

Creatine: Endogenous Metabolite, Dietary, and Therapeutic Supplement

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Key Words

muscle, brain, neurodegenerative diseases, inborn errors, amino acids, ergogenic action

Abstract

Creatine and phosphocreatine serve not only as an intracellular buffer for adenosine triphosphate, but also as an energy shuttle for the movement of high-energy phosphates from mitochondrial sites of production to cytoplasmic sites of utilization. The spontaneous loss of creatine and of phosphocreatine to creatinine requires that creatine be continuously replaced; this occurs by a combination of diet and endogenous synthesis. Vegetarians obtain almost no dietary creatine. Creatine synthesis makes major demands on the metabolism of glycine, arginine, and methionine. Large doses of creatine monohydrate are widely taken, particularly by athletes, as an ergogenic supplement; creatine supplements are also taken by patients suffering from gyrate atrophy, muscular dystrophy, and neurodegenerative diseases. Children with inborn errors of creatine synthesis or transport present with severe neurological symptoms and a profound depletion of brain creatine. It is evident that creatine plays a critical, though underappreciated, role in brain function.

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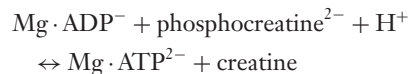
INTRODUCTION

Interest in creatine has increased markedly in recent years. Much of this interest has been due to the use of creatine as a supplement by athletes and bodybuilders. Recent information indicates that the annual consumption of supplemental creatine (as creatine monohydrate) amounts to some four million kilograms per annum with a value, in the United States alone, close to \$400 million. Creatine monohydrate is one of the more widely used dietary supplements. Readers who wish to access the literature on the efficacy of creatine in improving athletic performance will find that the article by Branch (6) provides a convenient entrée. Similarly, issues of the safety of sup-

plemental creatine are dealt with by Schilling et al. (67) and Graham & Hatton (25). This review article is concerned with the function of creatine and phosphocreatine in tissues, the synthesis of creatine (including the metabolic burden of this synthesis), the metabolic and therapeutic effects of supplemental creatine, and the inborn errors of creatine synthesis and transport. We have endeavored, as much as possible, to address recent work. An extensive coverage of the older literature is available in the review by Wyss & Kaddurah-Daouk (96). Readers should apply a cautious attitude to the many commercial and nonscientific Web sites devoted to creatine. However, a Web site maintained by the Office of Dietary Supplements of the National Institutes of Health (www.ods.od.nih.gov) is a valuable source of balanced information.

PHYSIOLOGICAL FUNCTION OF THE CREATINE SYSTEM

Creatine is involved in a single enzyme-catalyzed reaction, which is catalyzed by creatine kinase:



The conventional textbook description of this system is that it serves as a temporal high-energy phosphate buffer, so that adenosine triphosphate (ATP) may be rapidly replenished from adenosine diphosphate (ADP) and phosphocreatine. This is certainly true. However, we now appreciate that the creatine system plays a more complex role in energy metabolism. It is found in cells with high and fluctuating energy demand, such as skeletal muscle and the heart (96). It is not present in hepatocytes that have a constitutively high metabolic rate. It is also found in the brain. Although the oxygen consumption (and energy demand) of the brain as a whole is relatively constant (compared, for example, with the large excursions in energy demand exhibited by skeletal and cardiac muscle), it should be appreciated that individual, rapidly firing

neurons can exhibit quite a large variation in their energy needs (69).

The subcellular distribution of creatine kinase isoforms has stimulated a revision of the function played by the creatine system. Wallimann et al. (93) have provided a cogent account of the different creatine kinase isoenzymes. For the purposes of the present discussion, the key factor is their subcellular location. Mitochondrial creatine kinase is found at contact sites between the inner and outer mitochondrial membranes and, in the presence of creatine, ensures that much of the ATP produced by oxidative phosphorylation is readily converted to phosphocreatine. Cytosolic isoforms are found in the “bulk cytoplasm” and, most critically, at sites of high ATP demand, e.g., in myofibrils, sarcoplasmic reticulum, and plasma membranes (93). These and other observations have given rise to the proposal of a role for creatine and phosphocreatine in an energy shuttle of high-energy phosphates between the mitochondrial sites of ATP production and the cytosolic sites of ATP utilization (93). In this view, phosphocreatine (rather than ATP) diffuses from mitochondria to the major sites of ATP utilization and creatine (rather than ADP) diffuses back. Although the original arguments for such a shuttle were based on the fact that phosphocreatine (MW = 211) would diffuse faster than ATP (MW = 507) and creatine (MW = 131) would diffuse faster than ADP (MW = 427) (20), it is more likely that the key consideration lies in the differences between their free concentrations in the cytosol (ATP, 3–5 mM; ADP, 20–40 μ M; phosphocreatine, 20–35 mM; creatine, 5–10 mM) (93).

Temporal high-energy phosphate buffering and spatial high-energy phosphate transport are not mutually exclusive. Indeed, they probably coexist, to different degrees, in different cells, but depending on physiological requirements, one or the other may predominate. Thus, in fast-twitch (primarily glycolytic) muscles, the ATP buffer function predominates, whereas in slow-twitch, oxidative, or cardiac muscle, the energy transport

function is more important. Wallimann and coworkers (93) have identified a number of additional functions of the creatine/phosphocreatine system. These include buffering the products of ATP hydrolysis and maintaining cellular ATP/ADP ratios. ATP hydrolysis produces ADP, phosphate, and a hydrogen ion. During very rapid ATP hydrolysis, it is important that both ADP and the hydrogen ion are buffered. The creatine/phosphocreatine system prevents a rise in [ADP] that would both inhibit ATPases and lead to the metabolic loss of adenine nucleotides; elevated [ADP] leads to elevated [AMP], catalyzed by adenylate kinase, and this leads to increased deamination of AMP to IMP. Local or generalized cellular acidification, due to high rates of ATP utilization, is prevented by the requirement for a hydrogen ion when the creatine kinase reaction operates in the direction of ATP synthesis. Finally, it is apparent that by buffering both [ATP] and [ADP], the creatine kinase reaction maintains the constancy of the ATP/ADP ratio, which protects the thermodynamic efficiency of ATP hydrolysis. These different functional aspects of the creatine kinase system are illustrated in **Figure 1**.

Our knowledge of the function of individual creatine kinase isoforms has been enhanced by recent studies of knockout mice. These mice are viable and fertile and, indeed, when unstressed, often exhibit a relatively mild phenotype. This is often due to compensatory mechanisms as well as some redundancy. A good example is provided by mice deficient in both the homo-dimeric muscle creatine kinase, which is associated with sites of high ATP utilization, such as myofibrils and sarcoplasmic reticulum and the mitochondrial creatine kinase (MtCK), so that both ends of the phosphocreatine shuttle are ablated. These animals greatly expand their mitochondrial number near the longitudinal sarcoplasmic reticulum and the myosin filaments so that direct energy channeling via ATP and ADP is enhanced (37). Nevertheless, these mice exhibit a markedly reduced

MtCK:
mitochondrial
creatine kinase

AGAT:

L-arginine:glycine
amidinotransferase

GAMT:

guanidinoacetate
methyltransferase

voluntary running capacity (53). Knockout of MtCK results in left ventricular hypertrophy and dilatation (55). Mice in which both mitochondrial creatine kinase and the brain-specific creatine kinase were knocked out showed a number of neurological impairments, including severely diminished spatial learning (79).

CREATINE METABOLISM

Creatine Loss and Replacement

Both creatine and phosphocreatine spontaneously break down to creatinine that is quantitatively lost in the urine. The rate of loss is estimated to be about 1.7% of the total body pool per day (96). As more than 90% of the body's creatine and phosphocreatine is found in skeletal muscle, creatine losses (and creatinine excretion) vary as a function of gender and age. Creatinine excretion is at a maximum in the 18- to 29-year-old age group, with mean rates of $23.6 \text{ mg} \cdot \text{kg}^{-1} \cdot 24 \text{ h}^{-1}$. Mean rates for women are about 80% of rates for men. The rate of creatinine loss decreases almost linearly with age; men aged 70–79 years have mean excretion rates of $12.6 \text{ mg} \cdot \text{kg}^{-1} \cdot 24 \text{ h}^{-1}$ (11).

These data on creatinine excretion define the quantities of creatine that need to be provided by diet or by synthesis. The principal dietary sources of creatine are muscle meats and dairy products. We have employed United States Department of Agriculture data on the consumption of different foods, together with knowledge of the creatine content of different foods, to estimate dietary creatine (75). Our data indicate dietary creatine intakes of 7.9 and 5.0 mmol/day for men and women, respectively, in the 20- to 39-year-old age group. These intakes decrease somewhat with age. Food creatine has the same high bioavailability as does dissolved creatine (29), which has been estimated to be about 80% (49). These data permit us to tease out the quantity of creatine obtained from the diet and, therefore, the amount that must be synthesized. For ease

of comparison, our data are normalized to a 70-kg person. Vegans obtain virtually no dietary creatine and vegetarians very little. De novo synthesis must provide essentially all of their creatine. Ingestion of vegetarian diets is associated with decreased serum and muscle creatine levels (14, 47), which may indicate that creatine synthesis is insufficient in these subjects. For individuals ingesting a typical U.S. diet, we estimate rates of creatine synthesis of 7.7, 5.6, and 3.7 $\text{mmol} \cdot \text{day}^{-1}$ in males in the 20–39, 40–59, and 60+ age brackets, respectively. The rates for women were about 70%–80% of those in men.

Creatine synthesis occurs via a remarkably simple pathway (**Figure 2**). However, it involves three different amino acids: glycine, arginine, and methionine. In the first reaction, catalyzed by L-arginine:glycine amidinotransferase (AGAT), an amidino group is transferred from arginine to the amino group of glycine to produce guanidinoacetate and ornithine. The second reaction, catalyzed by guanidinoacetate methyltransferase (GAMT), employs S-adenosylmethionine to methylate guanidinoacetate, producing creatine and S-adenosylhomocysteine.

These rates of creatine synthesis permit us to estimate the metabolic burden it imparts. Synthesis of $7.7 \text{ mmol} \cdot \text{day}^{-1}$ (approximately 1.0 g) requires the same molar quantity of glycine, amidino groups, and methyl groups. The mean dietary intakes of these amino acids in U.S. men and women aged 31–50 are 48 mmol glycine, 27 mmol arginine, and 13 mmol methionine (17). Clearly, creatine synthesis makes major demands on amino acid metabolism. However, the nature of the demand varies among the three amino acids. As shown in **Figure 2**, the entire glycine molecule is incorporated into creatine so that creatine synthesis consumes about 16% of dietary glycine. In the case of methionine, only the methyl group is incorporated. Labile methyl groups are available in the diet from methionine, choline, and betaine. In addition, new labile methyl groups may be

produced by the process of methylneogenesis (54). Consensus estimates of the total transmethylation flux (i.e., the sum of all of the *S*-adenosylmethionine-requiring methylation reactions) are 17–23 mmol per day in young adults (54). Thus, creatine synthesis accounts for about 40% of all *S*-adenosylmethionine-derived methyl groups in young adults. This percentage decreases somewhat in the elderly. Direct evidence for the role of methylneogenesis in providing methyl groups for creatine synthesis is provided by the impairment of this process in patients with an inborn error of cobalamin metabolism (3).

It is still an open question whether creatine synthesis results in the loss of an entire arginine molecule. The key to this question depends on the fate of the ornithine produced by the AGAT reaction (**Figure 2**). Ornithine could be reconverted to arginine by the enzymes of the urea cycle (7). Since expression of carbamyl phosphate synthase I is restricted to the liver and the small intestine (7), AGAT expression would be required in these tissues. This is an unresolved issue. However, the 1953 study by Sandberg et al. (66) should be noted. After examining their catheterization studies across the human liver, these workers reported that the entire pathway of creatine synthesis occurred in this organ.

Creatine Synthesis and Transport: Tissue Sites and Regulation

The tissue localization of AGAT and GAMT is complex. In mammals, the kidneys express high activities of AGAT but low (or undetectable) activities of GAMT. This has given rise to the view that creatine synthesis is primarily an interorgan process in which guanidinoacetate produced by the kidney is methylated to creatine in the liver. This certainly occurs in the rat (96). However, the relative contribution of other tissues is uncertain. The possible occurrence of AGAT activity in the liver is enigmatic, in large part due to the difficulty of assaying the enzyme in tissues with high arginase activity. For example,

immunofluorescence microscopy has identified a protein that reacts to AGAT antiserum in rat liver (51), but unambiguous presence of enzyme activity in this tissue has yet to be demonstrated. It is clear that the pancreas and testes express both AGAT and GAMT and that AGAT is found in tissues such as lung, spleen, and brain. In summary, much of the body's creatine is synthesized via a renal-hepatic axis, but the importance of the synthetic enzymes that are expressed in other tissues remains to be delineated. We address the issue of brain creatine synthesis in Treatment of the Inborn Errors section below.

Regulation of creatine synthesis has been best studied in rats. Dietary supplementation of rats with creatine results in a marked decrease in AGAT activity and mRNA levels in the rat kidney (52), indicating regulation at a pretranslational level. The molecular mechanism whereby creatine down-regulates AGAT expression remains to be elucidated. Decreased hepatic creatine production follows from the decreased circulating guanidinoacetate levels.

By and large, the tissues that contain the largest quantities of creatine (e.g., skeletal and cardiac muscle) have essentially no capacity for its synthesis. A creatine transporter (CRT) has been identified that is responsible for the uptake of creatine into tissues such as skeletal muscle, cardiac muscle, kidney, and brain. Uptake occurs against a large concentration gradient; typical creatine concentrations in humans are 50–100 μ M in plasma and 5–10 mM in skeletal muscle. The transporter (SLC 6A8) is a member of the Na^+ -dependent neurotransmitter transporter family (closely related to γ -aminobutyrate, taurine, and betaine transporters), and this gene is found on the human X chromosome (27). A cDNA clone from human brain with an open reading frame of 1905 base pairs predicts a protein of 635 amino acids (\approx 70.5 kDa), 12 transmembrane-spanning domains, and putative phosphorylation and glycosylation sites (73). It has about 97% identity with homologous rat, rabbit, and bovine genes (72, 73). The transport

CRT: creatine transporter

properties of CRT have been determined. Creatine (a zwitterion) is cotransported with at least two Na^+ and one Cl^- . This transport therefore is electrogenic and an example of secondary active transport, driven by the sodium gradient established by the $\text{Na}^+ + \text{K}^+$ -ATPase (12). Creatine transport is enhanced by hormones (e.g., insulin) that activate the $\text{Na}^+ + \text{K}^+$ -ATPase and presumably increase the driving force for creatine uptake (72).

Studies on the size, tissue expression, and regulation of the CRT gene product have yielded confusing results; for example, estimates of its molecular weight vary from 40 kDa to 150 kDa. Some of this variation may be attributed to glycosylation and to the anomalous behavior of hydrophobic proteins in gels. The meticulous study of Speer et al. (74) clearly demonstrates that much of the difficulty has arisen as a result of the cross-reactivity of different antibodies against other proteins. Until this issue is resolved, data on the abundance, location, and regulation of CRT proteins must be treated with caution. Nevertheless, it is quite likely that different isoforms of CRT do exist as Northern blots have identified two different mRNAs (28).

Creatine transport may be regulated both acutely and chronically. Acute regulation may be brought about either by changes in the creatine concentration or in the sodium gradient or in the insertion of the transporter into the plasma membrane. Chronically, creatine transport may be regulated at the level of gene expression, translation, or post-translational modification. It is clear that creatine uptake is regulated by intracellular creatine concentrations. An inverse relationship exists between the intracellular creatine concentration and creatine uptake (18). It is known that an elevated extracellular creatine concentration leads to an initial increase in transport followed by a down-regulation. The mechanism of down-regulation is not known, but it seems to require protein synthesis (42).

Creatine is also absorbed in the intestine, reabsorbed in the kidney, and released, after

synthesis, by the liver. Tosco and coworkers (86) have demonstrated a creatine transporter, electrogenic and $\text{Na}^+ + \text{Cl}^-$ dependent, in jejunal apical membranes. In view of the small quantities of creatine found in the urine, it can be calculated that human kidneys must reabsorb approximately 13.5 mmol creatine (1.76 g) per day. A $\text{Na}^+ + \text{Cl}^-$ -dependent creatine transporter is known to occur in renal cortical brush-border membranes (24). Creatine synthesized by the liver is released into the blood. However, given the direction of the sodium gradient, this is likely to require a novel transporter that is yet to be identified. Recent work (74) has suggested that creatine transporters occur on mitochondrial membranes. Creatine uptake could be demonstrated by isolated mitochondria. However, the K_m for creatine is quite high (16 mM), the transport may be inhibited competitively by either lysine or arginine, and it is found in mitochondria from tissues (e.g., liver) that do not express creatine kinase (74). The occurrence of a mitochondrial creatine transporter challenges our understanding of creatine physiology. However, definitive demonstration of a physiological role for creatine inside the inner mitochondrial membrane has yet to be provided.

SUPPLEMENTAL AND THERAPEUTIC CREATINE INGESTION

Creatine monohydrate is one of the most common dietary supplements. It is taken by athletes and bodybuilders. A popular intake schedule involves a loading phase of 20 g per day (in four divided doses) for five days, followed by maintenance doses of 2–5 g per day. In addition to its use by athletes, creatine has been used therapeutically in patients suffering from a number of neurological and neuromuscular disorders.

Effects of Creatine Ingestion on Muscle Creatine Levels

Supplemental creatine is appreciably taken up by skeletal muscle over the first 2–3 days

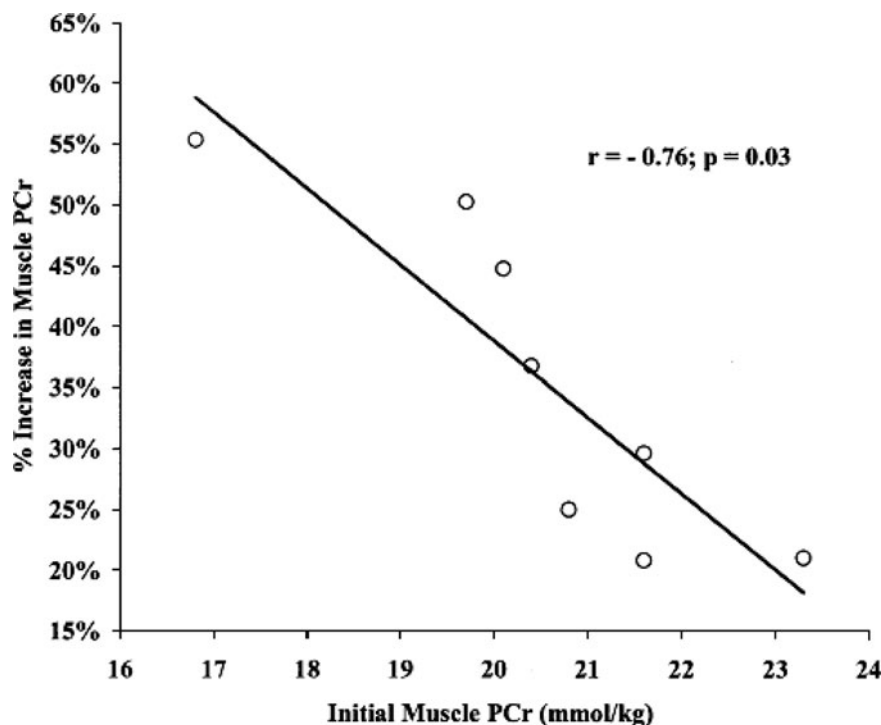


Figure 3

The increase in muscle phosphocreatine (PCr) after creatine supplementation is inversely related to presupplementation PCr levels. Reproduced from Volek & Rawson (90), with permission from Elsevier.

of supplementation. Decreased urine output during this time has been attributed to water retention because of the osmotic effects of creatine uptake (97). Creatine is frequently taken together with carbohydrate as this tends to increase its uptake into muscle, presumably as a consequence of insulin action. However, the amounts of carbohydrate required (~100 g per 5 g of creatine) are impractically large (26). Exercise is a potent stimulus for creatine uptake by skeletal muscle. This was elegantly demonstrated by studies in which a single leg was exercised, resulting in appreciably more creatine uptake than in the unexercised contralateral leg (30). Typical skeletal muscle levels, before supplementation, are about 85 and 41 mmol/kg dry matter, respectively, for phosphocreatine and creatine (30). Therefore, the combined concentration of creatine and phosphocreatine in muscle intracellular water, pre-supplementation, is of the order of 40–50 mM. Creatine supplementation increased total muscle creatine (creatine plus phosphocreatine) by about 25%, and when accompanied by

exercise, by an average of 37%. Clearly, these compounds are major osmotically active intracellular solutes. Creatine supplementation does not affect muscle ATP levels (30).

Considerable variation exists between individuals in the degree of creatine loading into muscles. Although this is not completely understood, it is clear that presupplement muscle creatine levels are an important factor (63). **Figure 3** shows that the increment in muscle phosphocreatine following supplementation in young (age 20–32) men was inversely related to their presupplementation levels. Muscle phosphocreatine levels in older (age 63–83) men were relatively refractory to supplementation, possibly because presupplementation levels were higher than those of the younger subjects (63).

The Ergogenic Effect of Creatine Supplementation

Creatine supplementation enhances our ability to do certain types of work or exercise. This

is clearly evident for high-intensity exercise of short duration. Evidence for a beneficial effect in endurance events is not compelling. A meta-analysis of 96 studies that examined the effect of creatine supplementation found marked effects on high-intensity exercise of short duration (≤ 30 s) but virtually no effect on performance tasks longer than 150 s (6). The effect of creatine supplementation on work performance and lean body mass was more pronounced in vegetarians than in non-vegetarians, presumably because their presupplementation levels of muscle creatine are lower (8). In recent years, the focus of research has shifted from performance evaluation to the elucidation of the mechanism(s) responsible for enhanced performance. Certainly, the elevated phosphocreatine levels are important because, via creatine kinase, they increase the ability to rapidly replete ATP during strenuous exercise. However, other mechanisms involving effects on muscle mass, the metabolism of carbohydrates, as well as metabolic regulation may also play a role. Because training can affect many of these parameters, and increased phosphocreatine levels permit more vigorous training, it is often difficult to determine whether the effects of creatine supplementation are direct or indirect. Studies *in vitro* avoid some of these problems but are, of course, less physiologically relevant. Comparison of the *in vivo* studies is often confounded by differences in the subject groups, in the duration or dosage of the creatine supplementation, in the extent to which muscle creatine levels are increased, and in any accompanying exercise protocols.

One of the most striking effects of creatine supplementation is an increase in muscle mass, especially when an exercise program accompanies supplementation. A meta-analysis of 96 studies reports a mean increase in lean body mass of 2.2 kg (6). Twelve weeks of resistance training coupled with creatine supplementation increased muscle fiber diameter by 35%, compared with a 6%–15% increase in a placebo group that also completed the

resistance exercises (89). It appears that creatine supplementation in the absence of an exercise program has little effect on muscle mass. An isotopic study of myofibrillar protein synthesis and degradation in subjects who did not exercise found no effects of creatine supplementation (45). The same group was unable to show any additional effect of creatine in subjects who underwent a program of resistance exercise (44). These results are at variance with the studies that found increased muscle mass, although the studies could be reconciled if there were effects of creatine supplementation on proteolysis; however, there are no data on the subject. In rats, creatine ingestion alone did not increase lean body mass; exercise alone increased lean body mass, and this effect was much larger in creatine-supplemented rats who exercised (23). With regard to mechanism, it should be noted that 12 weeks of resistance training of individuals who ingested creatine resulted in enhanced expression of both mRNA levels and protein content of myogenic regulatory factors (myogenin and MRF-4) compared with subjects who ingested a placebo during the exercise program (95).

Effects of creatine ingestion on carbohydrate metabolism have also been reported. In particular, muscle glycogen content increases by about 20% after five days of creatine loading (20 g per day), but this was not maintained during supplementation at 2 g per day. The increases in total muscle creatine and muscle glycogen were closely correlated. The mechanism for the increase in muscle glycogen may involve increased cellular hydration, secondary to total creatine accumulation (87). There is direct evidence that cell swelling can increase glycogen synthesis in rat skeletal muscle (46). The situation with regard to GLUT-4 is more problematic. The van Loon et al. (87) study, which reported elevated muscle glycogen, found that creatine supplementation caused no change in GLUT-4 mRNA or protein content. However, Derave et al. (15) found that creatine supplementation increased GLUT-4

expression during a six-week resistance exercise program that followed a two-week leg immobilization. Creatine supplementation of rats increases GLUT-4 expression as well as transcription factors that regulate GLUT-4 expression in muscle (36). There is uncertainty as to how an increase in GLUT-4 may be achieved. One suggestion is that creatine supplementation proportionately increases muscle creatine levels more than phosphocreatine levels. The AMP-activated protein kinase has been reported to be sensitive to the creatine/phosphocreatine ratio such that an increase in this ratio activates the AMP kinase (58), which in turn could bring about an increase in GLUT-4 (33). However, not every study reports that creatine loading alters the creatine/phosphocreatine ratio (87), and there is controversy as to whether phosphocreatine actually inhibits the AMP kinase (85).

Therapeutic Uses of Creatine

Creatine has been used therapeutically in a variety of human diseases as well as in animal models of human disease. Many, but not all, of these diseases include disorders of energy metabolism. The use of creatine in the treatment of inborn errors of creatine synthesis is addressed below.

Gyrate atrophy is an autosomal recessive disease characterized by chorioretinal degeneration and atrophy of type 2 muscle fibers. The molecular lesion involves mutations in ornithine aminotransferase with consequent hyperornithinemia (62). Plasma ornithine levels can be elevated 10- to 20-fold (0.65–1.35 mM); this elevation causes an inhibition of AGAT (K_i for ornithine is 0.25 mM) and therefore of creatine synthesis. Creatine therapy has been employed with mixed results. Muscle phosphocreatine levels were restored by the creatine therapy (31). However, although the skeletal muscle abnormalities were markedly reduced or even eliminated, the progression of visual impairment continued because the hyperornithinemia was not corrected (88).

Creatine supplementation has been used in a number of neurological and neurodegenerative diseases. Huntington's disease is caused by expanded CAG repeats in the gene that encodes huntingtin. It is one of nine known polyglutamine diseases. In general, fewer than 38 CAG repeats are not associated with pathology. However, beyond this number, a specific degenerative sequence begins in middle age; the longer the number of CAG repeats, the earlier the onset of symptoms. The function of the huntingtin protein is unknown, but it is thought that protein-protein interactions of either the mutant protein or of fragments arising from its proteolysis initiate a pathogenic program that ultimately leads to cell death. Dysfunctional cellular energy metabolism has been identified as one of the prominent early lesions in Huntington's disease and has been suggested to be an important component of the degenerative sequence (64). Positron emission tomography studies have shown reduced glucose metabolism both in presymptomatic and symptomatic patients (50). Striatal lactate is increased in symptomatic patients, and the increase is related to the CAG repeat number (35). Patients with Huntington's disease have reduced skeletal muscle ratios of phosphocreatine to inorganic phosphate (38). There is, therefore, considerable evidence that a defect in energy metabolism is an early event in these patients. There is also direct evidence for a significant reduction in the number of mitochondria and of their size in striatal caudate neurons of presymptomatic patients (64).

Extensive work has been carried out in animal models of Huntington's disease. R6/2 mice are a transgenic line that express *exon 1* of the human *HD* gene, containing 150 CAG repeats (41). These animals develop a sequence of motor and cognitive impairments, many of which recapitulate events in the human disease but in a highly compressed timeframe. Creatine supplementation of these animals prolonged life span, decreased brain atrophy, and delayed atrophy of striatal neurons and the formation of mutant huntingtin

protein aggregates (22). Creatine supplementation also reverses the decreased brain levels of creatine and ATP found in these mice; increases of 39% and 65% are found, respectively, upon creatine supplementation of these mice (13).

This preclinical work on creatine supplementation in an animal model has made a strong case for clinical trials. A number of small studies have shown that doses of 3–8 g per day are safe and tolerable (64). A significant (7.2%) increase in brain creatine was found in patients who ingested 5 g/day for four months (64). None of these human studies have as yet shown an effect on the progression or severity of the disease. However, a recent randomized, double-blind placebo-controlled trial of 64 subjects with Huntington's disease showed that serum levels of 8-hydroxy-2'-deoxyguanosine, an indicator of oxidative injury to DNA, which are markedly elevated in Huntington's patients, were reduced by supplementation of 8 g/day of creatine for 16 weeks (32). A strong case has been made for a large-scale trial of creatine supplementation in patients with Huntington's disease with sufficient statistical power to detect meaningful changes in the progression or severity of the disease. Creatine and the creatine kinase system have also been linked to pathological consequences in animal models of amyotrophic lateral sclerosis (94) and Alzheimer's disease (9).

There have also been trials of creatine supplementation in muscle and neuromuscular disorders. Low-dose creatine supplementation improves skeletal muscle function in patients with McArdle's disease (deficiency of muscle glycogen phosphorylase), but high doses do not improve function (91, 92). Tarnopolsky et al. (83) have shown that creatine supplementation of boys with Duchenne muscular dystrophy for four months (0.1 g/kg/day) was well tolerated and led to significant increases in handgrip strength and in fat-free mass. Louis et al. (43) reported increased muscle function (maximal voluntary contraction and resistance to fa-

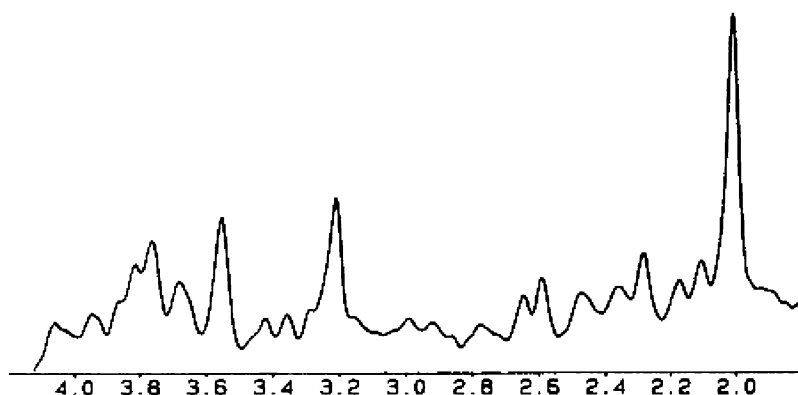
tigue) but no change in lean body mass in a group of boys suffering from either Duchenne or Becker muscular dystrophy. Bourgeois & Tarnopolsky (4) have reviewed the effects of creatine supplementation in mitochondrial cytopathies. Creatine ingestion has also been shown to reduce the plasma concentration of the atherogenic amino acid, homocysteine, in rats (75) and in humans (39), although one study was unable to confirm the finding (77). The decreased homocysteine is due to the down-regulation of endogenous creatine synthesis that, since it is responsible for 40% of all *S*-adenosylhomocysteine production in humans, is responsible for the production of 40% of the body's homocysteine (76).

INBORN ERRORS OF CREATINE SYNTHESIS AND TRANSPORT

Brain Creatine Depletion and Neurological Symptoms

Inborn errors involving mutations in each of the three proteins required for creatine synthesis and transport (AGAT, GAMT, and CRT) are now known. Although children with these mutations exhibit hypotonia, they present with little in the way of cardiac or skeletal muscular pathology (80). Rather, they present with a cluster of neurological symptoms that include mental retardation, speech delay, and epileptic seizures. Proton nuclear magnetic resonance spectroscopy reveals a massive depletion of brain creatine (80, 81) (**Figure 4**). Muscle creatine levels do not appear to be as severely depleted as do brain creatine levels in patients with deficiencies of either GAMT or CRT (21, 60). This may indicate the occurrence of redundant mechanisms for muscle creatine uptake. The creatine-deficiency diseases may be differentiated on the basis of biochemical findings. All of them are characterized by low urinary creatinine excretion and low plasma creatinine levels. However, these tests are not particularly reliable in very young infants and are not

A 22 Months



B 48 Months

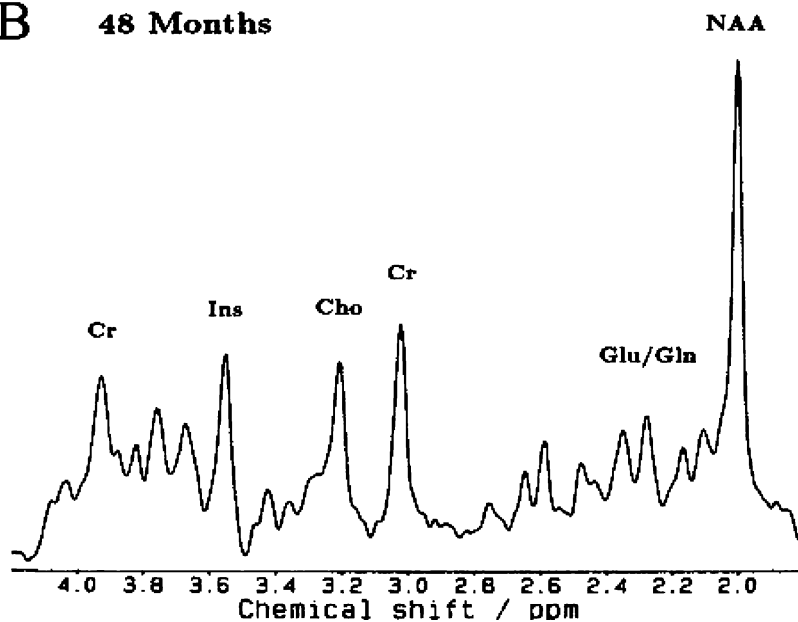


Figure 4

In vivo proton magnetic resonance spectroscopy of the brain of a patient with cerebral creatine deficiency due to guanidinoacetate methyltransferase deficiency. *Panel A* shows an absence of the creatine resonance; *Panel B* shows normalization of the creatine spectrum after six months of supplementation with creatine monohydrate. Cho, choline; Cr, creatine; Gln, glutamine; Glu, glutamate; Ins, inositol; NAA, *N*-acetylaspartate. Reproduced from Sykut-Cegielska et al. (81) with permission of the publisher.

absolutely specific for creatine-deficiency diseases; measurement of guanidinoacetate and creatine are more informative. AGAT deficiency is associated with very low plasma guanidinoacetate concentrations as well as low creatine levels. Patients with GAMT deficiency exhibit more severe symptoms, which include intractable epileptic seizures and a movement disorder. It is thought that the elevated guanidinoacetate levels exert

an independent pathological action (70). The CRT defect is characterized by elevated creatine and normal guanidinoacetate levels (65). The ultimate diagnosis of these diseases requires functional and genetic confirmation. Functional confirmation of AGAT and GAMT deficiency requires measurements of enzyme activity (80); for the transporter defect, it is necessary to show impaired creatine uptake, usually in cultured fibroblasts

(65). Recent work suggests that the creatine transporter defect may be relatively common, perhaps so common that it may occur in about 1% of males with mental retardation of unknown etiology (10, 56).

Treatment of the Inborn Errors

The fundamental objective in the treatment of children with these inborn errors is the restoration of brain creatine. The experience with AGAT and GAMT deficiency is that brain creatine may be restored, largely or completely, by creatine supplementation. This resulted in marked clinical improvement, but the children never fully recovered; learning and language functions remained impaired (80). Until recently, it has not been clear whether the neurological deficits could have been prevented if creatine supplementation had been instituted sufficiently early. Two recent studies suggest that a measure of guarded optimism may be justified. Battini et al. (2) have reported on a child, homozygous for the W149X mutation in AGAT, in whom an early diagnosis could be made since it was known that two older siblings harbored the same mutation. Treatment with creatine monohydrate was begun upon weaning, at four months of age. As of 18 months of age, the child's growth and development were entirely normal. A second study (71) concerns a child with GAMT deficiency; early diagnosis was also possible because of an older affected sibling. Treatment involved replacement of creatine as well as steps to reduce the synthesis of guanidinoacetate; these included reducing the substrates for AGAT (by feeding an arginine-restricted diet and providing benzoate to reduce glycine levels via the formation of hippuric acid) as well as inhibiting AGAT via provision of ornithine. Fourteen months of this treatment resulted in a normally developed child with none of the symptoms of GAMT deficiency.

Patients with deficiency of the creatine transporter are completely refractory to supplementation with creatine, and at present,

no effective therapy exists for this disorder. The severity of the creatine transporter deficiency presents something of a paradox, given that brain does possess a creatine synthetic capacity. Lunardi et al. (48) have suggested that this may be because the brain creatine transporter plays a dual role: the uptake of circulating creatine across the blood-brain barrier and the neuronal uptake of creatine that is synthesized in glia. Certainly, the brain uptake of creatine from the circulation is a slow process. It required six weeks of supplementation with creatine (4–8 g/day) to attain 50% of the normal brain creatine level in a GAMT-deficient infant (78). This contrasts with studies with mice that suggest a major role for the blood pool in providing creatine to the brain (57). The issue of the cell-specificity of brain creatine synthesis has not yet been clarified. Studies in mouse brain suggest very low GAMT activity in neurons but higher activities in oligodendrocytes, glia, and astrocytes; this has provided experimental support for the idea that glia may provide creatine to neurons (82). These results contrast with the studies of Braissant et al. (5), who find little evidence of mRNA for the creatine transporter in astrocytes associated with the blood-brain barrier. However, they find that both AGAT and GAMT are ubiquitously expressed in adult rat brain, and suggest, therefore, that the brain receives the bulk of its creatine via endogenous synthesis and that every cell in the central nervous system is capable of creatine synthesis. We now appreciate that how the brain, particularly the young brain, acquires its creatine is a crucial issue. Further work is needed to resolve these discrepancies.

Recently, Lunardi et al. (48) addressed the issue of providing creatine to the brain in the absence of a functional transporter. Studies with mouse hippocampal slices have shown that their creatine levels may be elevated by incubation with creatine benzyl ester and that this is not affected by an inhibitor of the creatine transporter (48). Presumably, the mechanism involves diffusion of the lipophilic creatine ester into the cell,

followed by hydrolysis by a broad-specificity esterase. Approaches along these lines have the potential of providing creatine to the brains of patients with creatine transporter deficiency (48).

PERSPECTIVE

Creatine and Brain Function

More than 90% of the body's store of creatine is found in skeletal muscle. Nevertheless, recent work has highlighted the crucial role of the creatine system in the brain. The most striking demonstration of the importance of creatine to the brain is provided by the devastating consequences that attend the creatine-deficiency diseases. However, other work also points to the importance of brain creatine. An intriguing study by Rae et al. (61) examined the effects of creatine supplementation on performance in a number of cognitive tests. The subjects, students from the University of Sydney, were either vegetarians or vegans as it was thought that their creatine status may be somewhat compromised because of very

low dietary intake of creatine. A double-blind, placebo-controlled, crossover trial was carried out in which the subjects ingested a daily dose of 5 g of creatine monohydrate or placebo for six weeks. Then, after a six-week washout period, the treatments were reversed. A number of cognitive tests that require speed of processing were administered at the beginning of the experiment and after each six-week period. Creatine ingestion resulted in a significantly improved performance on these tests. **Figure 5** illustrates performance on the Auditory Backward Digit Span test. This test requires subjects to listen to a series of numbers and then to recite them backward. It involves short-term storage and active memory, both of which have high energy requirements. The remarkable result, evident in **Figure 5**, is that at the end of the experiment, the subjects who had just completed six weeks of creatine intake could, on average, recite 8.5 digits backward, whereas those who had completed six weeks of placebo intake could, on average, recite 7 digits backward. Studies such as this should stimulate new work on the role of creatine in cognitive processes.

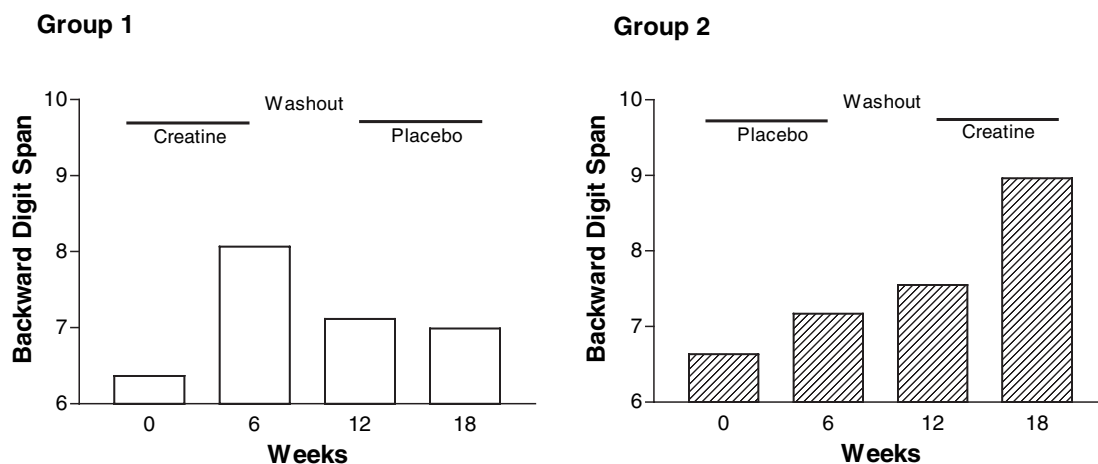


Figure 5

Effect of creatine monohydrate and placebo intake on performance on the Auditory Backward Digit Span test. Group 1 received creatine monohydrate for the first six weeks, followed by a six-week washout period, followed by placebo from weeks 12 to 18. Group 2 received placebo for the first six weeks, followed by a six-week washout period, followed by creatine monohydrate supplementation from weeks 12 to 18. Supplementation with oral creatine monohydrate significantly affected performance compared with the placebo ($P < 0.001$), with no effect of order. Adapted from Rae et al. (61).

MPT:
mitochondrial
permeability
transition

Nonclassical Mechanisms of Creatine Action

Creatine exerts many of its functions by increasing phosphocreatine levels, thereby permitting very rapid regeneration of ATP after a burst of ATP utilization. This is the “classical” function of creatine and is certainly responsible for the ergogenic effect of creatine supplementation in high-intensity, short-duration exercise. However, some of the effects of creatine supplementation may not be so readily explained. The neuroprotection offered by creatine in a mouse model of stroke affords a striking example. In these studies, mice fed a diet supplemented with creatine for three weeks underwent transient focal cerebral ischemia via occlusion of the cerebral middle artery for 45 min. The extraordinary result was that although only minor, nonsignificant changes were found in cerebral creatine, phosphocreatine, or adenine nucleotides, dietary creatine supplementation reduced infarct volume by about 40% (59). Although it is not possible to exclude a substantial increase in phosphocreatine in a small set of key cells, it seems unlikely that the classical mechanism can explain the results. Certainly, Prass et al. (59) suggest that neuroprotection occurred independent of changes in the bioenergetic status of the brain, possibly because of a cerebrovascular effect. It is appropriate, therefore, to review nonclassical actions of creatine.

Creatine and phosphocreatine are major intracellular solutes in muscle cells. Creatine supplementation, by increasing the levels of these metabolites, increases cell hydration. As outlined above, this acts as an anabolic signal that may be responsible for increased glycogen accretion. Creatine can also serve as a compatible osmolyte (similar to betaine, taurine, and myoinositol) in cultured muscle cells exposed to hypertonic stress. Exposure of C2C12 muscle cells to a hypertonic medium increased CRT mRNA about threefold and also increased creatine uptake into these cells (1). Creatine also exerts direct antioxidant effects, in particular toward radical

anions such as superoxide and peroxynitrite (40). It is tempting to relate this property to the neuroprotective action of creatine in a number of animal models of neurodegenerative disease (34).

Mitochondrial creatine kinase (either the sarcomeric MtCK found in striated muscle or the ubiquitous MtCK found in other tissues such as brain, kidney, and spermatozoa) plays a remarkable structural role in mitochondria (68). This enzyme occurs as an octamer at contact sites between the inner and outer mitochondrial membranes. These contact sites are formed by proteolipid complexes that contain, in addition to MtCK, the adenine nucleotide translocase (inner membrane protein) and porin (outer membrane protein). Physiologically, it is clear that this physical interaction provides a microcompartment that ensures close functional coupling between the substrates and products of creatine kinase. Porin, also known as the voltage-dependent anion channel, is an outer-membrane component of the system responsible for the mitochondrial permeability transition (MPT), the reversible opening of a large pore in the inner membrane that results in a general loss of small molecules from the matrix. The identity of all of the components of the MPT pore is not known, although the adenine nucleotide translocase has been implicated. Opening of the pore may be triggered by a number of signals, such as an increase in Ca^{2+} or reactive oxygen species; it may lead to cell death either through apoptosis (via the release of proapoptotic proteins such as cytochrome *c*) or necrosis (via energy depletion) (68). It is apparent that mitochondrial creatine kinase restrains the permeability transition. Direct evidence for this idea comes from elegant experiments involving transgenic mice that express the ubiquitous MtCK in their liver, an organ that does not normally express creatine kinase (19). Mitochondria from the transgenic livers were resistant to Ca^{2+} -induced MPT. Moreover, it is clear that MtCK played a functional role, not just a structural one, since the phenomenon required the correctly

localized, enzymatically active octameric protein as well as the substrates for creatine kinase (19). When MtCK is enzymatically active, it maintains a high matrix ADP concentration, which inhibits MPT pore opening (68). That creatine and phosphocreatine levels are able to restrain opening of the MPT pore may provide a mechanism for their neuroprotective effects, particularly in conditions associated with excessive production of reactive oxygen and reactive nitrogen species.

Finally, MtCK is involved in another pathological process that is modified by creatine levels. A number of events that affect energy metabolism in skeletal muscle (i.e., ischemia, experimental creatine depletion, and culture of adult rat myocytes in the absence of added creatine) lead to the appearance of enlarged mitochondria with arrays of paracrystalline inclusion bodies (68). These inclusions seem to consist, very largely, of MtCK. Similar inclusion bodies are also found in skeletal muscles of patients suffering from a va-

riety of mitochondrial cytopathies. In some circumstances, the occurrence of these inclusion bodies can be directly linked to creatine status. In the case of animals depleted of creatine by treatment with the CRT inhibitor β -guanidinopropionate, the inclusion bodies disappear upon withdrawal of this agent (16). A particularly intriguing case concerns an athletic patient with a novel cytochrome *b* mutation. Cells from this subject produced high basal rates of reactive oxygen species. A muscle biopsy showed paracrystalline inclusion bodies in mitochondria that disappeared after five weeks of treatment with creatine monohydrate at 10 g/day (84).

It is clear that creatine and creatine supplementation exert potent effects that go beyond the simple buffering of cytoplasmic ATP. Further understanding of these effects may be expected in the next few years. Certainly, the words of Hamlet are apposite: "There are more things in heaven and earth, Horatio, than are dreamt of in your philosophy."

SUMMARY POINTS

1. Creatine and phosphocreatine play important roles in both ATP buffering and high-energy phosphate shuttling in a variety of cells.
2. Because of the spontaneous and irreversible breakdown of both creatine and phosphocreatine to creatinine, creatine needs to be continuously replaced by a combination of diet and synthesis. Vegetarians receive very little creatine in their diet and must obtain almost all of it by synthesis. Creatine synthesis makes major demands on amino acid metabolism.
3. Creatine supplementation is used as an ergogenic agent, particularly by athletes involved in exercise of high intensity and short duration. It is also used therapeutically in the treatment of a number of neuromuscular and neurodegenerative diseases.
4. Though long recognized for its role in muscle, it is now clear that creatine is vital for normal brain function. This is evidenced by the neurological symptoms displayed by children with inborn errors of creatine synthesis or transport.

FUTURE ISSUES

1. How do neonates acquire brain creatine? What is the importance of synthesis within the brain compared with creatine supplied via the circulation?

2. What is the role of the liver in creatine synthesis? Is it capable of the entire synthesis of creatine or does it just methylate guanidinoacetate that it obtains from the circulation? What transporter is responsible for creatine release by the liver?
3. How does creatine exert its neuroprotective effect in animal models of neurodegenerative diseases? Is creatine supplementation as effective in Huntington's disease in humans as it is in animal models of this disease?

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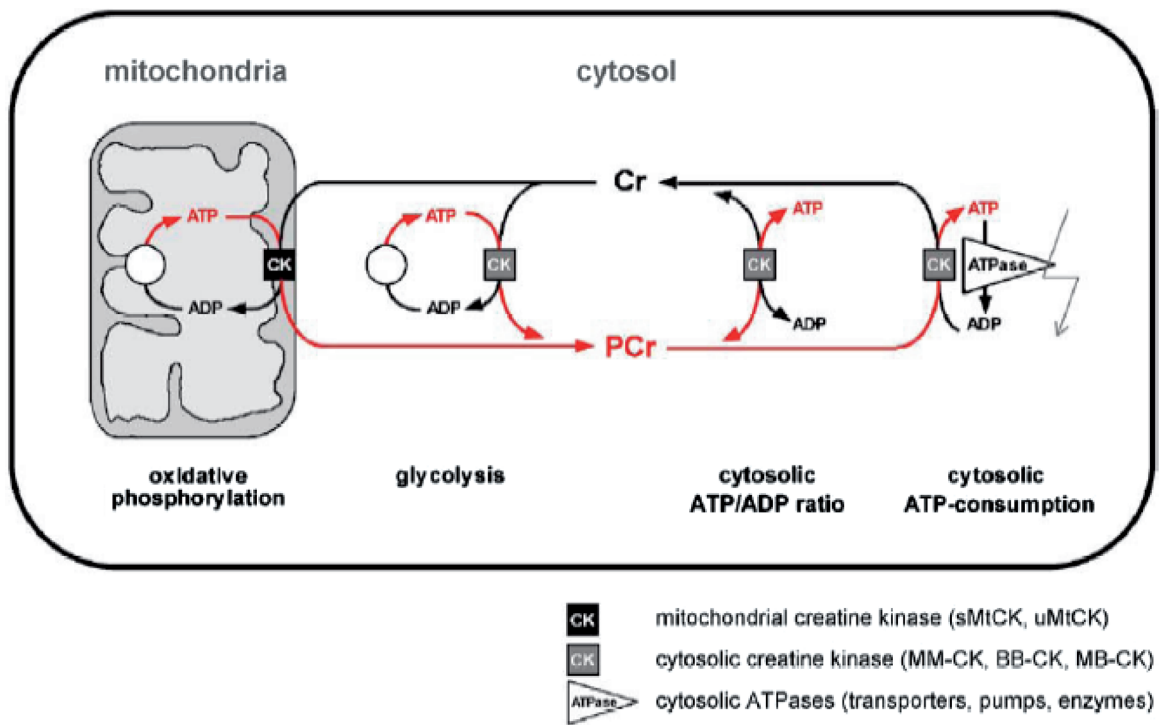
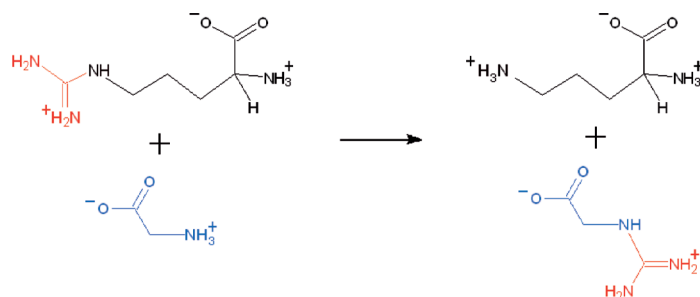


Figure 1

The creatine kinase/phosphocreatine system. ADP, adenosine diphosphate; ATP, adenosine triphosphate; CK, creatine kinase; CR, creatine; PCr, phosphocreatine. Reproduced from Schlattner et al. (68), with permission from Elsevier.

Arginine:Glycine Amidinotransferase
 Arginine + Glycine → Ornithine + GAA



Guanidinoacetate Methyltransferase
 AdoMet + GAA → Creatine + AdoHcy

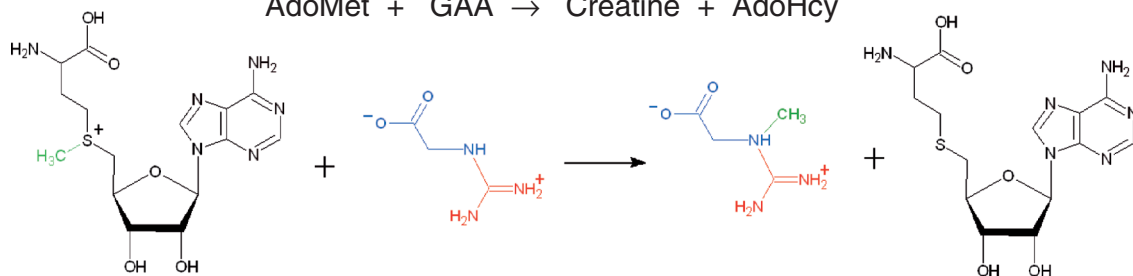


Figure 2

Pathway of creatine synthesis. GAA, guanidinoacetate.



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Errata

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