Pergamon

www.neuroscience-ibro.com

PII: S0306-4522(02)00088-X

*Neuroscience* Vol. 112, No. 2, pp. 243–260, 2002

0306-4522/02 $22.00+0.00

## COMMENTARY

# HEALTH IMPLICATIONS OF CREATINE: CAN ORAL CREATINE SUPPLEMENTATION PROTECT AGAINST NEUROLOGICAL AND ATHEROSCLEROTIC DISEASE?

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**Abstract**—Major achievements made over the last several years have highlighted the important roles of creatine and the creatine kinase reaction in health and disease. Inborn errors of metabolism have been identified in the three main steps involved in creatine metabolism: arginine:glycine amidinotransferase (AGAT), *S*-adenosyl-L-methionine:*N*-guanidinoacetate methyltransferase (GAMT), and the creatine transporter. All these diseases are characterized by a lack of creatine and phosphorylcreatine in the brain, and by (severe) mental retardation. Similarly, knockout mice lacking the brain cytosolic and mitochondrial isoenzymes of creatine kinase displayed a slightly increased creatine concentration, but no phosphorylcreatine in the brain. These mice revealed decreased weight gain and reduced life expectancy, disturbed fat metabolism, behavioral abnormalities and impaired learning capacity.

Oral creatine supplementation improved the clinical symptoms in both AGAT and GAMT deficiency, but not in creatine transporter deficiency. In addition, creatine supplementation displayed neuroprotective effects in several animal models of neurological disease, such as Huntington's disease, Parkinson's disease, or amyotrophic lateral sclerosis. All these findings pinpoint to a close correlation between the functional capacity of the creatine kinase/phosphorylcreatine/creatine system and proper brain function. They also offer a starting-point for novel means of delaying neurodegenerative disease, and/or for strengthening memory function and intellectual capabilities.

Finally, creatine biosynthesis has been postulated as a major effector of homocysteine concentration in the plasma, which has been identified as an independent graded risk factor for atherosclerotic disease. By decreasing homocysteine production, oral creatine supplementation may, thus, also lower the risk for developing, e.g., coronary heart disease or cerebrovascular disease.

Although compelling, these results require further confirmation in clinical studies in humans, together with a thorough evaluation of the safety of oral creatine supplementation. 

*Key words:* GAMT deficiency, AGAT deficiency, creatine transporter deficiency, neurological disease, hyperhomocysteinemia, creatine supplementation.

CONTENTS



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*Abbreviations:* 3-NP, 3-nitropropionic acid; AGAT, arginine:glycine amidinotransferase; AIA, amino-imidazo-azaarene; ALS, amyotrophic lateral sclerosis; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; B-CK, brain cytosolic isoform of CK; CK, creatine kinase; CPEO, chronic progressive external ophthalmoplegia; cCr, cyclocreatine; Cr, creatine; Crn, creatinine; CrT, creatine transporter; CSF, cerebrospinal fluid; EEG, electroencephalography; GA, gyrate atrophy of the choroid and retina; GAMT, *S*-adenosyl-L-methionine:*N*-guanidinoacetate methyltransferase; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; LHON, Leber's hereditary optic neuropathy; M-CK, muscle cytosolic isoform of CK; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; Mi-CK, mitochondrial CK; $Mi_a$-CK, ubiquitous isoform of Mi-CKM; $Mi_b$-CK, sarcomeric isoform of Mi-CKM; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRI, magnetic resonance imaging; MTHFR, methylenetetrahydrofolate reductase; NMDA, *N*-methyl-D-aspartate; PCr, phosphorylcreatine; ROS, reactive oxygen species; *S*-AdoMet, *S*-adenosyl-L-methionine; SCA1, spinocerebellar ataxia type 1; SOD, superoxide dismutase.

The last several years have seen an often rather emotional debate on the perceived and documented benefits and risks of oral creatine (Cr) supplementation. Primarily in the sports/fitness area, creatine supplementation has an outstanding success, and is advocated by some as the only truly effective ergogenic aid besides carbohydrate loading. Others, however, are scared by the rapid propagation of Cr supplementation and by its broad use even in adolescents. For these critics, oral Cr supplementation represents an imponderable health risk, and they emphasize that meaningful long-term studies on the safety of oral Cr supplementation are still lacking. Possibly for this reason, the French Agency of Medical Security of Food (AFSSA) released an opinion in January 2001 stating that the health risk associated with oral Cr supplementation is not sufficiently evaluated, and that Cr may be a potential carcinogen. Since there is at present no scientific basis for the latter assertion, this recommendation, in turn, has provoked a wave of protest, with suppliers and defenders of oral Cr supplementation asserting that Cr is absolutely safe.

In order to clarify this controversial issue, it is the intention of this article to summarize the scientific facts, to describe the relationships between the creatine kinase (CK)/phosphorylcreatine (PCr)/Cr system and brain function, to outline the potential benefits of oral Cr supplementation in neurological, neuromuscular and atherosclerotic disease, and to address the safety of oral Cr supplementation in humans.

## THE FIRST 150 YEARS OF CREATINE RESEARCH

Creatine (from the Greek *kreas*, flesh) was first isolated from meat extract by Chevreul (1835) (Table 1). It took almost another hundred years until Fiske and Subbarow (1927) and Eggleton and Eggleton (1927) discovered PCr which, because of its labile nature, was also called 'phosphagen'. Lundsgaard (1930) showed that muscle contraction is accompanied by PCr breakdown rather than lactate production, and therefore proposed that PCr plays a central role in energy supply for muscle contraction. Lohmann (1934) discovered the CK reaction, in which the γ-phosphate group of ATP is transferred to Cr to yield ADP and PCr.

For many years, the conception prevailed that PCr represents a storage form of high-energy phosphates and is utilized as an 'energy buffer' to replenish ATP at elevated workloads. However, the identification of distinct mitochondrial and cytosolic CK isoenzymes in the 1960s and 1970s led Saks et al. (1978) and Bessman and Geiger (1981) propose the 'PCr shuttle'. According to this hypothesis, the mitochondrial and cytosolic CK isoenzymes work in opposite directions. Mitochondrial CK (Mi-CK) catalyzes PCr synthesis from ATP produced through oxidative phosphorylation in the mitochondrial matrix. On the other hand, cytosolic CK catalyzes regeneration of ATP from PCr at sites of ATP consumption such as motor proteins (e.g., myosin ATPase) or ion pumps (e.g., sarcoplasmic $Ca^{2+}$-ATPase). Since PCr and Cr are present in most CK-containing tissues in higher concentrations than ATP and ADP, and since they also display higher diffusion coefficients, the CK/PCr/Cr system considerably increases the total capacity for intracellular high-energy phosphate transport.

Strong support for the PCr shuttle hypothesis was provided by Tombes and Shapiro (1985) through elegant experiments on sea urchin spermatozoa. Sea urchin spermatozoa are highly polar cells, with a single mitochondrion containing Mi-CK in the midpiece, and with a long sperm tail (containing cytosolic CK) in which dynein ATPase brings about the wave-like motility pattern of the sperm. When the CK isoenzymes in these spermatozoa were selectively inactivated with 2,4-dinitrofluorobenzene, only the proximal third of the sperm tail remained motile, whereas the distal two thirds became stiff.

## INSIGHTS FROM CK KNOCKOUT MICE: THE PROBLEM OF REDUNDANCY

Stimulated by the findings on sea urchin spermatozoa (see above), many researchers in the field expected CK knockout mice to confirm the critical importance of the CK/PCr/Cr system for high-energy phosphate metabolism and transport. Surprisingly, however, most CK knockout mice displayed rather mild phenotypes (for a review, see Wyss and Kaddurah-Daouk, 2000).

In higher vertebrates, four CK isoenzymes are present. The muscle cytosolic isoform of CK (M-CK) and sarco-

Table 1. Milestones of creatine research

| | |
|---|---|
| 1835 | Discovery of creatine (Chevreul) |
| 1927 | Discovery of PCr (Eggleton and Eggleton; Fiske and Subbarow) |
| 1934 | Discovery of the CK reaction (Lohmann) |
| 1981 | PCr shuttle hypothesis (Bessman and Geiger) |
| 1985 | Elegant experiments on CK function in sea urchin spermatozoa (Tombes and Shapiro) |
| 1993 | First CK knockout mice (van Deursen et al.) |
| 1994 | Discovery of first inborn error of Cr metabolism (GAMT deficiency; Stöckler et al.) |
| 2000 | First cases of AGAT deficiency (Bianchi et al.) |
| 2001 | First cases of Cr transporter deficiency (Cecil et al.; Salomons et al.) |

meric Mi-CK ($Mi_b$-CK) are found primarily in striated muscle, whereas the brain cytosolic isoform of CK (B-CK) and ubiquitous Mi-CK ($Mi_a$-CK) are more ubiquitously expressed. Mutants lacking M-CK were viable and fertile, and displayed no overt abnormalities and no alterations in absolute muscle force; however, they showed decreased burst activity at 1 Hz or 5 Hz stimulation (van Deursen et al., 1993). Mutants lacking $Mi_a$-CK or $Mi_b$-CK also displayed no abnormalities, except for an impaired stimulation of mitochondrial respiration by Cr and for an impaired maintenance of ATP concentration in cerebral gray matter during seizures and mild hypoxia (Steeghs et al., 1995, 1997; Kay et al., 2000; Kekelidze et al., 2001). Double mutants deficient in both M-CK and $Mi_b$-CK more closely approached a myopathy phenotype, with more pronounced disturbances in muscle performance than in single mutants. Tetanic force, power, work, and the rates of tension development and relaxation were all decreased, most likely due to abnormal $Ca^{2+}$ handling (see Watchko et al., 2000; Wyss and Kaddurah-Daouk, 2000; Gorselink et al., 2001).

Until very recently, knockout mice for B-CK were not available. B-CK is the first CK isoform expressed in embryonic development, and it is the primary isoform in brain, which left the possibility that B-CK knockout animals might not be viable. Although this hypothesis has now been disproved, single mutants lacking B-CK as well as double mutants lacking both $Mi_a$-CK and B-CK displayed a number of notable abnormalities: decreased weight gain; reduced life expectancy; disturbed fat metabolism (i.e., reduced white lipid metabolism and brown adipose tissue hypertrophy); impaired thermoregulation, resulting in sudden drops in body temperature; behavioral abnormalities (less sitting and less grooming relative to controls); and depressed learning capacity in the Morris water maze test (Wieringa, 2001). The double knockout mice had a slightly increased Cr concentration, but no PCr in the brain.

In recent years, the phenomenon of gene and functional redundancy has been shown to explain the surprisingly mild phenotypes of some CK knockout animals. Gene redundancy means that the genome of an organism encodes multiple proteins that carry out identical or similar functions. In particular, the occurrence of gene redundancy is observed with essential cell functions, such as intracellular production and transport of high-energy phosphates. Adenylate kinase, nucleoside diphosphate kinase, pyruvate kinase, or glycolytic phosphotransfer reactions may facilitate high-energy phosphate transport similarly to the PCr shuttle (e.g., Dzeja et al., 1996; Pucar et al., 2000, 2001; Janssen et al., 2000). Preferential associations may occur, such as between adenylate kinase-catalyzed phosphoryltransfer and glycolytic ATP generation; between CK-catalyzed phosphoryltransfer and ATP production through mitochondrial oxidative phosphorylation (Zeleznikar et al., 1995); between adenylate kinase-catalyzed phosphoryltransfer and ATP-sensitive potassium channels in the communication of metabolic signals from the mitochondria to the plasmalemma (Carrasco et al., 2001); or between CK and thrombin receptor signaling through the protease activated receptor-1 (Mahajan et al., 2000).

Most importantly, when one of the phosphoryltransfer systems is inhibited, either by selective inhibitors or by targeted disruption of the respective genes, the other phosphoryltransfer systems are able, at least in part, to take over the function of the defective system (Zeleznikar et al., 1995; Dzeja et al., 1996; Boehm et al., 2000; Janssen et al., 2000; Pucar et al., 2000; De Groof et al., 2001). These results underline the pronounced plasticity of intracellular high-energy phosphate transport.

## INBORN ERRORS OF CREATINE METABOLISM

In order to more fully comprehend the clinical manifestations associated with Cr biosynthesis disorders, it seems important to provide a short introduction into the basics of Cr metabolism in humans (for a review see Wyss and Kaddurah-Daouk, 2000). Cr is either taken up from the food by intestinal absorption, and/or is synthesized endogenously, primarily in kidney, pancreas, and liver. Arginine:glycine amidinotransferase (AGAT) catalyzes the reversible transamidination of the guanidino group from arginine to glycine to yield guanidinoacetic acid and ornithine. *S*-Adenosyl-L-methionine:*N*-guanidinoacetate methyltransferase (GAMT) subsequently catalyzes *S*-adenosyl-L-methionine (*S*-Ado-Met)-dependent methylation of guanidinoacetic acid to yield Cr and *S*-adenosyl-L-homocysteine. Cr is then transported through the blood and is taken up into Cr-requiring tissues against a large concentration gradient (plasma [Cr] $\sim$50 μM; intracellular [Cr+PCr] up to 40 mM). Uptake into the tissues is afforded by a $Na^+$- and $Cl^-$-dependent Cr transporter for which, so far, two genes have been described (see also Snow and Murphy, 2001). The creatine transporter 1 (CrT1) gene is localized on chromosome Xq28 and seems to be expressed ubiquitously. On the other hand, the CrT2 gene is localized on chromosome 16p11.1–11.2 and its expression seems to be restricted to the testis. Cr and PCr are non-enzymatically converted at an almost constant rate (overall $\sim$1.7%/day) into creatinine (Crn) which passively diffuses out of the cells and is excreted by the kidneys into the urine. The urinary Crn excretion therefore is a convenient indicator of the total Cr stores in the body. A 70-kg man contains $\sim$120 g Cr, of which $>$90% is found in muscle tissue.

The first inborn error of Cr metabolism, GAMT deficiency, was discovered in 1994 (Stöckler et al., 1994). Ten patients suffering from this disease have been identified so far (Schulze et al., 1997; Ganesan et al., 1997; van der Knaap et al., 2000; Leuzzi et al., 2000; Ensenauer et al., 2001; Schulze, 2001; see also von Figura et al., 2000; Wyss and Kaddurah-Daouk, 2000). GAMT deficiency is an autosomal recessive disorder and usually manifests during the first months of life as developmental delay, arrest, or even regression. Clinical symptoms are heterogeneous and include mental retardation, involuntary extrapyramidal movements, speech disability, epilepsy, muscular hypotonia and weakness, and, in older

patients, autism with self-injurious behavior (von Figura et al., 2000; Schulze, 2001). Experimental findings include increased guanidinoacetic acid concentrations (by the age of 3 days; Carducci et al., 2001) and either decreased Cr concentrations or no Cr in brain, cerebrospinal fluid (CSF), blood/serum, and urine. Crn concentration and excretion are decreased in serum and urine, respectively. Oral supplementation with 0.35–2.0 g/kg/day of Cr slowly increased the total Cr ( = Cr+PCr) concentration in the brain and normalized urinary Crn excretion. However, even after several months of treatment, total Cr concentrations in these patients' brains remained significantly below the normal range, and guanidinoacetic acid concentrations remained largely elevated in CSF, serum and urine (von Figura et al., 2000; Schulze et al., 2001). All patients benefited from Cr supplementation, although to different degrees. None has returned to a normal developmental level and all patients still lack active speech.

In order to further improve the clinical manifestations, combination treatments have been tried. Cr supplementation together with dietary arginine restriction failed to further decrease guanidinoacetic acid concentrations and to provide an additional clinical benefit (Schulze et al., 1998). On the other hand, combination of Cr plus ornithine supplementation with dietary arginine restriction further reduced the guanidinoacetic acid concentration in CSF, plasma, and urine, and almost completely suppressed epileptic seizures (Schulze et al., 2001). These findings therefore suggest an important epileptogenic activity of guanidinoacetic acid, a notion that is in line with earlier results (see Wyss and Kaddurah-Daouk, 2000). They also point to a beneficial effect of ornithine supplementation, whereby two alternative mechanisms of action are conceivable: (i) since the AGAT reaction is reversible ($K' \approx 1$), and since ornithine and guanidinoacetic acid are the two products of AGAT, ornithine supplementation may suppress guanidinoacetic acid formation according to the mass action ratio of the reaction. The fact that the initial dosage of ornithine chosen (0.1 g/kg/day) still did not fully normalize the plasma guanidinoacetic acid concentration (Schulze et al., 2001) may indicate that even higher amounts of ornithine are required to achieve an optimal therapeutic result. (ii) Dietary arginine restriction (without ornithine supplementation) resulted in only a *transient* reduction in plasma arginine concentration. In contrast, dietary arginine restriction plus ornithine supplementation yielded a sustained decrease in plasma arginine concentration which preceded the decrease in plasma guanidinoacetic acid concentration. This would indicate that lowering of the plasma arginine concentration is a crucial prerequisite for a further decrease in guanidinoacetic acid concentration.

AGAT deficiency was recently identified as an autosomal recessive disease in two sisters, 4 and 6 years of age (Bianchi et al., 2000; Item et al., 2001). The girls suffered from mild mental retardation and severe language delay. The serum concentrations of both Cr and guanidinoacetic acid were reported to be normal. On the other hand, guanidinoacetic acid excretion in the urine was largely decreased, and Cr and PCr were absent in the brain or at least below the detection limit of the methods employed. Cr supplementation at a rate of 400 mg/kg/day increased total Cr concentration in the brain to 40% and 80% of control within 3 and 9 months, respectively. With supplementation, a rapid progress in the acquisition of visual perceptual and fine motor skills was seen, together with a slower rate of general cognitive development. Language abilities also improved, but more slowly than non-verbal skills.

Finally, six male patients with X-linked CrT1 deficiency have been identified (Cecil et al., 2001; Salomons et al., 2001; Salomons, 2001). These patients initially presented with mild mental retardation, but with severe delay both in speech and in expressive language function. In addition, central hypotonia was observed, but gross and fine motor functions were normal. Unfortunately, this disease is progressive, so that by age 17, cerebral atrophy and an extremely low IQ (36) were noted (Cecil and Degrauw, 2001). Cr and PCr were absent in the brain, but Cr was increased in urine and plasma. Guanidinoacetic acid concentration in plasma and urine as well as Crn concentration in the blood were normal. The latter finding might indicate that despite CrT1 deficiency, Cr uptake into muscle may be normal. As a matter of fact, Cr has been observed in a patient's muscle (Cecil and Degrauw, 2001). Cr supplementation (0.34 g/kg/day) yielded no increase in brain Cr concentration and no clinical improvement.

The different Cr metabolism defects are highly instructive, allow interesting conclusions, and raise new challenging questions:

1. The finding of Cr in muscle of a patient with CrT1 deficiency (Cecil and Degrauw, 2001) is surprising, since (i) only two CrT genes have been described so far, (ii) CrT2 is expressed exclusively in testis, and (iii) CrT1 was shown to be expressed in both muscle and brain (see Wyss and Kaddurah-Daouk, 2000). Several alternative scenarios are conceivable: either there is even more heterogeneity in CrT isoproteins than considered so far; or Cr uptake into muscle is afforded as a side activity by another, muscle-specific, plasmalemmal transport system; or as a response to CrT1 deficiency, there is a compensatory up-regulation of CrT2 in muscle, but not in brain.
2. Several findings strongly suggest a limited permeability of the blood–brain barrier for Cr and, therefore, favor *de novo* Cr biosynthesis in the brain. Firstly, supplementation with high doses of Cr failed to normalize brain Cr concentration in both AGAT and GAMT deficiency (see above), despite normal or even supranormal concentrations of Cr in serum. Conversely, in laboratory animals, depletion of the bodily Cr stores by feeding of the Cr analogue β-guanidinopropionic acid progressively displaced Cr from muscle, but had only little influence on the Cr and PCr contents of brain (Holtzman et al., 1989). Secondly, in patients with AGAT or GAMT deficiency who ingest Cr, Cr uptake into the brain is a very slow process; steady-state concentrations are only reached

after many months, and they still remain below the normal range. Thirdly, the finding of a normal serum concentration of Cr in two sisters with AGAT deficiency (Bianchi et al., 2000) indicates that patients can efficiently take up dietary Cr (which in adults on average contributes ~50% to the daily Cr requirements) into the blood and most likely also into muscle tissue. The lack of Cr and PCr in these patients' brain, however, suggests that the normal brain is self-sufficient in terms of Cr biosynthesis. This latter conclusion is further corroborated by a number of previous studies showing that (i) the brain is capable of performing *de novo* Cr biosynthesis (see Wyss and Kaddurah-Daouk, 2000; Braissant et al., 2001), and (ii) astrocytes in general and, in particular, those contacting the capillary endothelial cells forming the blood–brain barrier do not express CrT1 (Braissant et al., 2001).

3. In sharp contrast to the above conclusions, the lack of Cr in the brain of patients with CrT1 deficiency (Cecil et al., 2001) questions the brain's capacity for *de novo* Cr biosynthesis. Similar to the rest of the body where the liver is the primary site of Cr biosynthesis, but contains only low levels of Cr itself, there may be a strict separation of Cr-synthesizing and Cr-accumulating cells in the brain as well. Only if this were the case, the Cr transporter would be required for significant accumulation of Cr in the brain. Although attractive, this latter hypothesis is, again, in contrast to a recent publication of Braissant et al. (2001). Based on *in situ* hybridization and immunohistochemistry experiments, these authors concluded that all cells of the brain are essentially capable of performing *de novo* Cr biosynthesis.

Clearly, more work is required to unravel the mysteries of Cr metabolism in the brain. Transgenic animals with targeted disruptions of the AGAT, GAMT and CrT gene(s) will be valuable tools to confirm the findings made in humans, to more systematically study the metabolic implications of the primary defect in the entire body, and to devise optimized treatment protocols. It will also be instructive to further investigate whether AGAT, GAMT or CrT1 deficiency also decreases Cr concentration in muscle or in other tissues.

In conclusion, four different primary defects affecting the functional capacity of the CK/PCr/Cr system (AGAT, GAMT and CrT1 deficiency in humans, and CK knockout mice) all resulted in developmental delay and learning disability. In all likelihood, the overlapping neurologic manifestations are therefore due to Cr deficiency rather than to secondary metabolic alterations. These findings therefore strongly support the crucial importance of the CK/PCr/Cr system for high-energy phosphate provision and transport in the brain. If the blood–brain barrier in fact impedes Cr uptake into the brain, significant increases in brain Cr concentration may only be possible either with high doses of oral Cr or with alternative concepts to bypass the blood–brain barrier.

## HEALTH—BENEFICIAL EFFECTS OF CREATINE AND ITS ANALOGUES: ORAL CREATINE SUPPLEMENTATION AS A MULTI-PURPOSE PREVENTION STRATEGY?

A considerable list of health benefits have been reported or proposed for oral supplementation with Cr and/or its analogues (Table 2; for a review, see Wyss and Kaddurah-Daouk, 2000). Based on this list, one might be inclined to consider Cr and its analogues promising pharmaceutical drugs. The following thoughts and arguments, however, are intended to help put the current knowledge on the potential benefits and limitations of oral Cr supplementation into context.

1. Since disruption of the CK/PCr/Cr system either in CK knockout animals or in AGAT, GAMT, or CrT1 deficiencies in humans produces rather mild phenotypes (see above), but, on the other hand, oral Cr supplementation increases the total Cr concentrations in muscle and brain of healthy subjects by only ~25% and 9%, respectively (Wyss and Kaddurah-

Table 2. Health benefits reported or proposed for orally administered creatine and/or creatine analogues (for a review, see Wyss and Kaddurah-Daouk, 2000)

| | Active compound | Selected recent references |
|---|---|---|
| Neuroprotection | Cr and Cr analogues | see text |
| Improvements in muscle strength and general well-being | Cr | Williams et al., 1999; Hespel et al., 2001a,b |
| Prevention/delay of strength decline in the elderly | Cr | Tarnopolsky, 2000; Wiroth et al., 2001 |
| Antitumor | primarily cCr | Kornacker et al., 2001 |
| Antiviral | Cr analogues; Cr? | Ness and McCarty, 2001 |
| Antidiabetic | Cr and Cr analogues | Vaillancourt et al., 2001; Larsen et al., 2001; Bajuk, 2001 |
| Anti-inflammatory | Cr | – |
| Inhibition of blood platelet aggregation | Cr | – |
| Lipid (cholesterol) lowering | Cr | (Volek et al., 2000; Schilling et al., 2001) |
| Protection against ischemic damage | Cr and Cr analogues | see text |
| Beneficial effects in (neuro-)muscular and cardiac diseases | Cr | Vorgerd et al., 2000; Walter et al., 2000; Passaquin et al., 2002; (Neubauer et al., 1998; Doherty et al., 2001) |
| Lowering of plasma homocysteine levels | Cr | see text |

References in parentheses report studies in which no beneficial effect of Cr supplementation was seen.

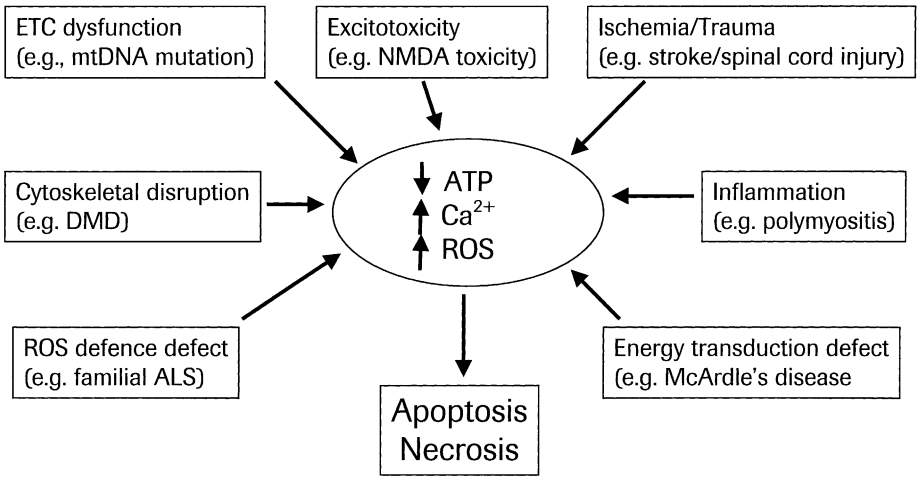


Fig. 1. Convergence of pathologic events in different neurological and neuromuscular diseases (Tarnopolsky and Beal, 2001). ETC, electron transport chain; mtDNA, mitochondrial DNA; DMD, Duchenne muscular dystrophy.

Daouk, 2000; Dechent et al., 1999), it is difficult to perceive how Cr supplementation should provide eminent health benefits.

2. The health benefits listed in Table 2 are not likely due to distinct, specific mechanisms of action of oral Cr supplementation. More probably, many of the health benefits may be due to a general strengthening of cellular energetics, making the cells and tissues more viable and resistant to metabolic and environmental challenges. If the effects of Cr supplementation were in fact pleiotropic and non-specific, a priori one would not expect only beneficial effects with Cr supplementation (see Safety of oral creatine supplementation).
3. Most attention in the public is paid at present to the ergogenic effects of Cr. However, the CK knockout animals as well as the AGAT, GAMT, and CrT1 deficiencies in humans have clearly shown that the impact of the CK system on brain function is more pronounced than on muscle function. Consequently, the neuroprotective role of Cr may potentially be much more relevant for mankind than the stimulation of muscle performance in particular protocols of maximal, intermittent exercise.

In conclusion, our knowledge of the health benefits and limitations of oral Cr supplementation, as well as of the underlying mechanisms of action, is still incomplete. Given its pleiotropic effects and the mostly small improvements in function associated with Cr supplementation, Cr is unlikely to become a successful therapeutic drug with a clearly defined mechanism of action. Rather, Cr may modulate the rate of disease progression and, in combination with other health beneficials, may be applied to the *prevention* of disease.

It would be beyond the scope of this article to discuss all health benefits of oral Cr supplementation in detail. Therefore, the following sections will focus on two areas of particular interest, namely the potential of oral Cr supplementation in neuroprotection and in lowering the plasma levels of homocysteine. For all other topics, the reader is referred to Wyss and Kaddurah-Daouk (2000) and to the references listed in Table 2.

## NEUROPROTECTIVE EFFECTS OF CREATINE

Despite the fact that neurological disorders are caused by many different primary defects, they often converge to display similar impairments in cellular energy metabolism in the brain. In these instances, intracellular concentration of ATP is decreased, resulting in cytosolic

Table 3. Reported cases of neuroprotective effects of oral creatine supplementation

| | |
|---|---|
| Animal models for: | |
| Huntington's disease | transgenic mouse models; 3-NP and malonate toxicity in the rat |
| Amyotrophic lateral sclerosis | wobbler mice and transgenic mice carrying the G93A SOD-1 mutation |
| Parkinson's disease | MPTP toxicity in mice |
| Excitotoxicity | intrastriatal injection of NMDA in rats |
| Head trauma | controlled cortical contusions in mice and rats |
| Spinocerebellar ataxia type 1 | transgenic mouse model with an expanded poly-Gln ataxin-1 |
| *In vitro* experiments: | |
| Glutamate, β-amyloid, and 3-NP toxicity | |
| 3-Hydroxyglutarate neurotoxicity | |
| Ischemia/anoxia in brain slices | |
| Human studies: | |
| AGAT, GAMT, and CrT1 deficiency | see text |
| Gyrate atrophy of the choroida and retina | primarily improvements in muscle performance reported |
| Mitochondrial cytopathies (MELAS, LHON) | |
| LCHD deficiency | |
| Myasthenia gravis | |

accumulation of $Ca^{2+}$ and stimulation of formation of reactive oxygen species (ROS). $Ca^{2+}$ and ROS, in turn, trigger apoptotic or necrotic cell death (see Fig. 1). For many of these disorders, impairments of brain Cr metabolism were described as well, i.e., decreases in total Cr concentration, PCr concentration, CK activity, and/or CrT content (for reviews see Wyss and Kaddurah-Daouk, 2000; Tarnopolsky and Beal, 2001; Butterfield and Kanski, 2001). Conversely, as discussed above, impairment of CK function either in CK knockout animals or in AGAT, GAMT or CrT1 deficiency in humans resulted in developmental delay and learning disability. Therefore, it is tempting to speculate that oral Cr supplementation may be a means of alleviating some of the clinical symptoms in neurological disease and delaying and/or slowing disease progression, although impairment of CK function is not the primary defect.

Over the last few months and years, a considerable body of scientific evidence supporting this conception has accumulated, both in animal models of neurodegenerative disease and in clinical studies (Table 3). In an animal model of Parkinson's disease, Cr protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopamine depletion and against loss of Nissl- and tyrosine hydroxylase-immunostained neurons in the substantia nigra of mice (Matthews et al., 1999). Wobbler mice and transgenic mice carrying the G93A superoxide dismutase-1 (SOD-1) mutation are two animal models of amyotrophic lateral sclerosis (ALS, motor neuron disease). In these mice, oral administration of Cr extended survival by up to 17% (from 144 to 169 days), improved motor performance, protected from loss of both motor neurons and substantia nigra neurons, retarded denervation muscle atrophy, and decreased both biochemical indices of oxidative damage and cortical glutamate concentrations (Klivenyi et al., 1999; Ikeda et al., 2000; Andreassen et al., 2001b).

Cr supplementation has also been investigated in four animal models of Huntington's disease: (i) malonate toxicity in the rat (Matthews et al., 1998; Malcon et al., 2000); (ii) 3-nitropropionic acid (3-NP) toxicity in the rat (Matthews et al., 1998; Shear et al., 2000); (iii) a transgenic mouse model (N171-82Q) produced by 82 polyglutamine repeats in a 171-amino acid N-terminal huntingtin fragment (Andreassen et al., 2001a); and (iv) a transgenic mouse model (line R 6/2) expressing exon 1 of the human huntingtin gene with an expanded CAG repeat (Ferrante et al., 2000). In the two transgenic mouse models of Huntington's disease, oral Cr supplementation improved survival by up to 19% (from 132 to 156 days, and from 98 to 115 days, respectively), delayed the onset of weight loss, slowed the development of motor symptoms, of brain atrophy, and of atrophy of striatal neurons, lessened the formation of intranuclear inclusions, attenuated reductions in striatal *N*-acetylaspartate concentration, and delayed the onset of diabetes. Similarly, in malonate and 3-NP toxicity in the rat, Cr reduced the volume of striatal lesions, protected against striatal lactate accumulation, and reduced biochemical indices of oxidative stress. In addition, Cr attenuated 3-NP-induced striatal atrophy, ventricular enlargement, spatial learning deficits and gait abnormalities on a balance-beam task.

In a transgenic mouse model of spinocerebellar ataxia type 1 (SCA1) caused by an expanded CAG trinucleotide repeat in the human SCA1 gene, oral Cr supplementation prevented or delayed Purkinje cell loss in the cerebellum, but neither delayed nor slowed progression of the ataxic phenotype (Kaemmerer et al., 2001). These results therefore question the hypothesis that energy impairment is a primary and early pathogenic mechanism in this mouse model of polyglutamine disease.

In order to test the effect of oral Cr supplementation on excitotoxic cell death, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid were injected into the striatum of rats. Cr attenuated striatal excitotoxic lesions produced by NMDA, but had no effect on lesions produced by AMPA or kainic acid (Malcon et al., 2000). In a supposed rat model of ischemic neuronal damage, inhibition of glutamate re-uptake (by intrastriatal administration of L-*trans*-pyrrolidine-2,4-dicarboxylate) together with induction of mitochondrial failure (by intraperitoneal injection of 3-NP) produced striatal damage. Pretreatment for 1 week with 1% Cr in the drinking water reduced the size of the striatal lesions by 32% (Massieu et al., 2001).

The susceptibility of the brain to hypoxia-induced seizures is highest in the newborn period. In rat and rabbit pups, hypoxia produces seizures most frequently (i.e., in 60–85% of pups exposed to 4% $O_2$ for 8 min) at 10–12 days of age and 15–20 days of age, respectively. Subcutaneous injection of Cr at a rate of 3 g/kg body weight for 3 days before hypoxia increased the PCr/nucleoside triphosphate ratio in the brain (Holtzman et al., 1998, 1999). The hypoxia-induced seizures were fully prevented at 10 days of age in the rat and at 15 days of age in the rabbit, and were reduced by 60% at 20 days of age in the rabbit by prior Cr injection. In 10-day-old rats, Cr also reduced hypoxia-induced mortality.

In animal models of traumatic brain injury obtained by controlled cortical contusions, intraperitoneal injection of Cr in mice and oral Cr supplementation in rats reduced cortical damage by up to 50% and preserved mitochondrial energetics (Sullivan et al., 2000). In injured rats, oral Cr supplementation increased the mitochondrial membrane potential, decreased intramitochondrial levels of ROS and $Ca^{2+}$, and maintained the concentration of ATP in cerebral cortex synaptosomes. Furthermore, in isolated non-synaptosomal mitochondria, oral Cr supplementation prevented induction of mitochondrial permeability transition which seems to be an important determinant for both necrotic and apoptotic cell death (see Crompton, 1999).

The above *in vivo* experiments were also complemented with *in vitro* studies. Glutaryl-CoA dehydrogenase deficiency is an autosomal recessive neurometabolic disorder characterized by accumulation of 3-hydroxyglutarate. The latter is a neurotoxin mediating excitotoxicity via NMDA receptors. In primary neuronal cultures from chick embryo telencephalons, Cr reduced neuronal damage and ROS formation when administered at least 6 h

before 3-hydroxyglutarate (Kölker et al., 2001). In cultured rat hippocampal and striatal neurons as well as in cortical and striatal astrocytes, Cr (and PCr) protected against glutamate, β-amyloid, and 3-NP toxicity (Deshpande et al., 1997; Brewer and Wallimann, 2000; Brustovetsky et al., 2001). In contrast, Cr had no significant protective effect in corticostriatal slice cultures when co-incubated with 3-NP (Gramsbergen et al., 2000). Finally, in hippocampal, striatal, or brainstem slices from rats and mice, Cr was shown to prevent anoxic ATP depletion, to delay anoxic depolarization, to protect from hypoxic (neuronal) damage, to delay and slow ischemia-induced dopamine release in striatal slices, and to protect the central respiratory network under anoxic conditions (Wilken et al., 1998, 2000; Balestrino et al., 1999; Toner and Stamford, 1999).

Several factors have been postulated to contribute to the neuroprotective effects of oral Cr supplementation: improvement of energy and calcium homeostasis, enhanced presynaptic glutamate uptake, or protection of mitochondria from mitochondrial permeability transition (see Brewer and Wallimann, 2000; Wyss and Kaddurah-Daouk, 2000; Kaemmerer et al., 2001). The relevance of these factors and the detailed mechanisms of action *in vivo* are not yet clear. The only consistent conclusion that can be drawn so far is that neuroprotection depends on increases in Cr and PCr concentrations in the brain. However, more Cr is not necessarily better: several animal studies have shown that most pronounced neuroprotection is observed at intermediate Cr dosages, while supplementation with more or less Cr yields inferior results (e.g., Matthews et al., 1998, 1999; Ferrante et al., 2000). Therefore, determination of the optimal dosage and mode of application is crucial for defining the full neuroprotective potential of Cr. This is particularly true for humans for whom no meaningful dose–response data are currently available.

In comparison to the considerable amount of literature describing animal or *in vitro* studies, only a few studies have addressed the effects of oral Cr supplementation in human diseases with neurological involvement. Clinical studies in Huntington's disease, Parkinson's disease, and ALS are currently on-going, but no results are yet available. Tarnopolsky et al. (1997) investigated oral Cr supplementation (10 g/day for 14 days, followed by 4 g/day for 7 days) in seven patients with mitochondrial cytopathies (mostly mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes, MELAS). Cr supplementation resulted in an increased high-intensity anaerobic and aerobic performance, but had no beneficial effects on activities of daily living such as cooking, housework, grooming, dressing, bathing, and walking. Subsequently, Tarnopolsky and Martin (1999) investigated Cr supplementation (5–10 g/day for 5 days) in 102 patients with neuromuscular disease (mitochondrial cytopathies, neuropathic disorders, inflammatory myopathies, dystrophies, congenital myopathies, etc.). Cr supplementation increased all indices of muscle strength measured. The muscle strength-enhancing effect of Cr supplementation (0.1–0.2 g/kg body weight for at least 3 months) was also seen in two patients with Kearns–Sayre syndrome, one patient with neuropathy, ataxia, and retinitis pigmentosa syndrome, and one patient with MELAS (Borchert et al., 1999). In addition, the patients and/or parents reported an improvement in the ability to perform daily activities and generally felt more motivated with a better ability to concentrate on individual tasks. These improvements were noticeable within 2–4 weeks of Cr supplementation.

In contrast to the previous findings, a placebo-controlled crossover trial of Cr supplementation (20 g/day for 4 weeks) in 13 patients with chronic progressive external ophthalmoplegia (CPEO) and three patients with mitochondrial myopathy yielded no significant improvements with regard to exercise performance, eye movements, or activities of daily life (Klopstock et al., 2000). This lack of benefit is not surprising, since only low-intensity exercise variables were tested, and since muscle Cr concentrations seem to be normal in CPEO – in contrast to most other mitochondrial diseases. Similarly, in 37 subjects with hereditary motor sensory neuropathy types I and II, oral Cr supplementation (5 g/day for adults, 0.1 g/kg/day for children) for 1 month had no significant effects on activities of daily living – as evaluated by a visual analogue activities of daily living questionnaire – on body weight, fat-free mass, as well as on all measures of muscle function analyzed (Doherty et al., 2001).

Gyrate atrophy of the choroid and retina (GA) is an autosomal recessive tapetoretinal dystrophy, beginning at 5–9 years of age with night blindness and myopia, and continuing with progressive constriction of the visual fields until, at age 20–45 years, the patients become practically blind. In addition, type II skeletal muscle fiber atrophy is seen with this disease. The underlying primary defect of GA is a deficiency in mitochondrial matrix L-ornithine:2-oxo-acid aminotransferase, resulting in accumulation of ornithine in the body which in turn inhibits the first enzyme of Cr biosynthesis, AGAT (for a review, see Wyss and Kaddurah-Daouk, 2000). Accordingly, the concentration of Cr is reduced in plasma, skeletal muscle, cerebrospinal fluid and brain of GA patients (see Näntö-Salonen et al., 1999). Brain magnetic resonance imaging (MRI) investigation revealed degenerative white matter lesions in 50% of GA patients, and 70% had premature atrophic changes, with an increase in the number of Virchow's spaces (Valtonen et al., 1999). Approximately 60% of GA patients had abnormal slow background activity, focal lesions or high-amplitude β-rhythm in electroencephalography (EEG). Oral Cr supplementation at a rate of 0.75–2.0 g/day for up to 15 years normalized the Cr concentration in skeletal muscle and prevented type II muscle fiber atrophy (Heinänen et al., 1999). In contrast, Cr supplementation only partially reversed the decrease in Cr concentration in the brain (Näntö-Salonen et al., 1999) and had no favorable effect on the MRI or EEG abnormalities (Valtonen et al., 1999). The latter may be due to the rather low daily dose of Cr used and to the limited permeability of the blood–brain barrier for Cr (see above).

Long-chain 3-hydroxyacyl-CoA dehydrogenase

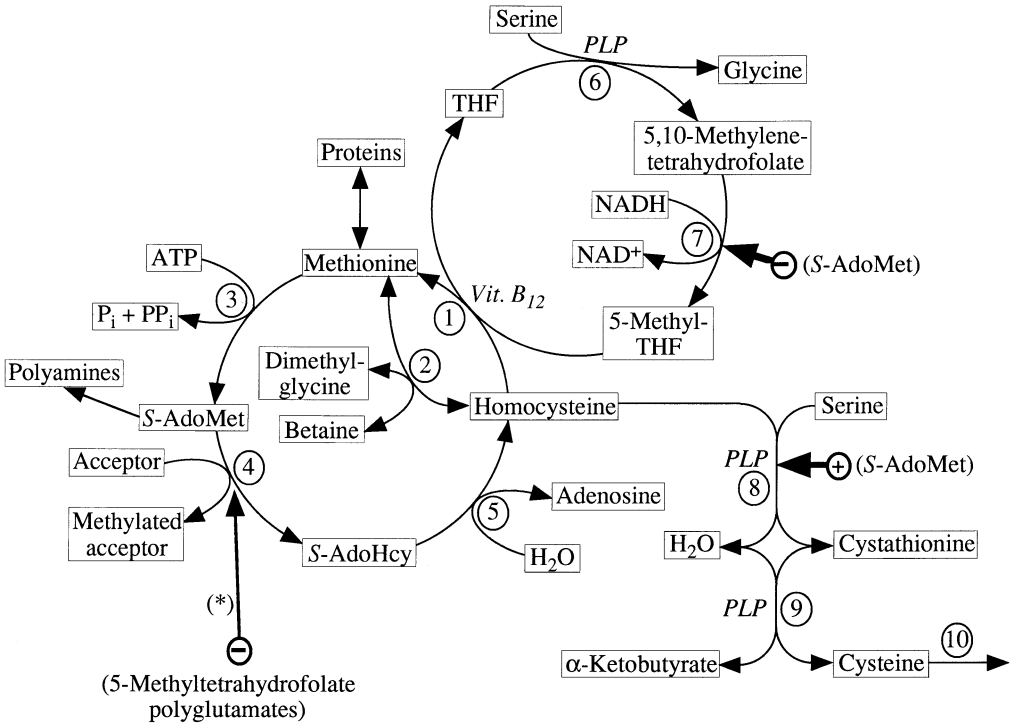


Fig. 2. Homocysteine metabolism in humans and animals. The enzymes involved are numbered: (1) 5-methyl-THF:homocysteine methyltransferase; (2) betaine:homocysteine methyltransferase; (3) ATP:methionine adenosyltransferase; (4) different *S*-AdoMet-dependent transmethylation enzymes, e.g., GAMT or glycine *N*-methyltransferase; (5) adenosylhomocysteinase; (6) serine hydroxymethylase; (7) methylenetetrahydrofolate reductase; (8) cystathionine β-synthase; (9) γ-cystathionase; (10) further metabolism/breakdown of cysteine. Bold arrows indicate primary regulation mechanisms: (+) stimulation of activity; (−) inhibition of activity; (*) inhibition of glycine *N*-methyltransferase by 5-methyltetrahydrofolate polyglutamates. The cofactors required for selected enzymes are indicated in italics.

(LCHAD) deficiency, a defect of mitochondrial β-oxidation, commonly presents with encephalopathy associated with hypoketotic hypoglycemia and cardiomyopathy. In a case report on a 6-year-old boy, supplementation with 4 g Cr daily was described to markedly lessen pain and improve exercise tolerance (Shortland et al., 2001). While before Cr supplementation, the patient required nine hospital admissions in one year, he had only one admission in the following year. Clearly, better controlled studies will be required to confirm the benefits of Cr supplementation in LCHAD deficiency.

Patients with myasthenia gravis typically exhibit skeletal muscle wasting, neuromuscular fatigue, and weakness. These symptoms are believed to be caused by a functional blocking or loss of postsynaptic acetylcholine receptors at the neuromuscular junction, as well as by a decreased PCr content in skeletal muscle (see Stout et al., 2001). In a case study on a 26-year-old man, Cr supplementation (5 g/day for 15 weeks) plus resistance training increased body weight and fat-free mass, and improved several measures of muscle performance by up to 37% (Stout et al., 2001). Finally, preliminary studies in ALS (Mazzini et al., 2001) and Huntington's disease (Kieburtz, 2001) yielded no evidence for a neuroprotective effect of oral Cr supplementation (3–5 g/day over 3–6 months), although it increased maximum isometric muscle strength (Mazzini et al., 2001).

Although Cr supplementation has been shown to modulate the manifestations of some neurological diseases, it is important to note that the human studies performed so far represent isolated cases, mostly with a small number of patients and, sometimes, questionable experimental designs. With few exceptions (for instance, genetic Cr metabolism defects – see above), the investigations were primarily directed towards studying the effect of Cr supplementation on muscle function and not on the neuroprotective role of Cr – leaving this avenue of treatment largely unexplored in man.

## POTENTIAL OF CREATINE SUPPLEMENTATION IN LOWERING PLASMA HOMOCYSTEINE CONCENTRATION

Homocysteine has been suggested to be an independent, graded risk factor for atherosclerotic disease affecting coronary, cerebral and/or peripheral vessels (for reviews, see Boers, 1998; Welch and Loscalzo, 1998; Selhub, 1999; Hankey and Eikelboom, 1999). For instance, a 5-μM increment in total homocysteine plasma level was found to be associated with an increased risk for developing coronary heart disease of 60% for men and 80% for women. Additional studies have demonstrated associations between elevated plasma homocysteine levels and increased risks of hypertension, neural tube defects, Alzheimer's disease, dementia, loss of cognitive function, and renal and liver disease. In line with

these epidemiological findings, studies in both normo- and hypertensive rats suggested increased levels of plasma homocysteine to cause multi-organ injury (Miller et al., 2000).

Homocysteine is formed from *S*-adenosyl-L-homocysteine (*S*-AdoHcy) which, in turn, is the product of *S*-AdoMet-dependent methylation reactions (Fig. 2). Homocysteine is either remethylated to methionine or catabolized to cysteine and further, with cystathionine β-synthase as the first enzyme in this (transsulfuration) pathway. The relative contributions of remethylation and transsulfuration to homocysteine metabolism primarily depend on the dietary methionine levels. When dietary methionine intake is low, homocysteine is primarily remethylated to methionine. However, when excess amounts of methionine are ingested, homocysteine is primarily degraded through the transsulfuration pathway.

Notably, a number of enzymes involved in homocysteine metabolism are cofactor-dependent. Cystathionine β-synthase, γ-cystathionase and serine hydroxymethylase have pyridoxal 5′-phosphate as a cofactor, whereas *N*-5-methyltetrahydrofolate:homocysteine methyltransferase is vitamin $B_{12}$-dependent.

In terms of regulation of homocysteine metabolism, the intracellular concentration of *S*-AdoMet plays a central role. When methionine intake is high, the concentration of *S*-AdoMet is also increased and acts both as an allosteric inhibitor of methylenetetrahydrofolate reductase (MTHFR) and as an activator of cystathionine β-synthase (see Selhub, 1999). Thereby, *S*-AdoMet suppresses the synthesis of a substrate (*N*-5-methyltetrahydrofolate) required for remethylation and stimulates catabolism of homocysteine through the transsulfuration pathway. When the concentration of *S*-AdoMet is low, MTHFR is activated whereas cystathionine β-synthase is not, so that remethylation of homocysteine is favored. A further component of the regulatory network is glycine *N*-methyltransferase which is abundantly present in liver and represents one of the reactions consuming *S*-AdoMet. Glycine *N*-methyltransferase is strongly inhibited by *N*-5-methyltetrahydrofolate polyglutamates. When the concentration of *S*-AdoMet is low and, consequently, *N*-5-methyltetrahydrofolate synthesis stimulated, further consumption of *S*-AdoMet by glycine *N*-methyltransferase will be suppressed, and vice versa.

The plasma homocysteine concentration normally is very tightly controlled and is kept close to 10 μM. However, hyperhomocysteinemia can be induced either by a genetic defect in one of the enzymes involved in homocysteine and/or cobalamin metabolism, or by a nutritional deficiency of one of the vitamins required as cofactors for these enzymes (see Welch and Loscalzo, 1998; Selhub, 1999; Hankey and Eikelboom, 1999).

A key question is the relationship between the plasma homocysteine concentration and Cr biosynthesis. GAMT, like glycine *N*-methyltransferase, is one of the *S*-AdoMet-dependent transmethylation enzymes. It has been estimated by Mudd et al. (1980) that Cr biosynthesis accounts for ~70% of the total utilization of 'labile methyl groups' (i.e., *S*-AdoMet) in the body. Based on these data, McCarty (2001) and Steenge et al. (2001) postulated that oral Cr supplementation, by suppressing endogenous Cr biosynthesis, has a sparing effect on *S*-AdoMet. This might result in activation of cystathionine β-synthase, would reduce the rate of formation of homocysteine, and might thereby contribute to a lowering of plasma homocysteine levels. That endogenous Cr biosynthesis may in fact be an important determinant of plasma homocysteine concentration is corroborated by the fact that in a number of studies, total plasma homocysteine concentration was positively correlated with the serum Crn concentration which, indirectly, is a measure of whole body Cr biosynthesis (see Lussier-Cacan et al., 1996; Dierkes et al., 2001; Rauh et al., 2001).

In order to test the above hypothesis, Steenge et al. (2001) tried oral Cr supplementation (20 g/day for 5 days, followed for 3 g/day for 8 weeks) in a limited number of healthy women aged 19–38 years and having a mean plasma homocysteine concentration of 10 μM. Cr supplementation resulted in a small (0.3 μM), but non-significant decrease in mean plasma homocysteine concentration, which led the authors to conclude: "...either ingesting Cr at the rates described here does not inhibit endogenous Cr synthesis or the rate of de novo Cr synthesis is not important in regulating plasma homocysteine homeostasis". Both conclusions seem inappropriate. The lack of an effect in young, healthy subjects with a well-balanced and fully functional regulatory network for homocysteine metabolism is neither proof nor indication that Cr supplementation will not have a favorable effect in subjects with compromised homocysteine homeostasis. Moreover, in a recent study on rats, supplementation of the diet with guanidinoacetic acid (stimulating endogenous Cr biosynthesis) resulted in a 49% increase in the plasma homocysteine concentration; in contrast, supplementation of diets with Cr (suppressing endogenous Cr biosynthesis) caused a 27% decrease in plasma homocysteine (Stead et al., 2001). Clearly, studies in both human subjects and experimental animals with elevated plasma homocysteine concentrations are required to sense the full potential of oral Cr supplementation for lowering plasma homocysteine concentrations and, thus, for indirectly lowering the risk of developing atherosclerotic disease.

## SAFETY OF ORAL CREATINE SUPPLEMENTATION

Little in the area of Cr metabolism has been discussed so controversially over the last several months and years as the safety of oral Cr supplementation. Rather than to further polarize the discussion, this section tries to provide a comprehensive overview of the perceived and potential risks of oral Cr supplementation. As such, this section may serve as a rational basis for *educated* decisions on when and how broadly to allow and/or advocate oral Cr supplementation for ergogenic or preventive purposes.

Based on the scientific literature as well as on discussions in popular science magazines and the lay press, the following aspects seem worth discussing (for recent reviews, see Graham and Hatton, 1999; Williams et al.,

1999; Terjung et al., 2000; Benzi, 2000; Benzi and Céci, 2001; Persky and Brazeau, 2001; Schilling et al., 2001):

- (Anecdotal) side effects of oral Cr supplementation
- Impact of oral Cr supplementation on renal function
- Safety questions arising from a comparison between Cr and its analogue, cyclocreatine (cCr)
- Carcinogenic potential of oral Cr: disproved fiction or potential fact?
- Are all sources of Cr equally safe?
- Consumer attitudes, perceptions, and education

The only fairly consistent side effect of oral Cr supplementation which is agreed upon by both advocates and critics is weight gain. In most cases, weight gain amounts to 1–2 kg; however, larger increases of 3–5 kg have occasionally been reported (see Wyss and Kaddurah-Daouk, 2000). Gastrointestinal distress, muscle cramps, muscle strains, dehydration, and heat intolerance have also been reported repeatedly as side effects. Most of these may be due to increased water retention in muscle during the initial days of Cr supplementation. Finally, anecdotal reports of adverse events include rash, dyspnea, vomiting, diarrhea, nervousness, anxiety, fatigue, migraine, myopathy, polymyositis, seizures, atrial fibrillation (see Wyss and Kaddurah-Daouk, 2000), and acute quadriceps compartment syndrome (Robinson, 2000). These reports represent isolated cases rather than a general pattern and lack a rational basis why they should be linked to Cr supplementation. Williams et al. (1999) are probably right when claiming that – with the exception of weight gain – none of the above side effects was scientifically proven in appropriately designed double-blind studies. These authors even conclude that there is no scientific evidence whatsoever to substantiate any safety concern. Again, this subjective view may not represent the full truth. Firstly, in controlled studies addressing potential side effects of oral Cr, appropriate countermeasures such as proper hydration may already have been taken. Secondly, Ray et al. (2001) observed a higher frequency of side effects typically assigned to Cr (i.e., muscle cramps, increased thirst, and stomach cramps) than of those not typically linked with Cr in a study on almost 700 high school athletes. Thirdly, minor adverse effects may be more prevalent than often believed. In the study of Juhn et al. (1999), 38 out of 52 baseball and football players reported at least one adverse side effect of oral Cr supplementation, most frequently diarrhea and muscle cramps. Admittedly, these studies can be criticized for the lack of a double-blind design and for not even involving an appropriate control group. One way or the other, the most likely side effects of oral Cr supplementation are mild in nature and easy to prevent: at least during the loading phase when 20 g Cr/day is consumed, subjects should take care to be properly hydrated and to avoid strenuous exercise.

The potential impact of oral Cr supplementation on renal function is less well established. Although a few articles were published showing that even 'long-term' oral Cr supplementation does not impair renal function in healthy athletes (Poortmans et al., 1997; Poortmans and Francaux, 1999, 2000), and even though in none of the clinical studies published to date (with the exception of the case reports discussed below), 'obvious' adverse effects of oral Cr supplementation on renal function have been observed (e.g., Mihic et al., 2000; Robinson et al., 2000), the relevance of these data is limited. Firstly, most of these studies involved a very limited number of subjects (usually $<10$), resulting in a low statistical significance of the data and an inability to detect small deteriorations in renal function. Secondly, in all of these clinical studies, young, healthy subjects were investigated, and serum Crn and urea concentrations as well as urinary Crn clearance were taken as primary measures of renal function. However, it is well known that these parameters are virtually unaffected in the initial stages of renal dysfunction, and become meaningful only when renal function is already depressed by 25–50%. Thirdly, 'long-term' in the context of the above studies corresponded to no more than 5 years. And fourthly, changes in parameters of renal function – although small in nature and possibly not of clinical relevance – have in fact been observed with oral Cr supplementation. Serum Crn concentration, probably mostly as a reflection of the increased bodily Cr pool, was increased by up to 60% (Bermon et al., 1998; Kreider et al., 1998; Robinson et al., 2000; Volek et al., 2000; Schilling et al., 2001). Increases in serum concentrations were also observed for urea, sodium and potassium (Robinson et al., 2000). In the study of Schilling et al. (2001), blood concentration of Crn and blood urea nitrogen tended to increase with the duration of Cr supplementation, although this tendency was not statistically significant.

Several theoretical and experimental indications exist how oral Cr supplementation *might* contribute to deterioration of renal function. As just mentioned, oral Cr supplementation results in a larger body pool of Cr which, in turn, gives rise to a higher serum concentration and urinary excretion rate of Crn. Through a series of reactions, Crn is metabolized to methylguanidine, production of which is favored at elevated Crn concentrations (for a review, see Wyss and Kaddurah-Daouk, 2000). Both Crn and methylguanidine were implied to be renal toxins. Nothing is known, however, about the minimum toxic concentrations of these metabolites, so that it is questionable whether they present a risk at all in healthy subjects undergoing Cr supplementation. In fact, an elevated serum concentration of Crn may even have the opposite effect: Crn may serve as a free radical scavenger and, thereby, protect the body from oxidative damage. In support of this view, reaction of Crn with the hydroxyl radical yields creatol, an intermediate in methylguanidine formation (see Wyss and Kaddurah-Daouk, 2000).

Yu and Deng (2000) reported that in mice, at least 25% of orally administered Cr is degraded to methylamine which, through semicarbazide-sensitive amine oxidase, yields formaldehyde, hydrogen peroxide, and ammonia. Accordingly, urinary formaldehyde levels almost doubled in mice after a single oral dose of Cr (50 mg/kg). Formaldehyde favors crosslinking of pro-

teins *in vivo* and is cytotoxic towards endothelial cells. In addition, chronic administration of methylamine causes oxidative and apparent renal damage in rats (see Yu and Deng, 2000).

Several isolated case reports and a study in rats also suggest that Cr supplementation *might* negatively impact renal function. In a patient with focal segmental glomerulosclerosis with normal renal function over the previous 5 years, a considerable deterioration of renal function was associated with the consumption of Cr (15 g/day for 1 week, followed by 2 g/day for 7 weeks; Pritchard and Kalra, 1998). Upon Cr consumption, plasma concentration of Crn increased from 100–110 μM to 180 μM, and glomerular filtration rate decreased from 122 to 54 ml/min/(1.73 $m^2$). When Cr supplementation was discontinued, both parameters returned to normal. In a previously healthy 20-year-old man, the development of acute interstitial nephritis was linked to the consumption of Cr (Koshy and Schneeberger, 1999). Derek Bell, an outfielder from the Houston Astros baseball team, was hospitalized twice in 1998 for renal dysfunction, and has publicly blamed Cr for his ailments (see Graham and Hatton, 1999). Finally, in Han:SPRD-*cy* rats, an animal model of human autosomal dominant polycystic renal disease, oral Cr supplementation (~0.3 g/kg/day for 7 days, followed by ~0.05 g/kg/day for 35 days) resulted in greater cyst growth and worsened renal function: renal weight, renal fluid contents, cyst scores and serum urea concentration were all increased, whereas Crn clearance was decreased (Edmunds et al., 2001).

Based on all these findings, we fully agree with Benzi (2000) and Benzi and Céci (2001) who stated that "... the evidence relating to the long-term effects of Cr on renal function is far less conclusive than some authors imply". Given the widespread use of Cr supplementation, a small number of isolated case reports – one in a subject with pre-existing renal disease – is neither proof nor sufficient evidence to conclude that oral Cr supplements adversely affect renal function in healthy individuals. However, based on current evidence, we concur with the opinion of other authors (e.g., Graham and Hatton, 1999) that Cr supplementation should be discouraged in subjects with or at risk for renal disease, or with other pre-existing diseases. In order to prove the safety and/or to quantify the risks of Cr supplementation, systematic, very carefully designed studies using the most sensitive and reliable methods for measuring glomerular filtration rate are required, both in humans and in laboratory animals having either normal or already compromised renal function. In addition, potential (positive or negative) synergistic interactions with other supplements should be investigated more carefully.

A comparison of the relative effects of Cr and cCr on cellular energy metabolism raises two questions with regard to the safety of oral Cr or cCr supplementation: (i) cCr was reported to have a series of beneficial effects, most notably in the inhibition of tumor growth. This latter effect is probably caused by decreasing the functional capacity of the CK system (for a review, see Wyss and Kaddurah-Daouk, 2000). cCr displaces Cr from the tissues, but is a poor CK substrate itself. Since displacement of Cr is not limited to the tumor, but affects other body tissues such as brain and muscle as well, cCr treatment might possibly cause muscle weakness and mental problems. (ii) Since cCr inhibits tumor growth, strengthening of cellular energetics by Cr supplementation might be postulated to favor tumor growth. Much to the contrary, none of the studies performed so far has shown tumor growth stimulation by Cr. In some studies, Cr had no effect on tumor growth, while in others, it even displayed antitumor activity (see Wyss and Kaddurah-Daouk, 2000; Kornacker et al., 2001).

The major concern of AFSSA, when releasing its negative opinion on oral Cr supplementation in 2001, seemed to be a purported carcinogenic effect of Cr. As described in detail by Wyss and Kaddurah-Daouk (2000), Cr and/or Crn might possibly be precursors of two classes of food mutagens: (i) amino-imidazo-azaarenes (AIAs) produced during cooking of meat; and (ii) nitrosation products of Cr or Crn that might be formed in the stomach. Based on current knowledge, the probability that nitrosation products of Cr(n) are formed in the stomach to any significant extent is close to zero. AIAs are preferentially formed at high temperatures (e.g. during frying or broiling of meat), but even then the concentrations of AIAs are so low that it is questionable whether they represent a significant cancer risk. At 37°C, AIA formation from Cr or Crn most likely does not occur or, at least, it is highly depressed. One single report (Manabe et al., 1992) mentioned AIA formation from Crn (not Cr!) *in vitro* at 37°C, but these findings have never been corroborated. Therefore, it would seem very unscientific at present to attribute any cancer risk to oral Cr supplementation.

In terms of quality of different commercial Cr preparations, the purity differs considerably between producers. Cr is produced by chemical synthesis, mostly from sarcosine and cyanamide. This reaction is prone to generation of variable amounts of contaminants such as dicyandiamide, dihydrotriazines, or Crn. Some producers fail to separate these contaminants from Cr during downstream processing. Since the toxicological profiles of these contaminants are not known, and since dicyandiamide liberates HCN when exposed to strongly acidic conditions (such as in the stomach?), only the purest preparations of Cr should be used and allowed for human consumption. Unfortunately, no quality labels are yet in place that would allow a consumer to judge the origin and quality of Cr in a given commercial product. Moreover, for most studies published so far, it is not possible to correlate the presence or lack of ergogenic, preventive, or adverse side effects with the quality of the Cr preparations used. Possibly, the reported anecdotal side effects are due to contaminants rather than to Cr itself.

Finally, when discussing the safety of oral Cr supplementation, it is important also to consider the consumer attitudes. Although Cr supplementation is not recommended below age 18, mostly because well-designed safety studies are lacking for children and adolescents, Cr use seems to be quite prevalent in middle and high schools in the USA. Children as young as 10 years old

used Cr to enhance their athletic performance. Although Cr use has been reported in 'only' 2–5% of children in grades 6–9, its prevalence increased to 5–44% in grades 10–12 (Smith and Dahm, 2000; Metzl et al., 2001; Ray et al., 2001). In studies on collegiate athletes, 28–41% reported Cr use, of which approximately one third had first used it when still in high school (LaBotz and Smith, 1999; Greenwood et al., 2000; Jacobson et al., 2001). For professional athletes, use rates of Cr of up to 75% have been reported for selected sports disciplines (see Smith and Dahm, 2000; Baylis et al., 2001). In general, Cr use and awareness about Cr are much higher in male than in female athletes, which is in line with the fact that the ergogenic effects of Cr are more consistent in the former than in the latter, and probably also because an improved physical appearance with a larger muscle mass is more important to men. Most frequent use of Cr is made in sports such as football, hockey, basketball, baseball, wrestling, gymnastics, and lacrosse (Smith and Dahm, 2000; McGuine et al., 2001; Metzl et al., 2001). The most common reasons cited for taking Cr were enhanced performance (74% of users) and improved appearance due to an increase in muscle size (61%; LaBotz and Smith, 1999; Metzl et al., 2001). In contrast, the most common reason for not taking Cr was safety concerns (46% of non-users; Metzl et al., 2001).

An alarming finding was that most athletes either did not know their dosing of Cr, or consumed Cr inconsistently with scientific recommendations (Juhn et al., 1999; LaBotz and Smith, 1999; Greenwood et al., 2000; Smith and Dahm, 2000; Ray et al., 2001; Schilling et al., 2001). In a study on 52 baseball and football players, for example, 39 athletes exceeded the recommended maintenance dose of 2–5 g Cr/day; 18 athletes took 9 g/day or more, and three athletes even took 17–20 g/day (Juhn et al., 1999). In another study on high school athletes, 44% of Cr users reported a maintenance dose of 5–10 g Cr/day, and 13% took 15–20 g/day (Ray et al., 2001). This excessive Cr consumption is, among other reasons, due to the fact that friends, teammates, media and athletic trainers or coaches, but not qualified dietitians, are often the primary sources of information on Cr (Juhn et al., 1999; LaBotz and Smith, 1999; Smith and Dahm, 2000; McGuine et al., 2001; Ray et al., 2001). A further point of concern, primarily when considering oral Cr supplementation in children and adolescents, is the finding of Smith and Dahm (2000) that Cr users are more likely to use other supplements as well. This supports the notion of Metzl et al. (2001) that Cr may serve as a gateway drug favoring consumption of other, possibly truly harmful nutritional supplements. The important lesson to be learned is that better education about oral Cr supplementation is required at all levels, namely suppliers, consumers, parents, trainers, coaches, dietitians, clinicians, politicians, regulatory bodies, etc.

In summary, based on current scientific knowledge, oral Cr supplementation at the recommended dosages (20 g/day or 0.3 g/kg/day for 5–7 days as a loading phase, and 2–5 g/day or 0.05 g/kg/day as a maintenance dose thereafter) must be regarded as safe. None of the studies in humans performed so far has suggested a significant health concern. In addition, toxicological studies have shown no acute or subacute toxicity of Cr in rats and mice, no mutagenic activity in the Ames test, and no negative effects in tolerance tests (skin irritation, eye irritation and sensitization tests in rabbits and guinea-pigs; Mertschenk et al., 2001).

However, oral Cr supplementation became popular and widespread only in the second half of the 1990s. This and the fact that the product quality of Cr is not regulated by the FDA (since it is a dietary supplement) may explain why well-designed and true long-term studies ($>5$ years) on the safety of chronic Cr consumption in humans are still lacking. In order to allow proper balancing between the benefits and potential risks of oral Cr supplementation, well-designed, meaningful safety studies involving considerably larger numbers of individuals are therefore required. Particular attention should be given to the long-term effects of oral Cr supplementation on renal function, because of its different purported modes of action that might result in a temporary or irreversible impairment of renal function. Finally, it is shocking how little consumers seem to know about proper supplementation regimes yet consume megadose amounts of Cr. Clearly, education of all people involved – from producers over consumers to the authorities – with a strong focus on scientifically proven facts is required to make best use of Cr.

## CONCLUSIONS

Although Cr was discovered 170 years ago, and despite its astounding success as an ergogenic aid since the 1990s, much is still unknown about its biological functions. This is particularly true for its potential in disease prevention. Recent progress in different areas of research has consistently shown that there may be a tight correlation between the capacity of the CK system and brain function. Inborn errors of Cr metabolism in humans and knockout mice lacking CK activity in the brain are characterized by developmental delay, mental problems and learning disability. On the other hand, oral Cr supplementation proved to be neuroprotective, particularly in animal models of Parkinson's disease, Huntington's disease, or ALS.

Still unproven is the hypothesis that oral Cr supplementation may decrease the plasma homocysteine concentration and thereby lower the risk of atherosclerotic disease. This hypothesis is based on a 20-year-old estimation that endogenous Cr biosynthesis accounts for 70% of the total consumption of 'labile' methyl groups (i.e., *S*-AdoMet) in the body which is the source of homocysteine.

In order to explore the full potential of oral Cr supplementation in the prevention of the above diseases, much more research is required. (i) The neuroprotective role of Cr needs to be confirmed in appropriately designed, well-controlled studies in humans. (ii) The extent to which the blood–brain barrier represents a block for the entry of orally administered Cr into the brain needs to be established. Since current evidence

indicates that Cr entry into the brain is limited, strategies for surpassing this inhibition or for circumventing the blood–brain barrier need to be explored. (iii) The optimal dosages, supplementation regimes, and the pharmacokinetics in general need to be better defined. In particular, a key question is whether Cr supplementation down-regulates the CrT to the extent that intermittent Cr supplementation, with alternating supplementation and washout periods, may provide better results than continuous Cr supplementation over several weeks and months (see Wyss et al., 1998; Guerrero-Ontiveros and Wallimann, 1998). (iv) The potential of oral Cr supplementation in lowering the plasma homocysteine concentration needs to be tested primarily in individuals with compromised homocysteine homeostasis. And (v), although current evidence indicates that oral Cr supplementation is safe, well-controlled long-term studies involving considerably larger numbers of subjects are required. In particular, attention should be paid to the effects of oral Cr supplementation on renal function. If all these lines of research provide favorable results, the future of oral Cr supplementation in disease prevention may be even brighter than that as an ergogenic aid.

## NOTE ADDED IN PROOF

In the context of the present article, three notable articles have recently been published. Watanabe et al. (2002) present evidence that Cr supplementation reduces mental fatigue when subjects repeatedly perform simple mathematical calculations. According to Jacobs et al. (2002), creatine supplementation enhances upper extremity work capacity in subjects with complete cervical-level spinal cord injury. And finally, Lawler et al. (2002) demonstrate direct antioxidant effects of creatine.

*Acknowledgements*—The participants of the 6th International Conference on Guanidíno Compounds in Biology and Medicine (Cincinnati, OH, USA, 31 August–3 September 2001) and, in particular, Joseph F. Clark, are gratefully acknowledged for stimulating discussions.

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(*Accepted* 20 *February* 2002)