



## Creatine: are the benefits worth the risk?

Mark A. Brudnak

*Vice President Technology MAK Wood, 1235 Dakota Drive, Units E-F, Grafton, WI 53024, USA*

### Abstract

Creatine monohydrate is a popular sports supplement used to maintain levels of high-energy phosphates during exercise. As a supplement, varying amounts are consumed per person corresponding to parameters such as body mass and level of training (i.e. maintenance versus loading doses). Numerous studies have reported beneficial effects including increased muscle mass during training and neural protection. However, negative reports have also been made of possible side effects, such as muscle cramping during exercise, and potential impurities. The present paper introduces the positive and negative aspects of creatine supplementation and focuses on the toxicological data of creatine, its metabolites and associated mutagenicity or carcinogenicity, genomeceutical effect(s), and any potential ‘contaminants.’ Additionally, the novel applications of creatine to the areas of neurology, cardiology, and diabetes are presented and discussed along with the representative data for sports nutrition. © 2004 Elsevier Ireland Ltd. All rights reserved.

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### 1. Introduction

Creatine monohydrate (Cr) is a popular supplement in the sports industry, used to maintain high-energy phosphates during strenuous exercise. With the discovery of phosphocreatine or creatine phosphate (CrP), and creatine kinase (CK) shortly after, Cr has been extensively studied since 1927 (Conway and Clark, 1996). During the 1990’s, and with the enactment of the Dietary Supplement Health and Education Act (DSHEA) studies on Cr rapidly grew, coinciding with an increase in the oral consumption of Cr. This was due, primarily, to a number of publications early in

the 1990’s, which found Cr to delay muscle fatigue. It was also reported that Cr could enhance muscle energy recovery (Finn et al., 2001).

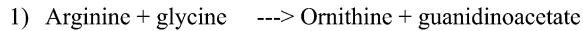
Varying amounts of Cr are consumed depending on what the athlete, the primary consumer, is trying to accomplish. The applications will be discussed below along with the negative aspects of oral Cr consumption. There are some anecdotal reports of varying detrimental effects such as heat exhaustion, muscle cramping, and stroke as the level of Cr consumption rises.

Creatine is an amino acid formed from arginine and glycine via an A:G transferase enzyme. Fig. 1A. shows a diagram of this. This produces ornithine and guanidinoacetate. Guanidinoacetate is then methylated via an S-adenosyl methionene. This forms creatine. In vivo, most of this is happening in the kidneys. The guanidinoacetate is then transferred to the liver where it is

*Abbreviations:* Cr or ‘creatine’, Creatine monohydrate; Crn, Creatinine; cCr, Cyclocreatine; tCr, Total Creatine; CrP, Creatine phosphate; AIA, amino-imidazo-azaarenes

*E-mail address:* [mbrudnak@wi.rr.com](mailto:mbrudnak@wi.rr.com) (M.A. Brudnak).

(A) Formed in a two-step (in vivo) reaction :



(A:G-amidinotransferase)

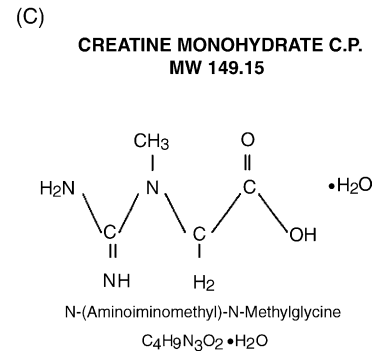
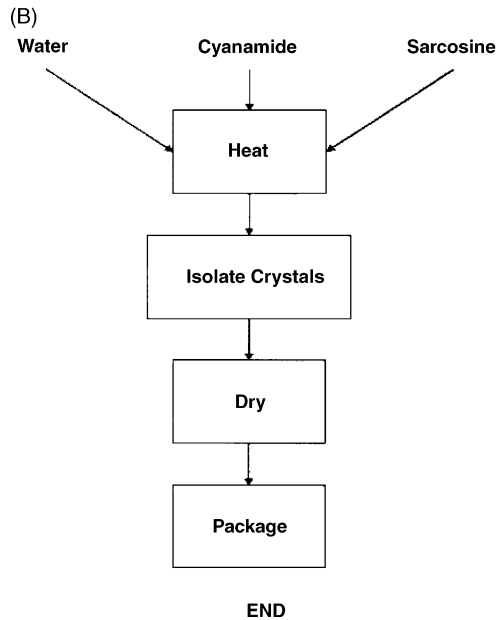


Fig. 1. (A–C) Production of creatine monohydrate. (A) The in vivo production of creatine from arginine and glycine via an A:G transferase enzyme. This produces ornithine and guanidinoacetate. Guanidinoacetate is then methylated via an *S*-adenosyl methionene, resulting in creatine. In vivo, most of this is happening in the kidneys. The guanidinoacetate is then transferred to the liver where it is methylated to form creatine. After this, the creatine traverses to the skeletal muscle where it is transported across the cell membrane via a  $Na^+$  dependant transport system. (B) The production of Cr commercially, takes cyanamide and sarcosine, adds heat and time, crystals precipitate out and are isolated and dried. (C) Structure of creatine monohydrate.

methylated to form creatine. After this, the creatine traverses to the skeletal muscle where it is transported across the cell membrane via a  $Na^+$  dependant transport system. Fig. 1B demonstrates how Cr is commercially processed in which cyanamide and sarcosine are reacted in the presence of heat and time, crystals are precipitated out, isolated and dried packaged.

The key feature of Cr is that it can bind a high-energy phosphate. This is relevant because when ATP is depleted, such as during times of high-intensity workouts, the CrP can donate a high-energy phosphate and rephosphorylate ADP to ATP.

Cr also acts as a temporal and spatial buffer. Fig. 2 shows a basic diagram of this occurring in a nerve cell

but this is true for striated tissue as well. Creatine by itself, in its unphosphorylated state, is transported into the cell where it becomes phosphorylated by mitochondrial associated kinases. The CrP can then move around the cell. In the cell, CrP can accumulated towards the synapse and assist with relatively high and stable synaptic potential energy.

Nervous tissue and the role of Cr supplementation is rapidly garnering more attention. There is mounting data in the literature supporting the use of Cr and Cr analogs in musculoskeletal disorders. Cr itself rapidly converts to creatinine, depending on the pH. At a pH greater than 6, the conversion is dramatically slowed. Cr normally converts to creatinine, which is

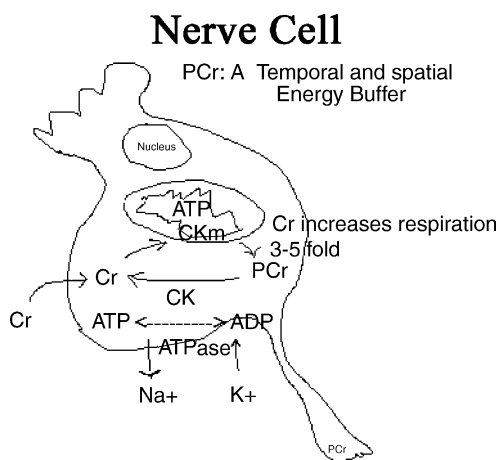


Fig. 2. Creatinephosphate as a eukaryotic temporal and spatial energy buffer. Relationship of Cr and PCr to ATP in the neuron, drawn largely from information on rat heart and chick brain. Exogenous Cr should be radially transported into neurons by a Cr transporter or synthesized if precursors are available. Under resting levels of ADP, ATP produced in the mitochondria is converted to PCr by mitochondrial CK (CKm) and released into the cytoplasm. The equilibrium constants for brain CK favors conversion of PCr to ATP. ATP is consumed to maintain ion homeostasis, e.g. via the plasma membrane ATPase and most importantly the sarcoplasmic-endoplasmic reticulum  $\text{Ca} + 2\text{-ATPase Ca} + 2$  pump. The resulting ADP is rapidly consumed in the regeneration of ATP from PCr by CKbb. PCr can be either transported from the soma to distant sites or generated at sites distant from the nucleus (nucleus), such as at synaptically localized mitochondria. At synapses, PCr can also be used directly as an energy source to load synaptic vesicles, especially under conditions of low potassium that would accompany high levels of synaptic activity.

not phosphorylatable, rapidly at acidic pH's. In vivo, once that conversion happens, the creatinine (Crn) is excreted in the urine. Cr is essentially stable at neutral to basic pH's.

### 1.1. Known and potential risks

There are varying types and degrees of risks involved in supplementation with Cr. One could have a 'non-effect' be a risk, if someone is supplementing the diet with something that does nothing, at the cost of not consuming something else that does something good. There could be a detrimental effect on some process or part of the body. The risk may even be a varying degree of both. Also, metabolites may present their own risks. In fact, discussed below is the fact

that there are several metabolites of Cr, which can be produced by various gut organism and to varying degrees, that are known carcinogens.

Reported side-effects include nausea, vomiting, diarrhea, nephritis, and cramping. However, blind studies do not seem to support those. In fact, when rechallenged, those that negatively responded do not repeatedly negatively respond. Muscle cramps/strains/damage/heat exhaustion are reported in individuals at high risk for such conditions. There have been some studies looking at long-term supplementation on cardiac health and post myocardial infarction. However, no benefit has been observed under the conditions of assay thus far. However, it is anticipated, that with correct conditions, a cardio-protective effect will be demonstrated. Nursing-mothers and children are contraindicated for supplementation. For the pediatrics, this is particularly important. This is because it is known that creatine has a genomeceutical effect in that it can alter gene expression. It is conceivable, such genomeceutical effects may have negative counter parts that have a deleterious effect. This, however, has not been shown.

Continuing with the genes, it is also known that downstream metabolites of creatine can form mutagens. In eukaryotes this doesn't appear to happen, but it does in prokaryotes. Prokaryotic metabolization of Cr is important because there are more bacteria living in and on the human body than there are human cells making up the body. At any given time, there are both beneficial and pathogenic bacteria in and on the body. While the probiotics tend as a group to detoxify pathogens, mutagens, and carcinogens and are precursors of the aforementioned, the pathogenic prokaryotes harbored by the body tend to do the opposite and activate those compounds and pathways.

A new class of mutagens has been intensively studied for the last 20 years since they were discovered in the food supply. These are the amino-imidazo-azaarenes (AIA) and can be found in cooled fish, chicken, beef, pork, and their extracts. It is not a coincidence that all these sources are also good sources of Cr. There are a plethora of the AIA compounds. The specifics of the AIA's mutagenicity are beyond the scope of this paper but it is sufficient to say they form DNA adducts and hence, interfere with proper functionality of the nucleic acids in the cells.

There is more and more mounting evidence that Cr and Crn are precursors for the AIA's. Short-term, this is probably not an issue with Cr supplementation. However, long-term, it is a very important issue. Couple that with the fact that it is common to supplement the diet with Cr at levels several hundred to often times several thousand times the level naturally present in the foods.

Considering the potential for mutagenicity, the picture for toxicology needs to be larger than one might at first think. That is to say, is any mutagenicity or potential mutagenicity preventable or even abolishable? Indeed it is, however, to what extent is not known. It is known that Vitamin C inhibits nitrosation reactions by reducing nitrite to NO. Antioxidants, flavonoids, chlorophylls, food-coloring agents, isothiocyanates, and capsaicin all reduce mutagenicity and carcinogenicity due to AIA mutagens.

Additionally, lactic acid bacteria are known to reduce the mutagenicity and carcinogenicity of Cr due to AIA's (Brudnak, 2001). This is discussed in more detail below.

### 1.2. Beneficial affects of Cr supplementation

Consumption of Cr linked with muscle load of Cr has been promoted for enhanced muscle energy recovery. This lead others to speculate that enhanced muscle development might be possible with oral supplementation of Cr (Harris et al., 1993). Subsequent studies have sought to define the various parameters involved with Cr supplementation (Balsom et al., 1994; Greenhaff et al., 1993). Well over 150 papers/articles have appeared (Greenhaff, 1995; Greenhaff et al., 1993, 1994; Birch et al., 1994). Cr supplementation enhances intermittent work performance. While there has been a pronounced beneficial effect for such exercises, there also seems to be a subpopulation of non-responders (Hespel et al., 2001). That is to say, individuals who derive no benefit as determined by various measurements, whether it is knee extensions or bench presses, etc.

Cr supplementation doesn't seem to affect endurance related exercises, at least not as they are measured. For instance, a sprint runner may benefit, where a long distance marathon runner will not. This is probably a combination of two factors. First, the measurable effect Cr has on ATP regeneration happens

within 10 s from initiation of the exercise. After which, ATP generation depends primarily on glycolysis and glycogenolysis. At that point, the energy production is at the mercy of enzyme kinetics. The second factor is the difficulty in measuring minute contributions to overall energy output via the techniques employed. It appears that the caveat for a beneficial contribution to performance seems to be that adequate periods of rest between bouts of work. Again, the reason for this is due to the kinetics of the reactions.

Most of the work demonstrating efficacious contributions (and some not so) has been reported in peer-reviewed literature. Negative aspects have not been so widely reported. This may be due to the nature of the problems, such as cramping, which is difficult to study under normal circumstances. However, negative effects have been reported in magazines and news stories. These often capture large headlines and captivate the audience with their tone. Some of the work has been reported in well-respected journals but it seems the anecdotal reports are the ones that get the most press often suggesting that one should look for certain brands and ask about the purity, without giving indication of country or what the purity should be. Such marketing techniques serve to terrorize the public into consuming a certain brand.

Several years ago, there was an article in a trade magazine that made an issue out of a theoretical artifact of the commercial creatine production process. This compound is dihydrotriazine (DHT).

Last year one US pharmaceutical company alone produced over 5 million kilograms of creatine, with every batch assayed, and DHT never showed up on a high-pressure liquid chromatography (HPLC) tracing (Wyss and Kaddurah-Daouk, 2000). Fig. 3A, show a typical HPLC batch analysis result. This was run at 210 nm for a wavelength, as is the generally accepted absorption wavelength for Cr analysis. One can see the creatine, dicyanamide (DC) and the creatinine, as indicated, with the later two being much smaller peaks. This is a batch that is 99.9% pure. In order to get dicyanamide to show up at all, the same sample was 'spiked' with a relatively large amount of DC. This is shown in Fig. 3B.

When the trade magazine reports came out, they didn't say what wavelengths they used for each sample or how much was used. In fact, they gave virtually no methodology. In fact, it is possible to skew the results

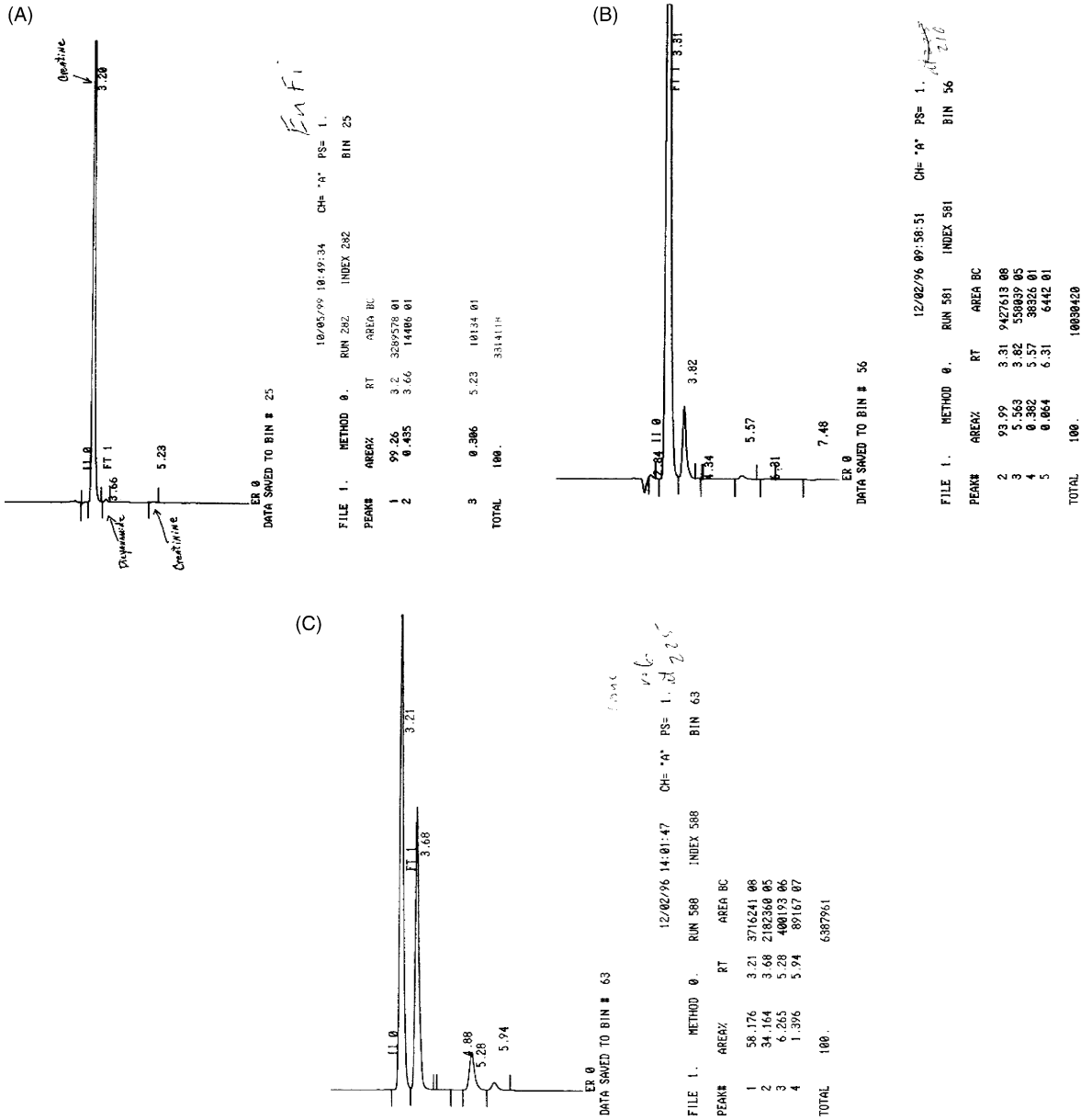


Fig. 3. (A and B) HPLC tracing of Cr. Absorbance was at 210 nm. Samples were prepared by sampling a standard production batch and serial dilutions were made with PBS as the diluents. Cr, dicyanamide, and Crn elute from the column, respectively. (C) Absorbance at 225 nm. Samples were prepared as in Fig. 3(A+B).

by altering the wavelength. Fig. 3C shows the same sample as in Fig. 3A, but at 225 nm instead of 210 nm. As can be seen, there is a large change in the relative absorption of the various ‘contaminants’ without any real change.

The real problem might be the metabolites of Cr. Typically, when Cr is used for things such as body building, there is an initial loading phase of 25–30 g/day for the first five or six days. After that, there is a maintenance dose of 2–5 g, typically 3 g,

per day. During that loading phase, the massive influx of Cr into the cell is accompanied with a concomitant influx of water. The net effect is that there is a swelling of the cells and associated tissues.

What has been ignored in most of the studies is that many pathogenic organisms such as *pseudomonas*, *E. coli*, yeast, etc. process Cr to known mutagens such as the imidazoquinolines and imidazoquinoxalines. That is not an all-inclusive list but covers most of the worst ones. The frying of foods rich in Cr and Crn can also create these compounds.

Some of the previously mentioned negative effects can be a matter of perspective. For instance, short-term creatine supplementation was shown not to alter hormonal responses to resistance training (Op't Eijnde et al., 2001). On an individual basis, that can be a good or bad thing. If one is interested in building lean striated muscle mass, then one might want to be able to increase the levels of testosterone via supplementation. Indeed, the study looked at testosterone. They also looked at growth hormone and saw no affect, but did observe a statistically significant effect on cortisol as compared to exercising placebo group.

Myocardial infarctions have been studied in relation to Cr level. Pronounced depletion of Cr post infarction has been observed. The addition of PCr competitor, such as guanidinophosphate, dramatically reduces the levels of PCr. The infarction plus GP almost completely ablates PCr in favor of the production of PGP. There has been some concern that creatine can have adverse cardiac function, as it has been speculated to affect left ventricular remodeling post ischemia. However, it has been shown that high-dose creatine feeding did not attenuate left ventricular remodeling (Horn, 1999). When heart, brain, liver, and kidney were looked at, post supplementation, it was observed that only liver and kidney accumulated Cr to any measurable extent (Horn et al., 1998).

Caffeine has been reported anecdotally to have a negative effect on the benefit derived from Cr supplementation. However, those same reports contradict themselves in that most of the work demonstrating a positive effect for Cr, used coffee as the delivery vehicle. Further recent studies suggest there is no discernible effect of caffeine, when torque is used for the analysis of strength change (Horn et al., 1998). However, it was observed that while torque was not affected, overall relaxation time was

with the co-ingestion of Cr and caffeine (Brudnak, 2001).

Previously, Cr was labeled as a genomeceutical. What that means is that it can beneficially affect gene expression. Readers are referred elsewhere for a more detailed explanation of what constitutes a genomeceutical (Brudnak, 2001; Op't Eijnde and Hespel, 2001). It was found that both during immobilization and rehabilitation, Cr supplementation increased GLUT4 expression. How it does this is not known. Coincident with GLUT4 up-regulation, one would expect a benefit to diabetics. In deed, it was found that Cr can significantly (400 mg/dl to 300 mg/d) attenuate blood glucose levels in diabetic mice (Blum et al., 2002).

Creatine is also being looked at for its neuroprotective effects. It is known that high-energy phosphates metabolism plays a critical role in progression of neurodegenerative diseases. Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis have all been looked at and appear to be beneficially affected by the consumption of Cr. Oral Cr produced significant protection against malonate lesions, and Cr protected against 3-nitropropionic (3-NP) acid neurotoxicity. Cr can decrease the lesion size by up to 80% (Menalled and Chesselet, 2002).

What is the mechanism of the protection? It is known that decreases in concentration of Cr, PCr, ATP, GDP, AMP, NAD, and ADP, are all caused by 3-NP, and are attenuated with Cr. Cr protects against malonate-induced increases in hydroxyl radical generation. Cr protects against 3-NP-induced increased in 3-nitrotyrosine, a marker of peroxynitrite-mediated oxidative injury. Cr pretreatment of cortical and striatal astrocytes, in vitro, delayed increase in intracellular  $Ca^{+2}$  produced by 3-NP. It appears to interact with the second messenger system.

What is particularly interesting is that Cr can reduce Huntingtin plaque formation in Huntington's Disease. At 2% of the diet, in a murine model, a significant reduction in Huntingtin deposition in the dorsomedial aspect of the neostriatum at the level of the anterior commissure was observed for 13 weeks of the trial. The R6/2 HD transgene mice suffered significant aggregates in number and size over the 13 weeks, whereas the Cr treated mice had greatly attenuated levels. Similarly, photomicrographs of the islets of Langerhan in the pancreas revealed

a marked attenuation due to the supplementation with 2% creatine (Ferro–Pfanstiehl Labs, personal communication).

None of the studies looked at the blood and tissue pH of the subjects. Granted, in a normal individual, blood pH does not change much; we have not been talking about the normal person. Rather we have been addressing individuals with mitochondrial cytopathies, neuropathic disorders, dystrophies, inflammatory myopathies, etc. There, the pH can alter significantly. This affects the availability of Cr to form PCr. If we consider a GI transit time of 24 h, and there is a state of acidosis, the luminal pH may easily be around 4–5 giving a couple percent conversion to creatinine. After the first 5 days of loading, the excess Cr is excreted in the urine. With an known measurable improvement of 5%, maximally, then it is easy to see how the pH can affect the parameters being looked at.

## 2. Discussion

Dietary consumption of foods and supplements containing creatine monohydrate is on the rise. At present, the individual consumption of Cr varies widely. It is not uncommon for athletes to ingest many times that of the levels found in natural sources such as red meats and fish. This has resulted in what appear to be minor side effects such as cramping and dehydration, those more serious consequences, such as strokes, have been reported in the popular press.

This paper has presented some of the rationales for both supplementation as well as the acute side effects. It was also presented that long-term, high-dose supplementation may have deleterious results, depending on the individual. Such results may be an increased risk for certain cancers. This is based on the fact that some pathogenic and natural inhabitants of the gut, such as *E. coli*, can produce metabolites from Cr that are known mutagens and/or carcinogens. It is very important to stress, however, that these organisms are normally present in very low numbers in a health individual. It is also of interest that those pathogenic organism can be kept in check through the regular consumption of beneficial organisms, or ‘probiotics.’ (Brudnak, 2003) For the athlete, the benefits of probiotic consumption go beyond the inhibition of

pathogenic organism in that they also aid in supporting proper peristalsis along the gastrointestinal (GI) tract. Additionally, they can digest much of the food, and therefore the calories from that food, an athlete ingests. Cr supplementation assisted with probiotics should, in theory, assist an athlete with maintaining healthy lean muscles by both siphoning off some of the food, as well as by shortening GI transit time and therefore the level of absorption, per unit of food and area of the GI, is decreased.

Taken as a whole, supplementation of the diet appears to be relatively safe with few, if any, real side effects. With proper attention to the complete diet, potential long-term side effects can be minimized, if not altogether eliminated. The toxicological data is still in its infancy. However, several novel parameters have been defined above which should also be monitored for a true analysis. It would be fruitful if various cancer marker, probiotic enumeration and identification we coupled with an analysis of the efficaciousness of Cr as a sports supplement.

It is anticipated that as more data is presented on the neuro- and cardio-protective effects of Cr, the use level and interest in Cr will also increase. It is apparent that Cr and associated compounds (e.g. cyclocreatine) will continue to be intensively studied and debated both for their efficacy and safety. Considering the beneficial effects of Cr on various muscle types and neurons, coupled with a lack of evidence for serious deleterious effects, it seems the answer to the initial question would be a qualified ‘yes.’ The qualification for the answer is that proper attention to total nutrition needs to be given. High intakes of water, probiotics, vitamins, minerals and antioxidants is a must. Without such attention, supplemental Cr would fall more heavily on the ‘cost’ portion of a cost-benefit curve.

Finally, the genomeceutical effect of creatine, as evidenced by its positive effect on GLUT4 expression deserves serious attention. It would be of benefit for a microarray analysis of the effect of Cr on various genes. Such simple and informative precautionary measures would help ensure the safety of Cr for both long-term and short-term supplementation. With potentially beneficial effects for diabetics, the application of Cr to the dietary supplement market is obviously widening, and at the same time refining, in its use.

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