

The metabolic burden of creatine synthesis

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Abstract Creatine synthesis is required in adult animals to replace creatine that is spontaneously converted to creatinine and excreted in the urine. Additionally, in growing animals it is necessary to provide creatine to the expanding tissue mass. Creatine synthesis requires three amino acids: glycine, methionine and arginine, and three enzymes: L-arginine:glycine amidinotransferase (AGAT), methionine adenosyltransferase (MAT) and guanidinoacetate methyltransferase (GAMT). The entire glycine molecule is consumed in creatine synthesis but only the methyl and amidino groups, respectively, from methionine and arginine. Creatinine loss averages approximately 2 g (14.6 mmol) for 70 kg males in the 20- to 39-year age group. Creatinine loss is lower in females and in older age groups because of lower muscle mass. Approximately half of this creatine lost to creatinine can be replaced, in omnivorous individuals, by dietary creatine. However, since dietary creatine is only provided in animal products, principally in meat and fish, virtually all of the creatine loss in vegetarians must be replaced via endogenous synthesis. Creatine synthesis does not appear to place a major burden on glycine metabolism in adults since this amino acid is readily synthesized. However, creatine synthesis does account for approximately 40% of all of the labile methyl groups provided by S-adenosylmethionine (SAM) and, as such, places an appreciable burden on the provision of such methyl groups, either from the diet or via de novo methylenogenesis. Creatine synthesis consumes some 20–30% of arginine's amidino groups, whether provided in the diet

or synthesized within the body. Creatine synthesis is, therefore, a quantitatively major pathway in amino acid metabolism and imposes an appreciable burden on the metabolism of methionine and of arginine.

Keywords Arginine · Glycine · Methionine · S-Adenosyl methionine · Methyl balance · Liver · Kidney

Introduction

Creatine plays a key role in cellular energetics, particularly in muscle and brain (Wyss and Kaddurah-Daouk 2000). Creatine deficiency is associated with severe neurological manifestations (Schulze 2003) which may be related to possible antioxidant effects or a possible role of creatine as a neuromodulator (Almeida et al. 2006). Quite high concentrations of creatine and creatine phosphate are maintained in skeletal and cardiac muscle, together with lesser, though still appreciable, quantities in the brain (Wyss and Kaddurah-Daouk 2000). It has been estimated that young 70 kg men contain about 120 g of total creatine (creatine and creatine phosphate), although it must be recognized that this quantity is markedly dependent on muscle mass. The maintenance of such a creatine pool requires its continuous synthesis. This synthesis represents a considerable metabolic burden, particularly on amino acid metabolism. The purpose of this paper is to give an account of this burden, paying particular attention to quantitative aspects.

Need for creatine synthesis

Both creatine and creatine phosphate are non-enzymatically converted to creatinine. While the spontaneous

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breakdown of creatine phosphate is somewhat more rapid than that of creatine, it is estimated that approximately 1.7% of the total creatine pool will be converted to creatinine per day. This estimate of the rate of the spontaneous conversion of creatine and creatine phosphate to creatinine is derived from the studies of their stability under defined “physiological” conditions (Wyss and Kaddurah-Daouk 2000). However, striking confirmation of this estimate has recently been provided by Kan et al. (2006). These investigators provided ^{13}C -creatine to humans and used magnetic resonance spectroscopy to follow the combined ^{13}C -creatine and ^{13}C -creatine phosphate signal in skeletal muscle for 105 days. This signal declined with a first-order rate constant of 1.6% per day.

We have employed the comprehensive data on creatinine excretion provided by Cockcroft and Gault (1976) to provide estimates of the loss of total creatine via its spontaneous conversion to creatinine. We estimate a loss of 14.6 mmol/day for a 70 kg male in the 20- to 39-year age group. Mean losses for women are about 80% that of men. The rate of creatinine loss decreases almost linearly with age; 70 kg men aged 70–79 years have a mean loss of 7.8 mmol/day (Brosnan and Brosnan 2007). We have also estimated dietary creatine intake from the NHANES III data on the consumption of different foods between 1988 and 1994 (Food and Nutrition Board 2005), together with the creatine content of these foods. These amount to 7.9 and 5.0 mmol/day for men and women, respectively, in the 19- to 39-year age group. Food creatine has the same high bioavailability as does dissolved creatine (Harris et al. 2002). Experiments involving the administration of ^{13}C -creatine have indicated a bioavailability of 80% (MacNeil et al. 2005). Using these data we estimate rates of uptake of creatine from diet of 6.3 mmol/day for omnivorous males in the 20- to 39-year age group, with a resultant rate of creatine synthesis of 8.3 mmol/day required to achieve a steady state. The rate for women would be about 70–80% of that in men. It should be emphasized that the principal dietary source of creatine is muscle meats, including fish. Creatine is also found in milk and dairy products but their creatine content is relatively small. An important consequence of this is that vegetarians, including lacto-ovo vegetarians, receive very little dietary creatine and consequently must provide the great bulk of their creatine via *de novo* biosynthesis.

The estimates of creatine synthesis provided above are calculated from balance data. Very recently Kasumov et al. (2009) have introduced an isotopic procedure in which the fractional synthesis of creatine was determined in a single human infused with [$1\text{-}^{13}\text{C}$] glycine. It is to be anticipated that the application of this approach to a variety of subjects will provide more direct estimates of creatine synthesis in humans.

Creatine synthesis: pathway, tissues, regulation

Creatine synthesis requires three amino acids, glycine, arginine and methionine, and three enzymes, methionine adenosyltransferase (MAT), arginine:glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT). Synthesis of a molecule of creatine incorporates an entire molecule of glycine, an amidino group from arginine and a methyl group from methionine (Fig. 1). The amidino group of arginine is transferred to the amine nitrogen of the glycine molecule to form ornithine and guanidinoacetate. A methyl group is then transferred from *S*-adenosylmethionine to guanidinoacetate (on to the amine nitrogen of the original glycine molecule) producing *S*-adenosylhomocysteine and creatine. The liver is the major organ for methionine catabolism and expresses relatively high activities of the liver specific isoform, MAT1/3. Thus the liver is capable of generating considerable *S*-adenosylmethionine.

The highest activities of AGAT are found in the kidney and pancreas, whereas the activity of GAMT is highest in the liver (Van Pilsum et al. 1972). Other tissues, such as the brain and testes, express lower activities. However, it is clear that much of creatine synthesis is an inter-organ process whereby guanidino acetic acid (GAA), produced by the kidney is converted to creatine in the liver (Wyss and Kaddurah-Daouk 2000; Edison et al. 2007). The role of the pancreas in creatine synthesis needs further investigation.

The interorgan transport of GAA means that the synthetic pathway must also include transporters for the release of GAA from the kidney and its uptake by the liver as well as for the release of creatine from the liver. GAA transporters have not yet been identified. Transporters responsible for the sodium-dependent uptake of creatine into tissues such as skeletal muscle are known (Walzel et al. 2002), but it is unlikely that these can be responsible for the release of creatine from cells such as the liver.

The regulation of creatine synthesis appears to occur primarily, if not exclusively, through changes in GAA production. Renal AGAT activity is upregulated by growth hormone and down-regulated by dietary creatine (Guthmiller et al. 1994). These effects are exerted at the pre-translational level. Creatine synthesis may also be regulated by substrate (particularly arginine) availability (Edison et al. 2007). There is little evidence for important regulatory effects at the level of GAMT. Rather, this enzyme responds to the GAA concentration, within the range of physiological GAA concentrations, as we have shown in isolated hepatocytes (Da Silva et al. 2009). Thus AGAT activity determines the rate of creatine synthesis, at least with respect to the renal-hepatic axis.

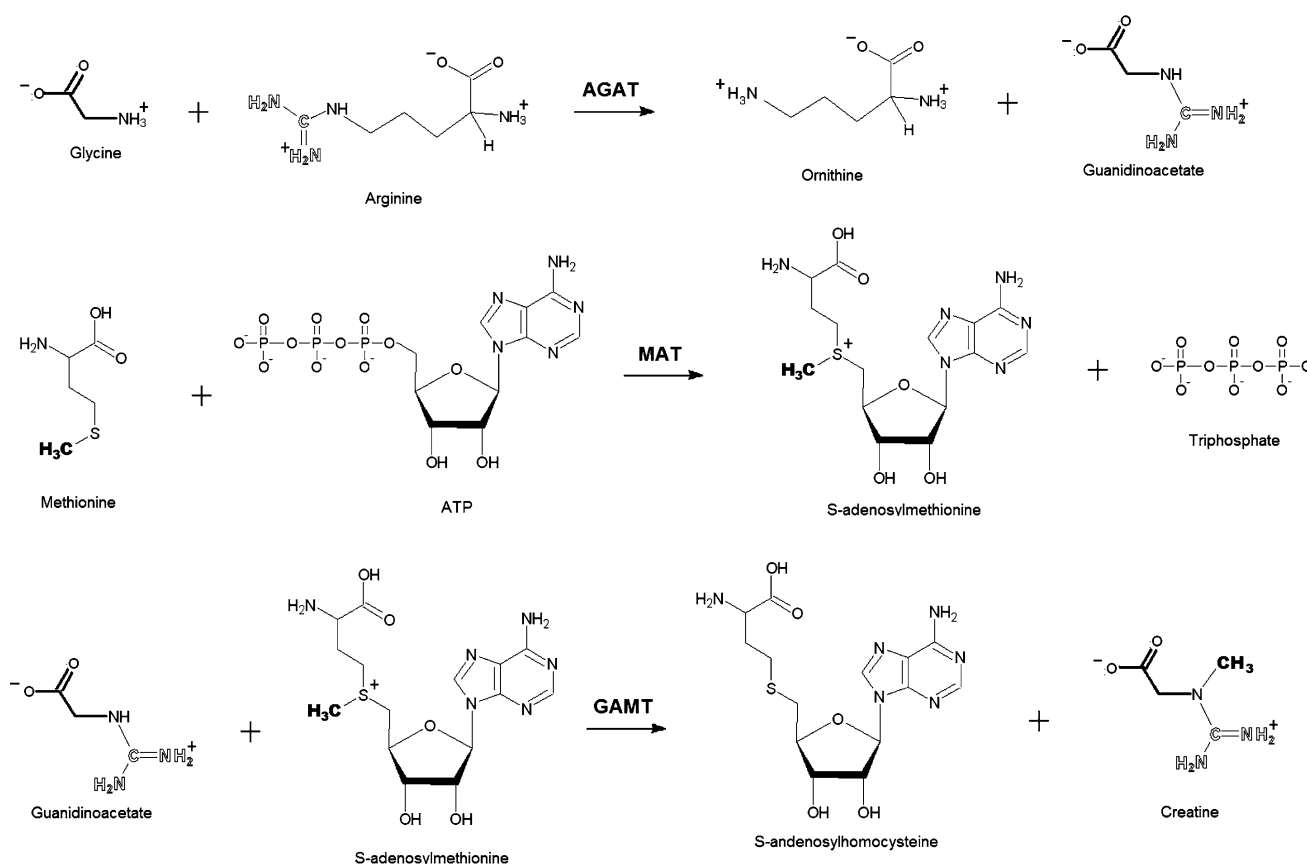


Fig. 1 Pathway of creatine synthesis. Glycine is represented in *bold print* to show that the entire glycine molecule is incorporated into creatine. The amidino group of arginine and the methyl group in SAM

are represented in *distinct typeface* so as to illustrate that it is these groups that are incorporated into creatine

Metabolic burden: glycine

Since the entire glycine molecule is incorporated into creatine it is instructive to compare the rate of creatine synthesis with that of glycine availability. Glycine, a non-essential amino-acid, is readily produced in our bodies. In addition to endogenous glycine synthesis, the mean daily glycine intakes of US males and females, respectively, in the 19–30 age bracket are 62 and 37 mmol, respectively; in the 71+ age bracket the mean daily glycine intakes are 39 and 30 mmol, respectively, for males and females (Food and Nutrition Board 2005). Of course much of this will be used for protein synthesis, especially collagen synthesis, but this will be matched by a comparable rate of proteolysis in adult humans in nitrogen balance. It is clear, therefore, that the dietary provision of glycine appreciably exceeds our calculated rates of creatine synthesis such that this synthesis imposes a minor burden on the provision of glycine. However, a recent paper by Melendez-Hevia (2009) argues for a limited capacity for glycine synthesis in humans so that this issue may need to be revisited.

Metabolic burden: methionine and methyl balance

Since the synthesis of creatine requires the provision of a methyl group from *S*-adenosylmethionine we must consider quantitative aspects of methyl group metabolism. Our diets are awash in methyl groups, in such compounds as alanine, the three branched-chain amino acids and ethanol. However, such methyl groups, which are linked to a carbon atom, are unreactive and we do not have the enzymatic machinery to make use of them. Rather, we must consider compounds that can provide methyl groups to *S*-adenosylmethionine, the universal methyl donor. These are often referred to as “labile methyl groups” and are linked to such electronegative (relative to carbon) atoms as nitrogen and sulfur. These may be obtained from the diet (i.e. methionine, betaine, or choline via betaine) or produced endogenously via methylneogenesis by a process requiring the successive actions of serine hydroxymethyltransferase (SHMT), methylene tetrahydrofolate reductase (MTHFR) and methionine synthase (MS). Methylneogenesis is remarkably dependent on B vitamin status. MTHFR uses NADPH as a reductant (niacin), contains FAD as a

prosthetic group (riboflavin) and produces methylene-tetrahydrofolate as a product (folate). In addition, MS is one of only two mammalian enzymes known to require vitamin B₁₂, in the form of methylcobalamin, as a prosthetic group.

Data on dietary intakes (Food and Nutrition Board 2005) of the US population reveal a mean daily intake of 17 mmol of methionine for males in the 19–30 age bracket and 10 mmol in females. The comparable data for males and females in the 71+ age bracket are 11 and 8 mmol, respectively. There are also data, acquired by means of stable isotope methodology, on the total transmethylation flux in humans, i.e. the sum of all methylation reactions that utilize SAM. These estimates, for a 70 kg person, are approximately 16.7–23.4 mmol/day in young adults and somewhat less in elderly subjects (15.5–21.7 mmol/day) (Mudd et al. 2007). Transmethylation rates in women may be 10% higher than in males (Fukagawa et al. 2000). Thus, creatine synthesis consumes a very substantial portion (about 40%) of the SAM used for all of the methyltransferase reactions in the body and, clearly, imposes a considerable burden on methyl balance and on methionine metabolism.

Metabolic burden: arginine

The mean dietary intake of arginine in US adults averages 34 and 20 mmol/day, respectively, in males and females in the 19- to 30-year age bracket, and 22 and 17 mmol/day, respectively, for males and females in the 71+-year old bracket. Of course, endogenously synthesized arginine must be added to these numbers. Arginine is synthesized via an intestinal renal axis whereby citrulline produced in the intestine is converted to arginine in the kidneys (Dhanakoti et al. 1990). Direct measurement of renal arginine production, by measuring arteriovenous differences across the kidney, indicate a rate of about 4 mmol/day (Tizianello et al. 1980), whereas isotopic estimates of the conversion of plasma citrulline to arginine suggest a rate of about 9 mmol/day (Castillo et al. 1993a). The sum of mean dietary arginine availability (the sum of intake and endogenous production) may, therefore, vary between about 20 and 45 mmol/day in young adults. To complicate matters further it appears that in humans as much as 40% of dietary arginine may be catabolized during first-pass metabolism in the gut (Castillo et al. 1993b). Despite the variability in these estimates, it is evident that creatine synthesis may impose an appreciable burden on arginine supply and metabolism.

The nature of this burden needs to be considered. AGAT only utilizes the amidino group from arginine, generating ornithine (Fig. 1). Ornithine has two principal metabolic

fates. It may be oxidized, a process that is initiated by ornithine aminotransferase, and it may also be reconverted to arginine, a process that requires carbamylphosphate synthetase 1 (CPS1), argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). CPS1 has a very limited distribution, being essentially confined to the liver and the small intestine. Notably, it is absent from the kidney (Goping et al. 1992), though ASS and ASL are highly expressed there (Dhanakoti et al. 1990). However, the kidney does express significant activities of ornithine aminotransferase, primarily in the proximal tubules (Levillain et al. 2000) which is also where renal AGAT is found (McGuire et al. 1986). Although this is not a settled issue it seems likely that ornithine produced by AGAT, at least in the kidney, may be catabolized rather than salvaged within the arginine family of amino acids.

Creatine synthesis in neonates

In addition to the replacement of creatine lost to creatinine, neonates also need to provide creatine to the expanding tissue mass. We have examined this issue in the suckling piglet, an animal with a remarkably rapid growth rate (Brosnan et al. 2009). Between day 4 and day 11 of age piglets acquire 12.5 mmol of creatine, of which 2.8 mmol is provided by the sow's milk. Thus, they need to synthesize 9.7 mmol/week. By comparison, in the same period they received 80 mmol of glycine in their milk and deposited approximately 360 mmol of glycine in their body protein. Clearly, in these animals, creatine synthesis is but a minor portion of the glycine economy. However, the situation is more finely balanced for methionine and arginine, whose intakes over the week were, respectively, 27.8 mmol and 47.7 mmol. Accumulation of arginine in tissue protein was approximately double that consumed, reflecting active de novo synthesis of this amino acid. However, methionine cannot be synthesized by mammals and its accumulation in tissue protein closely matched its dietary intake. Creatine synthesis, therefore, is an appreciable component of the whole body metabolism of methionine and arginine. Comparison of piglet rates of creatine synthesis with rates of whole body transmethylation flux (Riedijk et al. 2007) are of particular interest, revealing that creatine synthesis may consume some 63–77% of all of the labile methyl groups consumed by piglets (Brosnan et al. 2009). Given the extraordinarily rapid growth rates of piglets, these data should not be automatically taken to represent the situation with human neonates. However, they do make the point that creatine synthesis may impose a particularly large metabolic burden on rapidly growing young animals.

Situations with potentially impaired creatine synthesis

It is clear that creatine synthesis is a major metabolic process and can impose a particular metabolic burden, especially on methionine (methyl group) and arginine metabolism. The analysis of this burden presented above dealt with individuals who ingested the mean levels of glycine, methionine or arginine for the US population. There are a number of situations, however, where the burden may be even greater.

Low protein intake

The US data also provide quantitative information for those at the lower end of intakes (Food and Nutrition Board 2005). Those at the 10th percentile for methionine intake consume 12 and 7 mmol/day, respectively, for males and females in the 19- to 30-year bracket and 7 and 6 mmol/day, respectively, for males and for females in the 71+ year bracket. The comparable (10th percentile) data for arginine intake are 25 and 14 mmol/day, respectively, for males and females in the 19- to 30-year age bracket and 14 and 12 mmol/day, respectively, for males and females in the 71+-year bracket. Clearly, the metabolic burden experienced by individuals with such low amino acid intakes will be more severe, especially since it is likely that their creatine intake will be lower than normal.

Vegetarians

Creatine synthesis may offer a particular challenge to vegetarians. Since dietary creatine is exclusively of animal origin and since muscle meats are the primary source (dairy products generally supply, at most, 20% of dietary creatine), it is apparent that vegetarians need to synthesize all (vegans) or nearly all (lacto-ovo vegetarians) of their creatine. Thus, young adult vegetarian men may synthesize as much as 14 mmol creatine per day and young adult vegetarian women 11 mmol creatine per day. It should be noted that muscle creatine levels are reduced in vegetarians, compared with non-vegetarians (Venderley and Campbell 2006).

Elderly

Creatine synthesis may also be a particular burden for the elderly. Because of their lower muscle mass, elderly subjects need to replace somewhat lower losses of creatine than younger individuals. However, their amino acid intake is also appreciably decreased. A particular concern may relate to the occurrence of a relatively high incidence of Vitamin B₁₂-deficiency in this group (Smith 2008) since Vitamin B₁₂ is a cofactor for methionine synthase, a key

enzyme in the process of methylneogenesis. Folic acid deficiency might also be a concern, especially in countries without mandatory fortification.

Altered arginine synthesis or catabolism

Arginine availability may be compromised in a number of ways. Although produced in the body, it is usually classified as a conditionally essential amino acid since it is not always produced in sufficient amounts. In particular, endogenous arginine synthesis is insufficient to meet the needs of premature infants fed on TPN diets that do not contain this amino acid (Wilmore 2004). Arginine also becomes a dietary essential amino acid in patients who suffer from severe burns (Yu et al. 2001) due to greatly increased rates of arginine catabolism without a compensatory increase in endogenous production. Creatine synthesis has not been studied in these situations where dietary arginine becomes essential. However, we do know that renal GAA synthesis is exquisitely sensitive to physiological circulating arginine levels in rats (Edison et al. 2007). Clearly, creatine synthesis needs to be considered in these situations, particularly in situations such as thermal injury where the additional requirement for dietary arginine may be lengthy and in premature infants whose development is at so vulnerable a stage.

Inborn errors of urea synthesis

Arginine becomes a dietary essential amino acid in urea cycle defects other than arginase deficiency (Brusilow 1984; Brosnan and Brosnan 2010). These genetic disorders are associated with hypoargininemia and patients are generally supplemented with arginine. Decreased plasma levels of GAA and of creatine have been reported (Arias et al. 2004). Recently, decreased brain creatine levels have been reported in such patients, and it has even been suggested that this may contribute to the pathogenesis (Roze et al. 2007). However, it should be noted that decreased brain creatine is not a universal finding in these patients (Brosnan and Brosnan 2010).

Conclusions

Table 1 compares our estimates of creatine synthesis in young male adults with the dietary intakes of the three precursor amino acids, together with estimates of total body transmethylation rates. It should be emphasized that these estimates of amino acid intakes do not take into account the bioavailabilities of these amino acids. These vary between different protein sources, with the manner of food preparation and with the nature of an individual's gut

Table 1 Comparison of rates of creatine synthesis in young adults with dietary intakes of the three precursor amino acids and with the whole body transmethylation flux

Creatine synthesis (mmol/day)	8.3 ^a
Transmethylation flux (mmol/day)	16.7–23.4 ^b
Dietary glycine intake (mmol/day)	62 ^c
Dietary methionine intake (mmol/day)	17 ^c
Dietary arginine intake (mmol/day)	34 ^c

^a Current paper^b Mudd et al. 2007^c NHANES III (1988–1994), as reported by the Food and Nutrition Board 2005

microbiota. However, even without this correction, it is clear that creatine synthesis is a major metabolic process and imparts a considerable metabolic demand on arginine and methyl group metabolism. This metabolic burden is even greater in vegetarians and, possibly, in rapidly growing neonates. Arginine is a conditionally essential amino acid and its provision may be particularly critical in patients who have suffered thermal injuries. Creatine synthesis may also be compromised in some elderly subjects, many of whom have reduced protein intake and marginal vitamin status. For the most part, the fact that creatine synthesis may be decreased in these situations has not been considered; further work is necessary to determine whether this is so and whether creatine supplementation may be beneficial.

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