

Factors Influencing Creatine Loading into Human Skeletal Muscle

Rodney J. Snow and Robyn M. Murphy

School of Health Sciences, Deakin University, Burwood, Australia

SNOW, R. J., and R. M. MURPHY. Factors influencing creatine loading into human skeletal muscle. *Exerc. Sport Sci. Rev.*, Vol. 31, No. 3, pp. 154–158, 2003. *This review describes several factors involved in regulating skeletal muscle creatine uptake and total creatine content. Skeletal muscle total creatine content increases with oral creatine supplementation, although the response is variable. Factors that may account for this variation are carbohydrate intake, physical activity, training status, and possibly fiber type.*
Keywords: creatine transporters, creatine supplementation, nutritional supplement, ergogenic aid

INTRODUCTION

This review briefly outlines the importance of creatine (Cr) for muscle function in certain athletic performance. It then establishes that muscle Cr content is predominantly regulated by mechanisms involved with muscle Cr uptake, including extracellular Cr supply and the expression and activity of the Cr transporter (CreaT) proteins. Factors regulating the activity and the expression of CreaT proteins are discussed briefly. With this knowledge, the review then addresses strategies that may enhance human skeletal muscle Cr uptake during Cr supplementation such as exercise, training, prior period of dietary Cr abstinence (e.g., vegetarianism), and co-ingestion of Cr with carbohydrate. Where possible, how these factors act to stimulate muscle Cr transport is outlined. Finally, the review examines the evidence relating to the effect of fiber type and gender on human skeletal muscle Cr content and its accumulation with oral Cr supplementation.

CREATINE AND ITS CELLULAR FUNCTION

Cr is a nonessential dietary molecule that is synthesized from arginine, S-adenosyl-methionine, and glycine, primarily by the pancreas and liver. Cr is consumed in the diet predominantly in meat, fish, and other animal products. The Cr

degradation rate within humans is approximately 1.6% (2 g) per d. Therefore, to maintain whole body Cr stores, approximately 2 g of Cr needs to be synthesized or ingested daily. Approximately 50% of this requirement is obtained from an omnivorous mixed diet, and the remainder is synthesized endogenously. The degradation of the Cr pool involves the nonenzymatic, unregulated conversion of Cr and creatine phosphate (CrP) to creatinine, most of which is subsequently excreted from the body via the kidneys. Approximately 95% of the Cr stored within the body is found within skeletal muscle, with most of the remaining stores found in the heart, brain, and testes. In most cases, Cr is transported from the organs of synthesis (e.g., liver and pancreas), via the blood, to the tissues requiring Cr (e.g., skeletal muscle, brain, and heart). More than 90% of cellular Cr uptake occurs via a Na^+ and Cl^- -dependent CreaT protein against a very large concentration gradient (5,10). A proportion of Cr entering the cell is phosphorylated to form CrP that, via the creatine kinase reaction, contributes to the regeneration of ATP for energy requiring processes. In normal resting muscle, CrP levels make up approximately 65% of the total Cr pool. Cr and CrP content may also be involved in coupling aerobic metabolism with ATP demand (i.e., CrP shuttle), synthesis of specific muscle proteins, and acid-base balance.

There are a plethora of scientific studies investigating the potential ergogenicity of Cr. In summary, oral Cr supplementation can elevate human skeletal muscle TCr by 10–30% (4). Furthermore, this increase has been shown in most cases to enhance intermittent, high-intensity exercise performance and muscle strength gains in conjunction with resistance training. Performance during continuous endurance exercise is not altered or may decrease with weight-bearing activity such as running, whereas there appears to be an ergogenic effect of Cr supplementation during intermittent, sprinting

Address for correspondence: Rodney J. Snow, School of Health Sciences, Deakin University, 221 Burwood Highway, Burwood, 3125 Australia (E-mail: rsnow@deakin.edu.au).
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exercise that occurs during or after an endurance exercise bout. The findings that intense, intermittent exercise performance may be improved indicate that the regulation of cellular TCr content is important for muscular function, especially in times of metabolic stress induced by intense contractile activity.

The TCr content of cells is dependent on rates of Cr uptake, Cr trapping, and rates of Cr loss via creatinine. At present, there is a poor understanding of how cells regulate their TCr content. One of the major sites of regulation is Cr uptake. Dietary Cr serves as an end-product repressor for the first step in Cr biosynthesis, and this mechanism may act to attenuate the increase in Cr supply to cells, thereby lowering cellular Cr uptake. Regulation of CreaT protein activity and expression is also likely to be important in controlling Cr uptake and thus cellular TCr levels (10).

EXPRESSION OF THE CREATINE TRANSPORTER PROTEIN IN SKELETAL MUSCLE

There is clear evidence that Na^+ and Cl^- -dependent Cr transport occurs into skeletal muscle cells (5,12), and therefore the existence of CreaT proteins at the sarcolemma is well accepted. There is also strong evidence indicating that active Cr transport also occurs into mitochondria (13) and is likely to be mediated by a CreaT protein. Based on these data, Walzel *et al.* (13) have proposed a general scheme of cellular Cr transport (see Fig. 1) that involves three pools of Cr (serum, cytosol, and mitochondrial) that are connected by plasma membrane and mitochondrial CreaT proteins.

Several antibodies, designed to target specific amino acid sequences of the putative CreaT protein, have been developed by various laboratories. Although there is some circum-

stantial evidence that these antibodies are detecting *bona fide* CreaT proteins, there are unpublished data establishing that the antibodies employed by Walzel *et al.* (12,13) cross-react with subunits of pyruvate dehydrogenase. Consequently, the work determining cellular CreaT protein content using these CreaT antibodies needs to be revisited, and the remaining literature requires cautious interpretation until the specificity of the other available CreaT antibodies are established unequivocally. Given this doubt, the literature purporting to measure CreaT protein content is not discussed in the present review.

CREATINE TRANSPORTER KINETICS

Cr transport studies into incubated giant sarcolemmal vesicles (12) have found the K_m for Cr to be approximately 50 μM , whereas the K_m for mitochondrial Cr transport is approximately 16 mM (13). No studies have investigated Cr uptake kinetics into mature human skeletal muscle; however, several researchers have measured Cr transport in cultured human cells or cells transfected with the human CreaT1 gene and found the K_m to be between 15 and 77 μM (10). Unfortunately, maximal Cr transport rates (V_{max}) are difficult to compare between studies because they are rarely expressed in similar units. The fasting plasma Cr concentrations in humans is approximately 50 to 100 μM (4), indicating that the sarcolemmal Cr transporters may be working close to saturation. These data suggest that the amount of CreaT protein at the cell membrane is therefore important in determining intramuscular Cr levels. The possibility that the sarcolemmal transporters are working near saturating Cr levels *in vivo* needs to be treated cautiously, because Cr supplementation studies in humans (4) have demonstrated an aug-

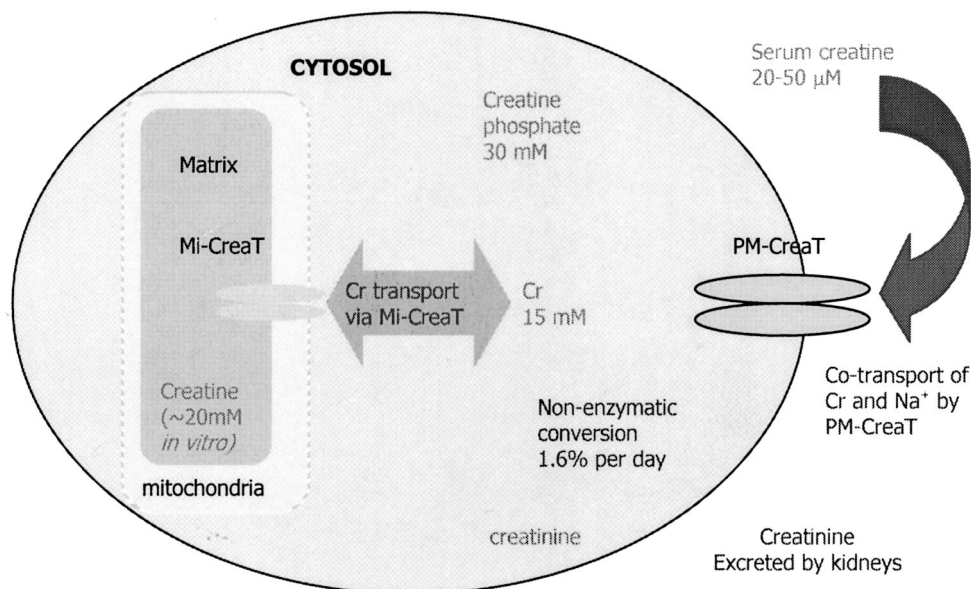


Figure 1. General scheme of cellular creatine (Cr) transport. A compartmentation of three Cr pools, that is, serum, cytosol, and mitochondria, are shown. These pools are interconnected via two different creatine transporters (CreaT), the high-affinity (low K_m) plasma membrane (PM-CreaT) and the low-affinity (high K_m) mitochondrial (Mi CreaT). [Adapted from Walzel, B., O. Speer, E. Zanolli, O. Eriksson, P. Bernardi, and T. Wallimann. Novel mitochondrial creatine transporter activity: implications for intracellular creatine compartments and bioenergetics. *J. Biol. Chem.* 277:37503–37511, 2002b. Copyright © 2002 American Society for Biochemistry and Molecular Biology. Used with permission.]

mented intramuscular TCr content, suggesting that an increased circulating Cr concentration may produce an elevation of sarcolemmal transporter activity.

REGULATION OF CREATINE TRANSPORTER ACTIVITY

Regulation of CreaT protein activity has been reviewed recently (10). In brief, regulation of the transporter's activity may involve the production of an unknown inhibitory protein; an increase in the number of the transporters at the plasma membrane (*i.e.*, translocation); changes in the driving force of the sarcolemmal CreaT protein, such as the Na^+ gradient; changes in phosphorylation or glycosylation states, or both, of the CreaT protein; allosteric inhibition of the CreaT protein; or alteration of the number of transporters expressed by the cell. Very little is known about the regulation of the mitochondrial CreaT proteins. Walzel *et al.* (13) demonstrated that mitochondrial Cr uptake is partly dependent on the energetic state of the mitochondria, but surprisingly, the mitochondrial Cr uptake is not inhibited by several amino acids and creatine analogues that are known to inhibit sarcolemma CreaT uptake.

There are many studies that have found an increased intracellular TCr accumulation associated with elevations in extracellular Cr levels. Most human studies have involved oral Cr supplementation. This nutritional regimen is known to result in markedly elevated plasma Cr levels (*e.g.*, 10- to 20-fold increases) (4). The increase in intracellular TCr content with oral Cr supplementation is best explained by an enhanced sarcolemmal CreaT activity. It should be noted that the initial increase in transporter activity accompanying elevated extracellular Cr levels is likely to be short lived, because data from both cell-culture studies (5) and *in vivo* human experiments where urinary Cr excretion has been determined (4) indicate that cellular Cr uptake is at least partly inhibited with short-term exposure to high extracellular Cr levels.

The mechanism by which sarcolemmal CreaT activity is inhibited remains unclear; however, there is evidence indicating that intracellular Cr, but not CrP levels, may be involved (5). It is reasonable to speculate that high extracellular Cr causes an initial increase in Cr uptake and an elevation in intracellular Cr concentration, which over time some how feeds back to inhibit Cr uptake. Recently, Wang *et al.* (14) provided evidence that this feedback may occur by reducing the activity of a nonreceptor protein tyrosine kinase that is closely associated with the plasma membrane known as c-Src kinase. These authors noted that a decrease in this kinase activity occurred in skeletal muscle after oral Cr supplementation and that this was related to a decreased tyrosine phosphorylation of sarcolemmal CreaT protein. They speculated that this change in tyrosine phosphorylation inhibited sarcolemmal CreaT activity.

REGULATION OF CREATINE TRANSPORTER EXPRESSION

Currently, there is very limited information available regarding factors influencing CreaT mRNA and protein ex-

pression. Clearly, the number of transcripts and transporter proteins results from the balance between rates of their synthesis and degradation. No research has been conducted on the regulation of CreaT mRNA or protein degradation rates. Regulation of the production of functional transporter proteins is complex and may involve transcriptional, translational, or posttranslational mechanisms, or a combination thereof.

As mentioned previously, an increase in intracellular Cr may be involved with signaling the acute inhibition of sarcolemmal CreaT activity; however, the signal(s) involved with altering cellular CreaT gene or protein expression has yet to be fully described for any tissue. Recent evidence (11) suggests that oral Cr supplementation does not alter the CreaT mRNA expression content in human skeletal muscle, despite a significant increase in muscle TCr, CrP, and Cr content. At present, there are no reliable data on the effect of Cr loading on the content, subcellular location, or activity of CreaT proteins in skeletal muscle.

FACTORS INFLUENCING CREATINE UPTAKE INTO SKELETAL MUSCLE

The maximum Cr concentration that can be attained by skeletal muscle and other Cr containing cells is unknown. The average TCr content in human skeletal muscle is approximately 120–130 $\text{mmol}\cdot\text{kg}^{-1}$ dry mass, but follows a normal distribution ranging from approximately 90 to 160 $\text{mmol}\cdot\text{kg}^{-1}$ dry mass. With dietary Cr supplementation we, and others (4), have measured muscle TCr contents in some individuals more than 180 $\text{mmol}\cdot\text{kg}^{-1}$ dry mass. Interestingly, there are also some healthy subjects who do not increase their muscle TCr stores despite undertaking a Cr supplementation regimen. It is unclear what factors contribute to this large individual variability in normal muscle TCr levels or muscle Cr loading response after supplementation; however, a number of factors have been investigated or postulated, some of which are discussed below.

Creatine and Carbohydrate Co-ingestion

In most cases, insulin has been found to stimulate muscle Cr uptake. Insulin probably acts by directly stimulating cell membrane Cr transport processes, rather than enhancing cell Cr delivery by elevating blood flow. Not surprisingly, therefore, co-ingestion of Cr with a very large amount of carbohydrate and the concomitant increase in circulating insulin levels has been shown to cause a greater human muscle TCr accumulation than when Cr was ingested alone (see Fig. 2) (3). The stimulatory effect of insulin on whole-body Cr disposal only occurs during the initial 24 h of Cr and carbohydrate co-ingestion, suggesting that this strategy is only likely to be effective in increasing muscle Cr loading on the first d of supplementation. The mechanism causing the reduction in insulin responsiveness is not known.

Creatine Supplementation and Exercise

Cr supplementation conducted in conjunction with prolonged, submaximal exercise clearly results in an enhanced Cr loading (see Fig. 3), but only into skeletal muscles engaged

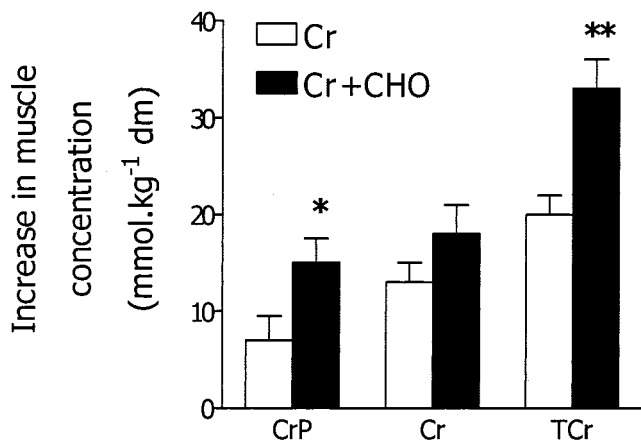


Figure 2. Increases in muscle concentrations of creatine phosphate (CrP), creatine (Cr), and total creatine (TCr) after 5 d of Cr supplementation (Cr ingestion) or after 5 d of Cr and carbohydrate (CHO) supplementation * $P < 0.05$; ** $P < 0.01$, significantly different between treatments. [Adapted from Green, A. L., E. Hultman, I. A. Macdonald, D. A. Sewell, and P. L. Greenhaff. Carbohydrate ingestion augments skeletal muscle creatine accumulation during creatine supplementation in humans. *Am. J. Physiol.* 271:E821–E826, 1996. Copyright © 1996 American Physiological Society. Used with permission.]

in the activity (4,9). How muscle activity causes this enhanced Cr uptake during Cr supplementation is unclear. Harris *et al.* (4) initially suggested that elevated muscle blood flow during contractile activity increases Cr delivery to the active muscles, thereby enhancing uptake. More recent research (9) indicates that altered muscle blood flow is not likely to explain the phenomenon. Robinson *et al.* (9) speculated that muscle contractile activity stimulates maximal Cr transport rates by allosteric activation of CreaT proteins, by recruitment or synthesis of new transporters, or by changes in the forces driving Cr transport such as the sodium gradient. Further research is required to determine whether any of these mechanisms actually play a role in causing the enhanced Cr uptake associated with exercise.

Training

There are reports of increases or no changes in skeletal muscle TCr stores after endurance, sprint, or strength training. In contrast, there are no human studies that have directly assessed the influence of sprint, endurance, or resistance training on muscle Cr uptake rates or the magnitude of muscle Cr loading after a period of Cr supplementation. Zange *et al.* (15), using ^{31}P -magnetic resonance spectroscopy, found that after a period of Cr supplementation, sports students increased their muscle CrP:ATP ratio to a greater extent than sedentary subjects. These data indicate that individuals involved in regular exercise (*i.e.*, training) may have a greater ability to load Cr. Although speculative, training may be expected to enhance rates of Cr uptake and muscle TCr accumulation because one or several bouts of exercise have been demonstrated to increase human muscle Cr loading (see above). Furthermore, training is associated with an improved insulin sensitivity and this may also augment muscle Cr uptake when extracellular Cr levels are elevated. The effect of training on muscle CreaT mRNA

content and protein expression or activity is currently unknown.

Chronically Reduced Dietary Creatine Intake (Vegetarianism)

Individuals consuming a vegetarian diet rely almost exclusively on endogenous Cr production to meet their daily Cr requirements, because their intake of Cr containing food, that is meat and meat products, is low. Delanghe *et al.* (2) reported that the daily urinary creatinine excretions rates were at least 30% lower in vegetarians, providing indirect evidence that vegetarians may have a reduced muscle TCr level (2). This finding indicated that endogenous Cr production was unable to compensate fully for the lack of dietary Cr. Interestingly, Lukaszuk *et al.* (6) recently demonstrated that skeletal muscle TCr content was 13% lower in a group of meat eaters who ingested a vegetarian diet for 3 wk. Importantly, these researchers did not find that the vegetarian diet augmented Cr uptake when supplemented with Cr in the fourth wk.

Muscle Fiber Type

The CrP content of human type II fibers is approximately 12% greater than in type I fibers. Only one study has determined the change in muscle CrP content in various human muscle fiber types after Cr supplementation. Casey *et al.* (1) found that the increase in CrP content in human quadriceps muscle type I and II fibers was similar (~15%) (1). Unfortunately, muscle Cr content was not measured, and the type II fiber pool must have contained a mixture of IIa and IIb

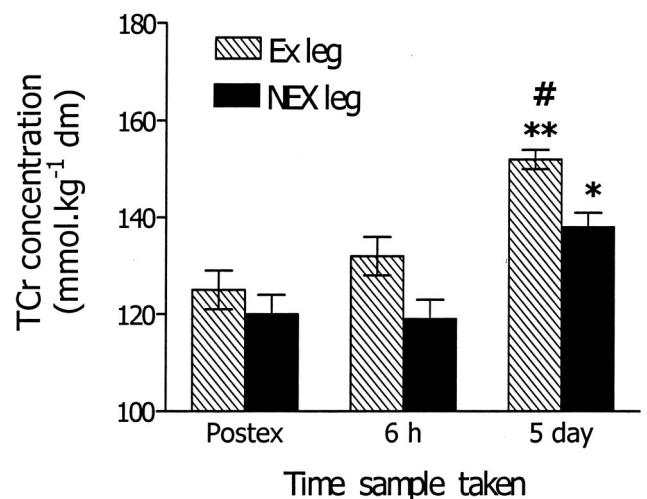


Figure 3. Muscle total creatine (TCr) concentrations from exhausted (Ex) and nonexhausted (NEX) limbs of creatine (Cr) and carbohydrate (CHO) supplemented subjects postexercise (Postex) and 6 h and 5 d after exercise. Over the entire 5-d experimental period, all subjects were supplemented with creatine (Cr) and carbohydrate (CHO). In subjects receiving only CHO supplements, no significant changes in TCr concentrations were seen (not shown). * $P < 0.05$; ** $P < 0.01$. Significant difference from Postex concentration. # $P < 0.01$. Significant difference between limbs after 5 d of supplementation. [Adapted from Robinson, T. M., D. A. Sewell, E. Hultman, and P. L. Greenhaff. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. *J. Appl. Physiol.* 87:598–604, 1999. Copyright © 1999 American Physiological Society. Used with permission.]

fibers. Therefore, the possibility that there may be a difference in Cr uptake between fiber types in humans cannot be dismissed solely on the work published to date. It should be noted that *in vitro* Cr transport rates are slower in fast twitch rat muscle compared with the slow twitch muscle at physiological Cr concentrations. Additionally, the magnitude of Cr loading appears to be greater in slow twitch, compared with fast twitch, rat muscle.

Gender

It is unlikely that gender affects skeletal muscle TCr content or Cr loading. Most (7,8) studies report that muscle TCr content is similar between the sexes. Furthermore, the magnitude of skeletal muscle Cr loading after a period of oral Cr supplementation was not different in males compared with females (4,8). Consistent with this observation is the fact that CreaT mRNA expression in skeletal muscle is also similar in males and females (7).

CONCLUSIONS

The regulation of muscle TCr content predominantly involves control of Cr uptake into muscle cells, which is dependent on extracellular Cr supply and the activity and expression of CreaT proteins. Oral Cr supplementation is able to elevate circulating Cr levels several fold and is therefore an effective strategy to increase Cr supply to muscle. A number of factors are known to alter CreaT protein activity; however, there is very little information available on the regulation of muscle CreaT protein expression. The magnitude of muscle TCr accumulation after Cr supplementation varies between individuals and is explained, at least in part, by diet, exercise, and possibly fiber type. Gender is unlikely to be an important factor.

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