (Terjung et al., 2000). Those activities that are repetitive in nature and those of high-energy output, which would stress the PCr system, would likely benefit from Cr supplementation (Terjung et al., 2000).

### B. Gyrate Atrophy

**1. Human Studies.** Gyrate atrophy (GA) is an autosomal recessive error that causes hyperornithinaemia and leads to chorioretinal degeneration and atrophy of Type 2 muscle fibers (Heinänen et al., 1999b). GA patients have lower levels of skeletal muscle PCr since ornithine inhibits the rate-limiting step of Cr biosynthesis (Heinänen et al., 1999b). Current therapy for GA can include diet modification to reduce plasma ornithine (Sipila et al., 1981). Sipila et al. (1981) supplemented seven patients with 1.5 g of creatine daily for 1 year. The diameters of Type 2 muscle fibers increased from 34.1 to 49.9  $\mu\text{m}$  (~ 45%) without a significant increase in the diameters of Type 1 fibers. Examination of the eyes revealed a slowing of impairment at an age normally associated with rapid progression of the disease. An-

other prospective study followed 13 GA patients for 5 years who were treated with 0.75 to 1.5 g (depending on age) of Cr per day (Vannas-Sulonen et al., 1985). The progression of the disease was unaffected by Cr but abnormalities in skeletal muscle such as tubular aggregates and Type 2 fiber atrophy disappeared. Discontinuation of Cr therapy in these patients caused reappearance of tubular aggregates. Patients supplemented with Cr (1.5–2.0 g/day) for 8 to 15 years were found to have a greater than 1.5-fold increase in PCr/P<sub>i</sub> ratio than patients receiving no Cr (Heinänen et al., 1999a). The supplemented group had nearly equivalent PCr/P<sub>i</sub> levels compared with healthy age- and sex-matched controls. The PCr/ATP ratio of Cr-treated patients was also similar to healthy controls. Additionally, patients supplemented with Cr precursors guanidinoacetate and methionine had increased muscle PCr although not as high as normal controls (Heinänen et al., 1999a).

### C. Diseases Affecting Mitochondria

Because Cr is involved in energy production and acts as a shuttle of ATP from the inner mitochondria to the cytosol, Cr was theorized to be useful in diseases of mitochondria where energy production is altered. Cr supplementation has been shown to be beneficial in diseases in which there is mitochondrial dysfunction such as Parkinson's, Huntington's, and myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS).

#### 1. Parkinson's Disease.

**a. Animal Studies.** Parkinson's disease is an idiopathic neurodegenerative disease characterized by depletion of dopamine levels in the brain. The loss of dopaminergic neurons may be caused by energy impairment resulting in cell death. MPTP neurotoxicity is used as a model for Parkinson's. MPTP is converted to MPP<sup>+</sup>, which inhibits complex I of the electron transport chain and impairs oxidative phosphorylation and subsequent ATP production. The administration of MPTP alone results in 70% depletion in brain dopamine levels in rodents (Matthews et al., 1999). Matthews et al. (1999) used this model and found that rats fed a 1% Cr diet (w/w diet) for 2 weeks showed less than a 10% brain dopamine loss when compared with nonsupplemented animals after exposure to MPTP/MPP<sup>+</sup>. There was a dose dependence from 0.25 to 1% Cr diet; however, this protection disappeared at 2 and 3% Cr diet. Interestingly, the Cr analog cyclocreatine was also neuroprotective at concentrations of 0.25 to 1% w/w diet. Histologically, there was no significant loss of nigral neurons in the Cr-treated group. There was no explanation for the inverted U-shaped response curve in dopamine protection or whether higher doses elicited additional beneficial or toxicological effects. Reasons for the inverted U-shape may be the result of changes in CreaT density, changes in intracellular osmotic pressure, or dysfunction in energy metabolism. Additionally, no intracellular Cr, tCr, PCr, or ATP levels were measured in this study.

## 2. Huntington's Disease.

*a. Animal Studies.* Huntington's disease results in the formation of lesions in the brain from an alteration in energy production. Matthews et al. (1998) used 3-nitropropionic acid (3-NP) to mimic changes in energy metabolism seen in Huntington's. 3-NP irreversibly inhibits complex II of the electron transport system and produces lesions caused by energy depletion. They reported that 1% Cr (w/w diet) after 2 weeks showed an 83% reduction in lesion volume as compared with untreated animals. Animals treated with the Cr analog cyclocreatine showed no protection and appeared to have exacerbated toxicity. Malonate can also be used to induce Huntington's-like lesions. In the same study, Matthews et al. found similar protection against malonate-induced toxicity with a U-shaped dose-response curve using a 1 and 2% Cr w/w diet demonstrating the most protection. In these studies, Cr-fed animals had higher striatal levels of PCr than control animals and Cr-treated animals exposed to 3-NP had higher levels of Cr, PCr, AMP, GDP, NAD, ATP, and lower levels of lactate than control animals treated with 3-NP. These changes would correlate with improved energy production. Cr-fed animals also showed reduced markers of oxidative damage caused by malonate or 3-NP. Again, no reason was given for the U-shaped response curve of Cr against lesion size.

Ferrante et al. (2000) used the transgenic R6/2 mouse model for Huntington's disease to examine the effect of Cr. There was a U-shaped dose-dependent increase of 9.4%, 17.4% for survival in mice fed a 1 and 2%, respectively. However, only a 4.4% increase in survival was found for a 3% w/w diet of Cr. Mice supplemented with Cr also showed increased rotarod performance when fed 1 and 2% Cr but not a 3% diet. Additionally, Cr maintained brain weight, reduced striatal atrophy, reduced striatal aggregates, and delayed the onset of diabetes. A recent study by Shear et al. (2000) supports the previous studies that Cr can attenuate anatomical abnormalities induced by 3-NP as well as improve motor performance variables.

## 3. Other Mitochondrial Pathologies.

*a. Animal Studies.* Other mitochondrial-related diseases can be affected by Cr supplementation. In a model for amyotrophic lateral sclerosis, GP3A transgenic mice (SOD1 mutation) had a life-span increased by 13 and 26 days when fed 1% or 2% Cr (w/w diet), respectively (Klivenyi et al., 1999). These animals also had no increase in 3-nitrotyrosine and other indicators of oxidative damage and showed increased motor performance, and Cr protected against loss of motor neurons and substantia nigra neurons. However, no levels of cellular tCr, Cr, PCr, ATP, or ADP were assessed in this study.

*b. Human Studies.* In a large study of 81 patients, Tarnopolsky and Martin (1999) investigated Cr supplementation in various neuromuscular diseases including mitochondrial cytopathies, neuropathic disor-

ders, dystrophies, congenital myopathies, and inflammatory myopathies. They found increases in high-intensity strength measurements such as isometric dorsiflexion, handgrip strength, and isokinetic and isometric knee strength in these patients following supplementation of 10 g/day for 5 days with 5 g/day for 5 to 7 days of maintenance. These patients also showed small but significant increases in body weight with supplementation. In the same investigation, 21 patients were supplemented in a single-blind placebo-controlled study and found results similar to that of the 81-patient study. Tarnopolsky's group also performed a short-term, randomized, crossover trial of Cr supplementation in patients with mitochondrial cytopathies (MELAS) (Tarnopolsky et al., 1997). Patients treated with Cr ( $2 \times 5$  g/day for 2 weeks with  $2 \times 2$  g/day for 1 week of maintenance) showed a 19% increase in hand-grip strength and a reduction in post-exercise cycle ergometry blood lactate. There were no differences in body composition, maximal voluntary contraction, resting energy expenditure, oxygen consumption, or rating of perceived exertion. It was concluded that Cr increased strength and high-intensity anaerobic and aerobic activities with no effect in lower intensity aerobic activity. Most of the patients in this study were already taking vitamin E and C and coenzyme Q<sub>10</sub> for treatment of their mitochondrial cytopathy.

## D. Other Brain Pathologies

*1. Animal Studies.* Hypoxia and energy-related brain pathologies (e.g., stroke) might benefit from Cr supplementation. Cr has been shown to protect the brainstem and hippocampus from hypoxia and that this protection may be attributable to the prevention of ATP depletion (Balestrino et al., 1999; Dechent et al., 1999; Wilken et al., 2000). Rodents supplemented with Cr ( $\sim 2$  g kg<sup>-1</sup> of body weight per day) showed increased brain Cr:choline levels with a slight decrease in apparent diffusion coefficient (ADC) during an acute ischemic challenge (Wick et al., 1999). ADC is associated with cytotoxic cellular swelling, and therefore a reduction in ADC may offer protection. Michaelis et al. (1999) found that Cr supplementation ( $\sim 2$  g kg<sup>-1</sup> of body weight per day) showed no differences in metabolic responses after global cerebral ischemia despite increased brain tCr. Due to increases in glucose and slight reductions in lactate found in the Cr-fed group, the authors concluded that neuroprotection may occur with more focal ischemia rather than global ischemia.

Cr has been found to be neuroprotective against *N*-methyl-D-aspartate and malonate excitotoxicity following a 1% (w/w) diet for 1 week in rats (Malcon et al., 2000). These investigators did not find protection against  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or kainic toxicity. In either case, no dose-response relationship was established. Cr has been shown

to protect hippocampal neurons from glutamate toxicity and partially protect embryonic neurons from  $\beta$ -amyloid toxicity (Brewer and Wallimann, 2000). This protection against  $\beta$ -amyloid was also seen in adult and aged neurons and therefore may attenuate the formation of senile plaques seen in Alzheimer's disease. In both cases, intracellular Cr and PCr were elevated when compared with toxin-treated neurons not supplemented with Cr.

**2. Human Studies.** There are few clinical data on the effect of Cr in the human brain. Stockler et al. (1994, 1996) report a treatable inborn error in Cr metabolism that causes tCr depletion in the brain and results in extrapyramidal movement disorders. Treatment with Cr in these patients restores Cr levels and improves neurologic symptoms. Other studies have found supplementation ( $4 \times 5$  g/day) for 4 weeks in human volunteers caused an 8.7% increase in brain tCr. The largest increases were seen in gray matter (4.7%), white matter (11.5%), cerebellum (5.4%), and thalamus (14.6%). Although no human studies have been done on Cr supplementation and resistance to brain injury, the increase in brain Cr may be relevant in ischemic injury similar to that seen in the rodent models.

### E. Muscular Disease

**1. Animal Studies.** Since 95% of Cr in the body is found in skeletal muscle, supplementation may be useful in treating myopathies. Duchenne's muscular dystrophy is a degenerative disease that causes mechanical instability of the sarcolemma leading to increased calcium leakage during periods of stress. Using *mdx* mice as a model for Duchenne's muscular dystrophy, Pulido et al. (1998) prepared a primary cell culture from hind-limb muscles. During myotube formation, cells were incubated with 20 mM Cr. After 12 to 14 days, cells were exposed to hypo-osmotic shock. Cells treated with Cr showed significantly lower intracellular calcium levels that were nearly equivalent to baseline calcium levels of control myotubes. This effect of Cr could be due to decreased sarcolemmal leakage or enhanced uptake by the sarcoplasmic reticulum. Further evidence from the Pulido study supported more of an effect on calcium uptake by sarcoplasmic reticulum  $\text{Ca}^{2+}$  ATPase. Intracellular PCr increased in both *mdx* and control myotubes with the former having a more pronounced increase.

**2. Human Studies.** In a double-blind crossover clinical study, Felber et al. (2000) examined Cr supplementation (10 g/day for adults and 5 g/day for children) for 8 weeks in 32 patients with various muscular dystrophies. At the end of the treatment period, the Cr group had a 3% increase in strength and a 10% increase in neuromuscular symptom score. There were no differences in clinical chemistries between groups. The authors concluded that long-term Cr supplementation in this population is needed.

In other studies related to muscle, patients with rheumatoid arthritis had strength improvements after sup-

plementation with 20 g of Cr/day for 5 days and then 2 g/day for the remaining 16 days but no change in physical functional ability or disease activity (Willer et al., 2000). This was an open study examining arthritis pre- and post-supplementation, but after supplementation there was a small increase in muscle Cr (~7%) and a decrease in both PCr (~24%) and tCr (~14.3%). The lack of change in muscle tCr may reflect the lack of change in functional ability and raises a more important question of why these patients did show the more typical increase of 20% seen in young healthy males. Patients with myophosphorylase deficiency (McArdle's disease) showed mild improvements from supplementation of 150 mg  $\text{kg}^{-1}$  for 1 week with maintenance doses of 60 mg  $\text{kg}^{-1}$  day<sup>-1</sup> in a placebo-controlled crossover trial (Vorgerd et al., 2000). These improvements consisted of lower self-reported severity and lower frequency of muscle pain and increased exercise performance including increased strength. Cr-treated patients showed increase in muscle PCr and increases in exercise performance during ischemia. This was the first study to examine the effects of Cr supplementation in McArdle's disease.

### F. Heart Disease

**1. Animal Studies.** The effects of Cr on cardiac tissue have been investigated. A study by Sharov et al. (1987) showed a protective effect of PCr on cardiac tissue following ischemia. Using rabbit hearts, PCr was administered intravenously either before and during cardiac artery ligation or 30 min post-ligation. These investigators found a reduction in necrotic zone under both PCr treatments compared with controls (Fig. 4). Ruda et al. (1988) found that PCr administration reduced ventricular arrhythmia after acute myocardial infarctions, but the effects of Cr on cardiac tissue are still unclear. Other studies have also shown PCr to possess anti-arrhythmic activities (Rosenshtraukh et al., 1988). Feeding Cr to healthy rats or rats after a myocardial infarction failed to increase intramuscular Cr (Horn et al., 1998). The  $\beta$ -blocker bisoprolol has been shown to increase total cardiac Cr up to 40% (Laser et al., 1996). The ability to increase Cr and related energetics in heart tissue may be one beneficial mechanism of the action of  $\beta$ -blocker therapy (Laser et al., 1996). Ingwall et al. (1985) have also shown that diseased myocardium has lower Cr content. Supplementation with Cr has also provided protection to cardiac tissue from metabolic stress (Constantin-Teodosiu et al., 1995).

**2. Human Studies.** Gordon et al. (1995) investigated the effect on ingestion of Cr in patients with congestive heart failure in a double-blind, placebo-controlled study (20 g/day for 10 days). Ejection fraction at rest and at work did not change but increased exercise performance in regard to both strength and endurance. Another study in patients with congestive heart failure showed that Cr supplementation improved skeletal muscle metabolism with reductions in ammonia and lactate accumulation

(Andrews et al., 1998). Recently, Neubauer et al. (1999) showed that hearts with dilated cardiomyopathy had 50% less tCr compared with healthy hearts as well as 30% less CreaT. Cr supplementation also has been shown to lower total plasma cholesterol and triglycerides (Earnest et al., 1996). These results were similar in humans and rodents and may suggest a therapeutic benefit of Cr supplementation.

### G. Use of Creatine Analogs

Analogs of Cr were used initially to study Cr metabolism and uptake. These analogs are currently being investigated as a treatment for Huntington's disease, anti-tumor agents, and as antiviral agents. The most commonly used analogs are  $\beta$ -guanidinopropionic acid and cyclocreatine. This class of compounds has been shown to inhibit replication of several viruses including human and simian cytomegaloviruses and varicella zoster virus (Lillie et al., 1994), to protect neurons from 3-NP toxicity disease (Matthews et al., 1998), and reduce tumor size (Bergnes et al., 1996). A recent article by Wyss and Kaddurah-Daouk (2000) reviews the use and potential use of Cr analogs.

## VI. Side Effects

Side effects from Cr supplementation have been reported both anecdotally and in the scientific literature. Possible side effects of Cr supplementation have been previously reviewed by Juhn and Tarnopolsky (1998b). Briefly, Cr supplementation has been documented as being associated with weight gain, gastrointestinal distress, and renal dysfunction and anecdotally reported to cause muscle cramps and hepatic dysfunction.

Typically weight gain is between 1 and 2 kg and is initially brought on by water retention, but may be maintained by changes in amount of lean body mass. Athletes generally desire this effect. Gastrointestinal distress has been reported anecdotally but little to no studies have documented nausea, vomiting, or diarrhea. This may be a function of single large doses of Cr or subsequent ingestion of large amounts of carbohydrates. Muscle cramps have been reported anecdotally, but published studies have yet to find muscle cramps associated with supplementation.

In a double-blind, crossover study, subjects were supplemented with Cr at 20 g/day ( $4 \times 5$  g/day) for 5 days with a 28-day washout between treatments (Kamber et al., 1999). Supplementation had no effect on hepatic function as indicated by no changes in blood liver enzymes (i.e., creatine kinase, urea, aspartate aminotransferase, alanine aminotransferase,  $\gamma$ -glutamyl transferase, lactate dehydrogenase). This study indicates that short-term supplementation may be safe, but the effect of long-term supplementation is still unknown. Cardiovascular function as assessed by changes in systolic and diastolic blood pressure was unaffected by Cr (Mihic et

al., 2000). Finally, Cr has been implicated in renal dysfunction. In two isolated cases, one patient presented with interstitial nephritis that improved upon termination of Cr use (Koshy et al., 1999), and another patient with focal glomerular sclerosis showed a reduction in GFR with Cr supplementation that returned upon termination of supplementation (Pritchard and Kalra, 1998). Before the diagnosis of focal glomerular sclerosis, the patient had relapsing steroid-responsive nephrotic syndrome and was currently on cyclosporin. It was recently found that cyclosporin inhibits Cr uptake in vitro and may explain the nephropathy brought on by Cr (Tran et al., 2000). Although these pathologies are serious, these were isolated incidences including one patient that had a history of kidney disease. Studies have shown that renal function and glomerular filtration are not effected by supplementation despite slight increases in plasma creatinine (Poortmans et al., 1997; Poortmans and Francaux, 1999). In one of these studies (Poortmans et al., 1997), subjects were self-supplementing with 2 to 30 g of Cr for 10 months to 5 years, and no changes in renal responses to creatinine, urea, or albumin were observed.

It was recently hypothesized that Cr supplementation could be cytotoxic (Yu and Deng, 2000). Cr can be ultimately converted to formaldehyde and hydrogen peroxide by the reaction illustrated in Fig. 1. Formaldehyde has the potential to cross-link proteins and DNA leading to cytotoxicity. The investigators did find increased urine formaldehyde after Cr administration; however, they did not measure markers of protein or DNA cross-linking or indicators of oxidative stress.

## VII. Products

Cr products may be purchased from supermarkets, nutrition stores, and via the Internet. Because Cr falls under the Dietary Supplement Health Education Act of 1994, the Food and Drug Administration does not regulate the quality of dietary supplements but does regulate structure/function claims. Therefore, there is some concern of the quality of products available. A recent review by Benzi (2000) discusses some product quality issues, some of which are discussed briefly here. Commercial Cr is produced from the reaction of sarcosine and cyanamide. This process can yield several possible contaminants such as creatinine, dicyandamide, dihydrotriazines, and ions such as arsenic. The ion contaminants as well as dicyandamide could be a potential health hazard. Therefore, good manufacturing practices need to be employed to protect the consumer. The ultimate goal for product quality research is to establish a monograph for the United States Pharmacopoeia (USP).

## VIII. Conclusion

It has been nearly 170 years since the discovery of Cr, but it was not until the 1990s that athletes began to

supplement themselves to enhance exercise performance and muscle mass. Research has corroborated the reports from athletes that Cr can increase exercise performance and muscle mass especially in conjunction with resistance training. Since then, the use of Cr has been extended to the medical field for the treatment of energy-related and neuromuscular-related diseases. Recent advances in molecular biology has allowed the location and cloning of the creatine transporter, which can further our understanding of Cr physiology and possibly allow a target for pharmacological intervention.

As research explores further applications for the therapeutic use of Cr or Cr analogs, it will be necessary to establish pharmacokinetic information for purposes of dosing and the possible prediction of physiological effects via pharmacokinetic/pharmacodynamic modeling. It will also be necessary to establish good manufacturing practices to ensure product quality to the users. Other concerns need to be addressed regarding long-term Cr use, the identification of side effects, and populations to exclude from supplementation.

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#### REFERENCES

- Andrews R, Greenhaff P, Curtis S, Perry A and Cowley AJ (1998) The effect of dietary creatine supplementation on skeletal muscle metabolism in congestive heart failure. *Eur Heart J* **19**:617–622.
- Balestrino M, Rebaudo R and Lunardi G (1999) Exogenous creatine delays anoxic depolarization and protects from hypoxic damage: Dose-effect relationship. *Brain Res* **816**:124–130.
- Balsom PD, Harridge SD, Söderlund K, Sjödén B and Ekblom B (1993) Creatine supplementation per se does not enhance endurance exercise performance. *Acta Physiol Scand* **149**:521–523.
- Balsom PD, Söderlund K and Ekblom B (1994) Creatine in humans with special reference to creatine supplementation. *Sports Med* **18**:268–280.
- Balsom PD, Söderlund K, Sjödén B and Ekblom B (1995) Skeletal muscle metabolism during short duration high-intensity exercise: Influence of creatine supplementation. *Acta Physiol Scand* **154**:303–310.
- Becque MD, Lochmann JD and Melrose DR (2000) Effects of oral creatine supplementation on muscular strength and body composition. *Med Sci Sports Exerc* **32**:654–658.
- Benzi G (2000) Is there a rationale for the use of creatine either as nutritional supplementation or drug administration in humans participating in a sport? *Pharmacol Res* **41**:255–264.
- Bergnes G, Yuan W, Khandekar VS, O'Keefe MM, Martin KJ, Teicher BA and Kaddurah-Daouk R (1996) Creatine and phosphocreatine analogs: Anticancer activity and enzymatic analysis. *Oncol Res* **8**:121–130.
- Bermon S, Venembre P, Sacht C, Valour S and Dolisi C (1998) Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults. *Acta Physiol Scand* **164**:147–155.
- Berneis K, Ninnis R, Haussinger D and Keller U (1999) Effects of hyper- and hypoosmolality on whole body protein and glucose kinetics in humans. *Am J Physiol* **276**:E188–E195.
- Bessman SP and Carpenter CL (1985) The creatine-creatine phosphate energy shuttle. *Annu Rev Biochem* **54**:831–862.
- Brannon TA, Adams GR, Conniff CL and Baldwin KM (1997) Effects of creatine loading and training on running performance and biochemical properties of rat skeletal muscle. *Med Sci Sports Exerc* **29**:489–495.
- Brewer G and Wallimann T (2000) Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. *J Neurochem* **74**:1968–1978.
- Casey A, Constantin-Teodosiu D, Howell S, Hultman E and Greenhaff PL (1996) Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *Am J Physiol* **271**:E31–E37.
- Constantin-Teodosiu D, Greenhaff PL, Gardiner SM, Randall MD, March JE and Bennett T (1995) Attenuation by creatine of myocardial metabolic stress in Brattleboro rats caused by chronic inhibition of nitric oxide synthase. *Br J Pharmacol* **116**:3288–3292.
- Crim M, Calloway D and Margen S (1976) Creatine metabolism in men: Creatine pool size and turnover in relation to creatine intake. *J Nutr* **106**:371–381.
- Dangott B, Schultz E and Mozdziaik PE (2000) Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. *Int J Sports Med* **21**:13–16.
- Dash A, Miller D, Huai-Yan H, Carnazzo J and Stout J (1999) Evaluation of creatine monohydrate permeability using Caco-2 monolayers as an in vitro model for intestinal absorption (Abstract). *PharmSci* **1**:4222.
- Dechent P, Pouwels PJ, Wilken B, Hanefeld F and Frahm J (1999) Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. *Am J Physiol* **277**:R698–R704.
- Demant TW and Rhodes EC (1999) Effects of creatine supplementation on exercise performance. *Sports Med* **28**:49–60.
- Dodd JR, Zheng T and Christie DL (1999) Creatine accumulation and exchange by HEK293 cells stably expressing high levels of a creatine transporter. *Biochim Biophys Acta* **1472**:128–136.
- Earnest CP, Almada AL and Mitchell TL (1996) High-performance capillary electrophoresis-pure creatine monohydrate reduces blood lipids in men and women. *Clin Sci (Colch)* **91**:113–118.
- Earnest CP, Snell PG, Rodriguez R, Almada AL and Mitchell TL (1995) The effect of creatine monohydrate ingestion on anaerobic power indices, muscular strength and body composition. *Acta Physiol Scand* **153**:207–209.
- Febbraio MA, Flanagan TR, Snow RJ, Zhao S and Carey MF (1995) Effect of creatine supplementation on intramuscular TCR, metabolism and performance during intermittent, supramaximal exercise in humans. *Acta Physiol Scand* **155**:387–395.
- Felber S, Skladal D, Wyss M, Kremser C, Koller A and Sperl W (2000) Oral creatine supplementation in Duchenne muscular dystrophy: A clinical and 31P magnetic resonance spectroscopy study. *Neurol Res* **22**:145–150.
- Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, Kaddurah-Daouk R, Hersch SM and Beal MF (2000) Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* **20**:4389–4397.
- Fitch C and Sinton D (1964) A study of creatine metabolism in diseases causing muscle wasting. *J Clin Invest* **43**:444–452.
- Fitch CD and Shields RP (1966) Creatine metabolism in skeletal muscle. I. Creatine movement across muscle membranes. *J Biol Chem* **241**:3611–3614.
- Fitch CD, Shields RP, Payne WF and Dacus JM (1968) Creatine metabolism in skeletal muscle. 3. Specificity of the creatine entry process. *J Biol Chem* **243**:2024–2027.
- Fry DM and Morales MF (1980) A reexamination of the effects of creatine on muscle protein synthesis in tissue culture. *J Cell Biol* **84**:294–297.
- Gordon A, Hultman E, Kaijser L, Kristjansson S, Rolf CJ, Nyquist O and Sylven C (1995) Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance [see comments]. *Cardiovasc Res* **30**:413–418.
- Graham AS and Hatton RC (1999) Creatine: A review of efficacy and safety. *J Am Pharm Assoc (Wash)* **39**:803–810; quiz 875–877.
- Green AL, Hultman E, Macdonald IA, Sewell DA and Greenhaff PL (1996a) Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am J Physiol* **271**:E821–E826.
- Green AL, Simpson EJ, Littlewood JJ, Macdonald IA and Greenhaff PL (1996b) Carbohydrate ingestion augments creatine retention during creatine feeding in humans. *Acta Physiol Scand* **158**:195–202.
- Greenhaff P (1997) The nutritional biochemistry of creatine. *Nutr Biochem* **8**:610–618.
- Greenhaff PL, Bodin K, Söderlund K and Hultman E (1994) Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. *Am J Physiol* **266**:E725–E730.
- Guerrero-Ontiveros ML and Wallimann T (1998) Creatine supplementation in health and disease. Effects of chronic creatine ingestion in vivo: Down-regulation of the expression of creatine transporter isoforms in skeletal muscle. *Mol Cell Biochem* **184**:427–437.
- Guimbal C and Kilimann MW (1993) A Na(+)-dependent creatine transporter in rabbit brain, muscle, heart, and kidney. cDNA cloning and functional expression. *J Biol Chem* **268**:8418–8421.
- Hagenfeldt L, von Döbeln U, Solders G and Kaijser L (1994) Creatine treatment in MELAS [letter]. *Muscle Nerve* **17**:1236–1237.
- Harris R and Lowe J (1995) Absorption of creatine from meat or other dietary sources by dog. *Vet Record* **137**:595.
- Harris RC, Hultman E and Nordesjo LO (1974) Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scand J Clin Lab Invest* **33**:109–120.
- Harris RC, Söderlund K and Hultman E (1992) Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci (Colch)* **83**:367–374.
- Haugland RB and Chang DT (1975) Insulin effect on creatine transport in skeletal muscle. *Proc Soc Exp Biol Med* **148**:1–4.
- Haussinger D, Lang F and Gerok W (1994) Regulation of cell function by the cellular hydration state. *Am J Physiol* **267**:E343–E355.
- Heinonen K, Nanto-Salonen K, Komu M, Erkintalo M, Alanen A, Heinonen OJ, Pulkki K, Nikoskelainen E, Sipilä I and Simell O (1999a) Creatine corrects muscle 31P spectrum in gyrate atrophy with hyperornithinaemia. *Eur J Clin Invest* **29**:1060–1065.
- Heinonen K, Nanto-Salonen K, Komu M, Erkintalo M, Heinonen OJ, Pulkki K, Valtonen M, Nikoskelainen E, Alanen A and Simell O (1999b) Muscle creatine phosphate in gyrate atrophy of the choroid and retina with hyperornithinaemia—clues to pathogenesis. *Eur J Clin Invest* **29**:426–431.
- Horn M, Frantz S, Remkes H, Laser A, Urban B, Mettenleiter A, Schnackerz K and Neubauer S (1998) Effects of chronic dietary creatine feeding on cardiac energy metabolism and on creatine content in heart, skeletal muscle, brain, liver, and kidney. *J Mol Cell Cardiol* **30**:277–284.
- Hultman E, Söderlund K, Timmons JA, Cederblad G and Greenhaff PL (1996) Muscle creatine loading in men. *J Appl Physiol* **81**:232–237.

- Ingwall J, Kramer M, Fifer M, Lorell B, Shemin R, Grossman W and Allen P (1985) The creatine kinase system in normal and diseased human myocardium. *N Engl J Med* **313**:1050–1054.
- Ingwall JS (1976) Creatine and the control of muscle-specific protein synthesis in cardiac and skeletal muscle. *Circ Res* **38**:1115–1123.
- Ingwall JS, Morales MF and Stockdale FE (1972) Creatine and the control of myosin synthesis in differentiating skeletal muscle. *Proc Natl Acad Sci USA* **69**:2250–2253.
- Ingwall JS, Morales MF, Stockdale FE and Wildenthal K (1975) Creatine: A possible stimulus skeletal cardiac muscle hypertrophy. *Recent Adv Stud Card Struct Metab* **8**:467–481.
- Ingwall JS, Weiner CD, Morales MF, Davis E and Stockdale FE (1974) Specificity of creatine in the control of muscle protein synthesis. *J Cell Biol* **62**:145–151.
- Ingwall JS and Wildenthal K (1976) Role of creatine in the regulation of cardiac protein synthesis. *J Cell Biol* **68**:159–163.
- Jacobs I (1999) Dietary creatine monohydrate supplementation. *Can J Appl Physiol* **24**:503–514.
- Juhn MS and Tarnopolsky M (1998a) Oral creatine supplementation and athletic performance: A critical review. *Clin J Sport Med* **8**:286–297.
- Juhn MS and Tarnopolsky M (1998b) Potential side effects of oral creatine supplementation: A critical review. *Clin J Sport Med* **8**:298–304.
- Kamber M, Koster M, Kreis R, Walker G, Boesch C and Hoppeler H (1999) Creatine supplementation—part I: Performance, clinical chemistry, and muscle volume. *Med Sci Sports Exerc* **31**:1763–1769.
- Karlsson J, Ungell A, Grasjo J and Artursson P (1999) Paracellular drug transport across intestinal epithelia: Influence of charge and induced water flux. *Eur J Pharm Sci* **9**:47–56.
- Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, Mueller G, Wermer M, Kaddurah-Daouk R and Beal MF (1999) Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med* **5**:347–350.
- Koshy KM, Grisdwold E and Schneeberger EE (1999) Interstitial nephritis in a patient taking creatine [letter]. *N Engl J Med* **340**:814–815.
- Kraemer WJ and Volek JS (1999) Creatine supplementation. Its role in human performance. *Clin Sports Med* **18**:651–666.
- Kreider RB, Ferreira M, Wilson M, Grindstaff P, Plisk S, Reinardy J, Cantler E and Almada AL (1998) Effects of creatine supplementation on body composition, strength, and sprint performance. *Med Sci Sports Exerc* **30**:73–82.
- Ku CP and Passow H (1980) Creatine and creatinine transport in old and young human red blood cells. *Biochim Biophys Acta* **600**:212–227.
- Kushmerick MJ, Moerland TS and Wiseman RW (1992) Mammalian skeletal muscle fibers distinguished by contents of phosphocreatine, ATP, and Pi. *Proc Natl Acad Sci USA* **89**:7521–7525.
- Laser A, Neubauer S, Tian R, Hu K, Gaudron P, Ingwall JS and Ertl G (1996) Long-term beta-blocker treatment prevents chronic creatine kinase and lactate dehydrogenase system changes in rat hearts after myocardial infarction. *J Am Coll Cardiol* **27**:487–493.
- Lillie JW, Smees DF, Huffman JH, Hansen LJ, Sidwell RW and Kaddurah-Daouk R (1994) Cyclocreatine (1-carboxymethyl-2-iminoimidazolidine) inhibits the replication of human herpes viruses. *Antiviral Res* **23**:203–218.
- Loike JD, Simes M and Silverstein SC (1986) Creatine uptake, metabolism, and efflux in human monocytes and macrophages. *Am J Physiol* **251**:C1218–C1235.
- Lowe J, Murphy M and Nash V (1998) Changes in plasma and muscle creatine concentration after increases in supplementary dietary creatine in dogs. *J Nutr* **128**:2691S–2693S.
- Maganaris CN and Maughan RJ (1998) Creatine supplementation enhances maximum voluntary isometric force and endurance capacity in resistance trained men. *Acta Physiol Scand* **163**:279–287.
- Malcon C, Kaddurah-Daouk R and Beal M (2000) Neuroprotective effects of creatine administration against NMDA and malonate toxicity. *Brain Res* **860**:195–198.
- Marescau B, De Deyn P, Wiechert P, Van Gorp L and Lowenthal A (1986) Comparative study of guanidino compounds in serum and brain of mouse, rat, rabbit, and man. *J Neurochem* **46**:717–720.
- Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, Kaddurah-Daouk R and Beal MF (1999) Creatine and cyclocreatine attenuate MPTP neurotoxicity. *Exp Neurol* **157**:142–149.
- Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah-Daouk R and Beal MF (1998) Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci* **18**:156–163.
- McNaughton LR, Dalton B and Tarr J (1998) The effects of creatine supplementation on high-intensity exercise performance in elite performers. *Eur J Appl Physiol* **78**:236–240.
- Meyer RA, Brown TR and Kushmerick MJ (1985) Phosphorus nuclear magnetic resonance of fast- and slow-twitch muscle. *Am J Physiol* **248**:C279–C287.
- Meyer RA, Sweeney HL and Kushmerick MJ (1984) A simple analysis of the "phosphocreatine shuttle". *Am J Physiol* **246**:C365–C377.
- Michaelis T, Wick M, Fujimori H, Matsumura A and Frahm J (1999) Proton MRS of oral creatine supplementation in rats. Cerebral metabolite concentrations and ischemic challenge. *NMR Biomed* **12**:309–314.
- Mihic S, MacDonald JR, McKenzie S and Tarnopolsky MA (2000) Acute creatine loading increases fat-free mass, but does not affect blood pressure, plasma creatinine, or CK activity in men and women. *Med Sci Sports Exerc* **32**:291–296.
- Moller A and Hamprecht B (1989) Creatine transport in cultured cells of rat and mouse brain. *J Neurochem* **52**:544–550.
- Mujika I and Padilla S (1997) Creatine supplementation as an ergogenic acid for sports performance in highly trained athletes: A critical review. *Int J Sports Med* **18**:491–496.
- Nash SR, Giros B, Kingsmore SF, Rochelle JM, Suter ST, Gregor P, Seldin MF and Caron MG (1994) Cloning, pharmacological characterization, and genomic localization of the human creatine transporter. *Receptors Channels* **2**:165–174.
- Neubauer S, Remkes H, Spindler M, Horn M, Wiesmann F, Prestle J, Walzel B, Ertl G, Hasenfuss G and Wallimann T (1999) Downregulation of the Na(+)-creatine cotransporter in failing human myocardium and in experimental heart failure. *Circulation* **100**:1847–1850.
- Odom JE, Kemp GJ and Radda GK (1996) The regulation of total creatine content in a myoblast cell line. *Mol Cell Biochem* **158**:179–188.
- O'Gorman E, Beutner G, Wallimann T and Brdiczka D (1996) Differential effects of creatine depletion on the regulation of enzyme activities and on creatine-stimulated mitochondrial respiration in skeletal muscle, heart, and brain. *Biochim Biophys Acta* **1276**:161–170.
- Parise G, Mihic S, MacLellan D, Yarasheski K and Tarnopolsky M (2000) Creatine monohydrate supplementation does not increase whole body or mixed muscle fractional protein synthetic rates in males and females (Abstract). *Med Sci Sport Exerc* **32**:S289.
- Pitts RF (1934) The clearance of creatine in dog and man. *Am J Physiol* **109**:532–541.
- Poortmans JR, Auquier H, Renaut V, Durussel A, Saugy M and Brisson GR (1997) Effect of short-term creatine supplementation on renal responses in men. *Eur J Appl Physiol* **76**:566–567.
- Poortmans JR and Francaux M (1999) Long-term oral creatine supplementation does not impair renal function in healthy athletes [see comments]. *Med Sci Sports Exerc* **31**:1108–1110.
- Pritchard NR and Kalra PA (1998) Renal dysfunction accompanying oral creatine supplements [letter] [see comments]. *Lancet* **351**:1252–1253.
- Pulido SM, Passaquin AC, Leijendekker WJ, Challet C, Wallimann T and Rugg UT (1998) Creatine supplementation improves intracellular Ca<sup>2+</sup> handling and survival in mdx skeletal muscle cells. *FEBS Lett* **439**:357–362.
- Rawson E, Gunn B and Clarkson P (2001) The effects of creatine supplementation on exercise-induced muscle damage. *J Strength Cond Res*, in press.
- Rawson ES and Clarkson PM (2000) Acute creatine supplementation in older men. *Int J Sports Med* **21**:71–75.
- Rawson ES, Wehnert ML and Clarkson PM (1999) Effects of 30 days of creatine ingestion in older men. *Eur J Appl Physiol* **80**:139–144.
- Ren JM, Semenkovich CF and Holloszy JO (1993) Adaptation of muscle to creatine depletion: Effect on GLUT-4 glucose transporter expression. *Am J Physiol* **264**:C146–C150.
- Robinson TM, Sewell DA, Hultman E and Greenhaff PL (1999) Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. *J Appl Physiol* **87**:598–604.
- Rosenshtaukh LV, Anyukhovsky EP, Beloshapko GG, Undrovinas AI, Fleidervish IA, Paju AY and Glukhovtsev EV (1988) Some mechanisms of nonspecific antiarrhythmic action of phosphocreatine in acute myocardial ischemia. *Biochem Med Metab Biol* **40**:225–236.
- Ruda M, Samarenko MB, Afonskaya NI and Saks VA (1988) Reduction of ventricular arrhythmias by phosphocreatine (Neoton) in patients with acute myocardial infarction. *Am Heart J* **116**:393–397.
- Schedel JM, Tanaka H, Kiyonaga A, Shindo M and Schutz Y (1999) Acute creatine ingestion in human: Consequences on serum creatine and creatinine concentrations. *Life Sci* **65**:2463–2470.
- Schloss P, Maysor W and Betz H (1994) The putative rat choline transporter CHOT1 transports creatine and is highly expressed in neural and muscle-rich tissues. *Biochem Biophys Res Commun* **198**:637–645.
- Schnirring L (1998) Creatine supplements face scrutiny: Will users pay later? *Phys Sportsmed* **26**:15–23.
- Sharov VG, Saks VA, Kupriyanov VV, Lakomkin VL, Kapelko VI, Steinschneider A and Javadov SA (1987) Protection of ischemic myocardium by exogenous phosphocreatine. I. Morphologic and phosphorus 31-nuclear magnetic resonance studies. *J Thorac Cardiovasc Surg* **94**:749–761.
- Shear DA, Haik KL and Dunbar GL (2000) Creatine reduces 3-nitropropionic-acid-induced cognitive and motor abnormalities in rats. *Neuroreport* **11**:1833–1837.
- Sims E and Seldin D (1949) Reabsorption of creatine and guanidoacetic acid by the renal tubules. *Am J Physiol* **157**:14–20.
- Sipila I, Rapola J, Simell O and Vannas A (1981) Supplementary creatine as a treatment for gyrate atrophy of the choroid and retina. *N Engl J Med* **304**:867–870.
- Smith SA, Montain SJ, Matott RP, Zientara GP, Jolesz FA and Fielding RA (1998) Creatine supplementation and age influence muscle metabolism during exercise. *J Appl Physiol* **85**:1349–1356.
- Snow RJ, McKenna MJ, Selig SE, Kemp J, Stathis CG and Zhao S (1998) Effect of creatine supplementation on sprint exercise performance and muscle metabolism. *J Appl Physiol* **84**:1667–1673.
- Sora I, Richman J, Santoro G, Wei H, Wang Y, Vanderah T, Horvath R, Nguyen M, Waite S, Roeske WR, et al. (1994) The cloning and expression of a human creatine transporter. *Biochem Biophys Res Commun* **204**:419–427.
- Steenge GR, Lambourne J, Casey A, Macdonald IA and Greenhaff PL (1998) Stimulatory effect of insulin on creatine accumulation in human skeletal muscle. *Am J Physiol* **275**:E974–E979.
- Steenge GR, Simpson EJ and Greenhaff PL (2000) Protein- and carbohydrate-induced augmentation of whole body creatine retention in humans. *J Appl Physiol* **89**:1165–1171.
- Stockler S, Hanefeld F and Frahm J (1996) Creatine replacement therapy in guanidinoacetate methyltransferase deficiency, a novel inborn error of metabolism. *Lancet* **348**:789–790.
- Stockler S, Holzbach U, Hanefeld F, Marquardt I, Helms G, Requart M, Hanicke W and Frahm J (1994) Creatine deficiency in the brain: A new, treatable inborn error of metabolism. *Pediatr Res* **36**:409–413.
- Tarnopolsky M and Martin J (1999) Creatine monohydrate increases strength in patients with neuromuscular disease [see comments]. *Neurology* **52**:854–857.
- Tarnopolsky MA, Roy BD and MacDonald JR (1997) A randomized, controlled trial of creatine monohydrate in patients with mitochondrial cytopathies [see comments]. *Muscle Nerve* **20**:1502–1509.
- Terjung RL, Clarkson P, Eichner ER, Greenhaff PL, Hespel PJ, Israel RG, Kraemer WJ, Meyer RA, Spriet LL, Tarnopolsky MA, Wagenmakers AJ and Williams MH

- (2000) American College of Sports Medicine roundtable. The physiological and health effects of oral creatine supplementation. *Med Sci Sports Exerc* **32**:706–717.
- Thorell A, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JF, Dufresne SD, Horton ES, Ljungqvist O and Goodyear LJ (1999) Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. *Am J Physiol* **277**:E733–E741.
- Tran TT, Dai W and Sarkar HK (2000) Cyclosporin A inhibits creatine uptake by altering surface expression of the creatine transporter. *J Biol Chem* **275**:35708–35714.
- Vanakoski J, Kosunen V, Meririnne E and Seppala T (1998) Creatine and caffeine in anaerobic and aerobic exercise: Effects on physical performance and pharmacokinetic considerations. *Int J Clin Pharmacol Ther* **36**:258–262.
- Vandenbergh K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L and Hespel P (1997) Long-term creatine intake is beneficial to muscle performance during resistance training. *J Appl Physiol* **83**:2055–2063.
- Vandenbergh K, Van Hecke P, Van Leemputte M, Vanstapel F and Hespel P (1999) Phosphocreatine resynthesis is not affected by creatine loading. *Med Sci Sports Exerc* **31**:236–242.
- Vannas-Sulonen K, Sipila I, Vannas A, Simell O and Rapola J (1985) Gyrate atrophy of the choroid and retina. A five-year follow-up of creatine supplementation. *Ophthalmology* **92**:1719–1727.
- Volek J and Kraemer W (1997) Creatine supplementation: Its effect on human muscular performance and body composition. *J Strength Cond Res* **10**:200–210.
- Volek JS, Duncan ND, Mazzetti SA, Putukian M, Gomez AL and Kraemer WJ (2000) No effect of heavy resistance training and creatine supplementation on blood lipids. *Int J Sport Nutr Exerc Metab* **10**:144–156.
- Volek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gomez AL, Pearson DR, Fink WJ and Kraemer WJ (1999) Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc* **31**:1147–1156.
- Vorgerd M, Grehl T, Jager M, Muller K, Freitag G, Patzold T, Bruns N, Fabian K, Tegenthoff M, Mortier W, Luttmann A, Zange J and Malin JP (2000) Creatine therapy in myophosphorylase deficiency (McArdle disease) a placebo-controlled crossover trial. *Arch Neurol* **57**:956–963.
- Walker J (1979) Creatine: Biosynthesis, regulation, and function. *Adv Enzym* **50**:117–242.
- Walter MC, Lochmuller H, Reilich P, Klopstock T, Huber R, Hartard M, Hennig M, Pongratz D and Muller-Felber W (2000) Creatine monohydrate in muscular dystrophies: A double-blind, placebo-controlled clinical study. *Neurology* **54**:1848–1850.
- Wick M, Fujimori H, Michaelis T and Frahm J (1999) Brain water diffusion in normal and creatine-supplemented rats during transient global ischemia. *Magn Reson Med* **42**:798–802.
- Wilken B, Ramirez JM, Probst I, Richter DW and Hanefeld F (2000) Anoxic Atp depletion in neonatal mice brainstem is prevented by creatine supplementation. *Arch Dis Child Fetal Neonatal Ed* **82**:F224–F227.
- Willer B, Stucki G, Hoppeler H, Bruhlmann P and Krahenbuhl S (2000) Effects of creatine supplementation on muscle weakness in patients with rheumatoid arthritis. *Rheumatology* **39**:293–298.
- Willott CA, Young ME, Leighton B, Kemp GJ, Boehm EA, Radda GK and Clarke K (1999) Creatine uptake in isolated soleus muscle: Kinetics and dependence on sodium, but not on insulin. *Acta Physiol Scand* **166**:99–104.
- Wyss M and Kaddurah-Daouk R (2000) Creatine and creatinine metabolism. *Physiol Rev* **80**:1107–1213.
- Yu PH and Deng Y (2000) Potential cytotoxic effect of chronic administration of creatine, a nutrition supplement to augment athletic performance. *Med Hypotheses* **54**:726–728.
- Zeisel SH (1999) Regulation of “nutraceuticals” [see comments]. *Science (Wash DC)* **285**:1853–1855.