Is it time to reevaluate methyl balance in humans?1–3

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ABSTRACT
S-Adenosylmethionine (AdoMet) is the major biological methyl donor. AdoMet’s methyl group arises both from the diet (eg, methionine, choline, and betaine) and from de novo synthesis by the process of methylneogenesis. At least 50 AdoMet-dependent methylation reactions have been identified in mammals, and genomic analyses suggest that the final number will be much higher. Such methylation reactions play major roles in biosynthesis, regulation, and detoxification. Creatine synthesis is thought to account for the use of >70% of AdoMet-derived methyl groups in humans. This is not consistent with recent studies in mice, in which the phosphatidylethanolamine methyltransferase gene was deleted (PEMT−/−). Loss of this hepatic enzyme resulted in a 50% decrease in plasma homocysteine, which suggests that it accounts for a major component of whole-body AdoMet utilization. A reexamination of human creatine metabolism showed that dietary creatine can account for as much as 50% of daily creatine requirements in nonvegetarians and, therefore, that estimates of creatine synthesis need to be reduced. We suggest that creatine synthesis is responsible for a smaller proportion of AdoMet-derived methyl groups than has been suggested and that phosphatidylethanolamine synthesis via phosphatidylethanolamine methyltransferase is a major consumer of these methyl groups. Am J Clin Nutr 2006;83:5–10.

KEY WORDS Homocysteine, methyltransferase, S-adenosylmethionine, creatine, phosphatidylcholine, methylation demand

INTRODUCTION

In 1887, Wilhelm His reported the results of experiments in which he administered pyridine to dogs and found that they excreted N-methylpyridine in their urine (1). Today, ≈50–100 methyltransferases are known and, in addition, a bioinformatics analysis of the published genomes of various organisms suggests that ≈0.6–1.6% of open reading frames in animal, plant, and microbial genomes may code for methyltransferases (2).

Methionine, a sulfur-containing essential amino acid, participates in complex and far-reaching metabolism in mammals. As outlined in Figure 1, dietary methionine can be taken up by tissues and adenylated to form S-adenosylmethionine (AdoMet), which is the methyl donor in virtually all known biological methylation reactions (3). AdoMet donates its methyl group to a methyl acceptor via numerous methyltransferases, thereby forming a methylated product and S-adenosylhomocysteine (AdoHcy). (Only 2 of these methyltransferases are illustrated in Figure 1.) AdoHcy is subsequently cleaved to homocysteine, which lies at an important metabolic branch point. Homocysteine can enter the transsulfuration pathway, thus removing sulfur from the methionine conservation cycle and forming other products, such as cysteine. Alternatively, homocysteine can be remethylated to reform methionine via either betaine-homocysteine methyltransferase or methionine synthase or can be exported to the extracellular space. The complete methionine cycle and transsulfuration pathway, as outlined in Figure 1, exists primarily in liver, kidney, intestine, and pancreas (4); however, all cells possess some capacity to form homocysteine and to remove it, either via remethylation, transsulfuration, or both.

Labile methyl groups can supply the methyl group for AdoMet. Only a small number of processes are known to do this. Indeed, most dietary methyl groups (eg, in fatty acids, alanine, and the branched-chain amino acids) cannot supply these groups to AdoMet. As far as is known, labile methyl groups are provided by dietary methionine, betaine, and choline (via betaine) and produced endogenously (methylneogenesis) by methyltetrahydrofolate reductase in the form of 5’-methyltetrahydrofolate (Figure 1).

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Several roles of methionine are well described, such as protein and polyamine synthesis. In recent years, a great deal of attention has been focused on homocysteine, because an elevation of this nonprotein amino acid in plasma is associated with an increased risk of cardiovascular disease (5, 6), Alzheimer disease (7), and fractures (8). However, AdoMet consumption and methyl balance remain a relatively understudied aspect of the methionine-homocysteine metabolism. We outline here several lines of evidence that suggest that creatine synthesis (via the AdoMet-dependent methylation of guanidinoacetate in liver) may not be as dominant a consumer of AdoMet as is currently believed and that a revision of our concepts of methyl balance data in humans may be timely.

CREATINE SYNTHESIS

For the past 25 y, it has been widely accepted that creatine synthesis is the major consumer of AdoMet-derived methyl groups in humans. This is based on the elegant labile methyl balance studies that were conducted in humans by Mudd and Poole and Mudd et al (3, 9). In their first experiment, Mudd and Poole maintained normal adults on both normal diets and on diets curtailed in methionine and choline (and thus curtailed in labile methyl groups). Outflows of methyl groups were calculated by measuring urinary excretions of creatine, creatinine, and sarcosine and with the use of previously published data on methylated compounds excreted in human urine. Among the significant findings was that de novo methyl group synthesis must be significant (and thus the homocysteine conservation cycle normally active), because the excretion of creatinine and other methylated compounds in the urine exceeded methyl group intake, as calculated from the methionine and choline content of normal diets. Their data suggested that 70% of available AdoMet was used for creatine synthesis and that methylneogenesis was enhanced when labile methyl group intake was curtailed.

Mudd and Poole (3) noted that the oxidation of methionine and choline methyl groups via sarcosine would be underestimated in their study, because little sarcosine is found in urine when it is metabolized via sarcosine dehydrogenase (3). In an effort to refine these estimates, Mudd et al (9) studied 2 sarcosinemic patients on several dietary regimens, although complete data could be obtained for only one of the patients. This patient was deficient in sarcosine dehydrogenase, and the authors judged that the metabolic block in the patient was sufficiently complete to estimate sarcosine formation in humans. Thus, they assumed that the sarcosine that was excreted in the urine of this patient reflected endogenous sarcosine synthesis (and subsequent
degradation) in normal humans and provided an estimate of methionine and choline methyl group oxidation through sarcosine. This patient was placed on various dietary regimens, and the patient’s urinary creatine, creatinine, sarcosine, and total sulfate excretions were measured. With these data, the authors concluded that 72% of the available AdoMet from normal diets was consumed in creatine synthesis. Of the remaining 28%, they estimated that 14% was used in phosphatidylcholine synthesis, 4% was used in polyamine synthesis, and 10% was used for other methylation reactions. An unavoidable limitation of estimates of AdoMet consumption that are based on the urinary excretion of methylated products is that they do not capture situations where substrates are methylated and subsequently demethylated in vivo (10).

Note that Mudd et al (9) measured creatinine excretion in men and women aged 18–24 y. Creatinine excretion reflects muscle mass and, therefore, displays considerable variation. For example, it is evident from the findings of Mudd et al (9) that men excrete more creatinine than do women. Creatinine excretion (and, therefore, body creatine pools) is at a maximum in the 18–29 y age group; mean creatinine excretion rates of 23.6 mg · kg⁻¹ · 24 h⁻¹ have been reported for men in that age group (11). This rate decreases almost linearly with age; creatinine excretion rates of 12.6 mg · kg⁻¹ · 24 h⁻¹ have been reported for men aged 70–79 y (11).

A second point relates to diet. Mudd et al (3) placed their subjects on both normal diets and on semisynthetic diets that were virtually devoid of creatine. Therefore, excreted creatinine was used as an index of creatine synthesis. They did not account for dietary creatine in their analysis of the subjects who were on normal diets. However, in a real-life, nonexperimental situation, creatine synthesis is likely to be appreciably less than creatinine excretion; the difference is due to creatine and creatinine ingestion. We estimated the quantitative contribution of dietary creatine. Creatine is found only in animal products, principally in meat, poultry, fish, milk, and milk products. Estimates of food intake in the United States show that men in the 20–39 y age group ingest an average of 293 g of meat, poultry, and fish per day (12). (Because the creatine content of organ and luncheon meats is low, we excluded their intakes from these calculations.) The intakes for men in the 40–59 y age group and in the ≥60 y age group were 272 g and 218 g, respectively. Women ingest an average of 171 g (20–39 y age group), 167 g (40–59 y age group), and 156 g (≥60 y age group). These muscle meats contain approx. 30 mmol creatine/kg, but as much as one-third of this can be converted to creatinine during cooking (13).

Milk creatine must also be taken into account. US data (12) on the intake of milk and milk products (predominantly as fluid milk) show intakes of 261g, 245g, and 252g for men aged 20–39 y, 40–59 y, and ≥60 y, respectively. Intakes for women aged 20–39 y, 40–59 y, and ≥60 y were 208g, 190g, and 207g, respectively. The creatine content of cow milk is approx. 0.6 mmol/L (14). These data permitted us to estimate dietary creatine intake in these age groups: for the 20–39 y age group, the intake was 7.9 mmol for men and 5.0 mmol for women; for the 40–59 y age group, the intake was 7.4 mmol for men and 4.8 mmol for women; and for the ≥60 y age group, the intake was 6.3 mmol for men and 4.7 mmol for women. Food creatine has the same high bioavailability (≈90%) as does dissolved creatine (15). Combining these results with those of Cockroft and Gault (11) for age-dependent, daily creatinine excretion, and normalizing for a 70 kg person, we estimated that the actual daily rates of creatine synthesis in men were ≈7.7 mmol for the 20–39 y age group, ≈5.6 mmol for the 40–59 y age group, and ≈3.7 mmol for the ≥60 y age group. The corresponding estimates for women were ≈70–80% those for men. These estimates contrast those that were obtained by Mudd et al (15.4–15.8 mmol for men and 9.9–10.6 mmol for women) (3). It should be emphasized that we have no disagreement with Mudd’s estimates for his experimental subjects (ie, young persons who were on creatine-free diets). However, these data must not be extrapolated to the population at large. We suggest that our estimates are more representative of a free-living population who ingest a Western-type diet. Furthermore, we emphasize the effect of age on the rate of creatine synthesis. Our new estimates challenge the idea that creatine synthesis is the dominant reaction that uses AdoMet.

The stable isotope approach, which was pioneered by Storch et al (16), is an independent means of estimating AdoMet-requiring reactions. With this approach, methionine, which is labeled with 13C in the carboxyl carbon and 3H in the hydrogen atoms of the methyl group, is used to measure whole-body rates of transmethylation, transsulfuration, and remethylation. The earliest application of this approach in humans suggested daily rates of transmethylation (16.6 mmol/d) (16) that were, effectively, identical to those calculated by Mudd and Poole (14–18 mmol/d) (3). These estimates provided independent support for the whole-body rate of AdoMet utilization calculated by Mudd and Poole (3). However, 2 points need to be emphasized. First, the 2 studies were not comparable because the subjects in the Mudd and Poole study (3) ate a diet that contained little or no creatine, whereas the subjects in the Storch et al study (16) were not controlled with regard to creatine ingestion. We anticipate that isotopic measurement of transmethylation in the subjects of the Mudd and Poole study would provide higher rates of transmethylation because of their need to synthesize all their creatine. Second, the Storch model was refined by MacCoss et al (17). One of the uncertainties of the original model was that it required an estimate of the intracellular isotopic enrichment of methionine. In the absence of a measure of this variable, it was assumed to be 80% of plasma methionine enrichment, a figure that was arrived at by analogy with leucine metabolism. MacCoss et al (17) evaluated the plasma isotopic enrichment of homocysteine and of cystathionine as indexes of intracellular methionine enrichment. Remarkably, the isotopic enrichment of these 2 molecules in plasma was identical, which indicated that they both reflected metabolic processes in the same cells. The transsulfuration pathway is found primarily in the liver, kidneys, pancreas, and intestine but is most active in the liver, which is the dominant tissue for methionine catabolism as well as the site of the high-flux methytransferases (18). When the isotopic enrichment of plasma homocysteine reached a plateau, it was only ≈58% that of methionine. Using this new approach, MacCoss et al (17) calculated that previous estimates of transmethylation should be increased by ≈40% (to ≈24 mmol/d). The isotopic studies carried out thus far have used an intravenous infusion of the tracer. This is a concern, because a substantial proportion of dietary amino acids may be catabolized within the intestine (19, 20). Although no information is available for intestinal methionine catabolism in adults, it seems that as much as 30–50% of dietary methionine is catabolized by the intestine in piglets (19). It is quite possible that this component of methionine catabolism would be largely, or
even entirely, undetected when the tracer methionine is administered intravenously. When piglets are fed parenterally, rather than enterally, methionine requirements are reduced by 30% (21), which provides evidence that circulating methionine is not readily available for intestinal metabolism. Therefore, intravenous tracers may only detect nonintestinal methionine metabolism and may underestimate whole-body metabolism. Also note that isotopically measured whole-body transmethylation rates are slightly higher in women than in men, when expressed on a body-weight basis, and even higher in a comparison between the muscle mass of men and women. In addition, a comparison of methionine kinetics between young men and women (31–35 y of age) (22) and elderly men and women (3 age: 79 y) showed that the transmethylation rates in the elderly were at least as high as in the young adults (23). Both of these findings contrast what would be expected if creatine synthesis were the dominant sink for AdoMet-derived methyl groups (22). These data raise an obvious question: if creatine synthesis is not the dominant consumer of AdoMet, what other methyltransferases account for the high rates of AdoMet consumption?

**PHOSPHATIDYLCHOLINE BIOSYNTHESIS**

We recently published data from studies of mice that suggested that a significantly greater proportion of AdoMet was used in phosphatidycholine biosynthesis than was previously thought. We investigated the role of phosphatidylethanolamine N-methyltransferase (PEMT), a liver-specific enzyme, on homocysteine metabolism using the PEMT knockout mouse (24). We hypothesized that PEMT contributed significantly to homocysteine production by the liver, because 3 methyl groups are required to produce 1 phosphatidylcholine molecule. Remarkably, we observed a 50% decrease in plasma homocysteine in the knockout mice compared with wild-type controls. Key enzymes of homocysteine metabolism (ie, AdoMet synthase, cystathionine β-synthase, methylenetetrahydrofolate reductase, betaine-homocysteine methyltransferase, and methionine synthase) were unchanged, which indicated that the lack of PEMT was responsible for the observed differences. We calculated that a 20 g mouse secreted ≈30 μmol phosphatidylcholine into bile per day. Approximately one-third of phosphatidylcholine in murine liver is derived from PEMT [the rest is synthesized via the cytidine diphosphate–choline (ie, Kennedy) pathway, which is not AdoMet dependent] (25, 26). Ten μmol of PEMT-derived phosphatidylcholine corresponds to 30 μmol of AdoMet consumption, because 3 AdoMet molecules are required to generate 1 phosphatidylcholine molecule from phosphatidylethanolamine. Therefore, ≈30 μmol AdoHcy were generated in a 24 h period to satisfy phosphatidylcholine requirements for bile. These data suggested that phosphatidylethanolamine methylation not only contributes significantly to plasma homocysteine concentrations but may also represent the dominant methylation reaction in mice. Clearly, these data contrast Mudd’s low estimates of phosphatidylcholine synthesis (3).

The calculation of AdoMet consumption by PEMT in the study conducted by Mudd et al (9) required accurate information on the oxidation of choline to sarcosine in the body and recovery of this compound in the urine. However, when sarcosine was administered to a patient who was deficient in sarcosine dehydrogenase, only 60–80% was recovered in the urine. One possible explanation for the incomplete recovery of sarcosine may be due to its metabolism by enzymes other than sarcosine dehydrogenase. The discovery of a peroxisomal pipelic acid oxidase that can also oxidize sarcosine (27) may provide an example of such an enzyme. In addition, the well-established mitochondrial sarcosine dehydrogenase could also explain the incomplete recovery. In any case, the incomplete recovery of sarcosine in the urine in these experiments underestimated its formation and therefore the contribution of the PEMT reaction. These measurements were likely additionally complicated by the nonaqueous destinations of PEMT-derived phosphatidylcholine and by bile secretion into the intestine, both of which may be poorly reflected in urinary values. In fact, current estimates indicate that a typical human secretes ≈5 mmol phosphatidylcholine into bile each day (28–30). Approximately one-third of this phosphatidylcholine (1.65 mmol) could be generated by the PEMT reaction (25, 26). Hence, the PEMT reaction may consume 5 mmol AdoMet/d to satisfy phosphatidylcholine production. This estimate for bile secretion alone is much higher than the estimate for total PEMT-dependent AdoMet consumption obtained by Mudd et al (2.5 mmol/d) (3, 9). Note that the use of bile phosphatidylcholine secretion to estimate newly formed hepatic phosphatidylcholine may be problematic because of the enterohepatic circulation of biliary lipids. Absorption of enteral phosphatidylcholine exceeds 90%; of the absorbed fraction, 20% is intact phosphatidylcholine, 40% is lysophosphatidylcholine, and 40% is degraded to glycerolphosphocholine and phosphocholine, which is taken up via the portal vein (31, 32). Intestinal phosphatidylcholine (intact phosphatidylcholine and reacylated lysophosphatidylcholine) is used to form chylomicrons and is secreted into the lymphatic system, which bypasses the liver. Therefore, it is unclear how biliary phosphatidylcholine reabsorption influences hepatic phosphatidylcholine biosynthesis. In addition to bile synthesis, PEMT-synthesized phosphatidylcholine is secreted in lipoproteins and used for the synthesis of hepatic membranes. Taken together, these data indicate an important role of PEMT in determining AdoMet consumption in humans.

Our calculations suggest that creatine synthesis is quantitatively less significant than is phosphatidylcholine synthesis via PEMT in AdoMet consumption. It must be emphasized that our values were based on estimates of PEMT-derived phosphatidylcholine synthesis in rodents, but several lines of evidence indirectly support the numbers we presented above. The effects of creatine supplementation on plasma homocysteine have been studied by us in rats (33) and by others in humans (34, 35). Creatine synthesis begins in the kidney with the formation of guanidinoacetate via l-arginine-glycine amidinotransferase. Guanidinoacetate is transported to the liver where it is methylated by AdoMet-dependent guanidinoacetate methyltransferase to form creatine; l-arginine-glycine amidinotransferase is the primary regulatory site in the pathway. Dietary creatine (and guanidinoacetate) suppresses the activity of the amidinotransferase. We hypothesized that provision of creatine in the diet would suppress guanidinoacetate synthesis and thereby both attenuate methylation demand and decrease plasma homocysteine concentrations. We observed a remarkable suppression of l-arginine-glycine amidinotransferase (≈90%) but only a ≈25% reduction in plasma homocysteine, much less than might be anticipated if creatine synthesis accounts for >70% of AdoMet utilization. Note that the hepatic activities of cystathionine β-synthase and methionine synthase, enzymes responsible for
removing homocysteine, were not changed by creatine supplementation (33). The effect of creatine supplementation on plasma homocysteine in humans has also been examined. One study (34) found no effect; however, a second study (35) found a small, but statistically significant, decrease (≈10%) in plasma homocysteine after creatine supplementation. Such creatine supplementation in young volunteers decreases plasma guanidinoacetate by 30–50% (36). Given this degree of down-regulation of endogenous creatine synthesis, the small decreases observed in plasma homocysteine are hardly consistent with creatine synthesis being responsible for >70% of AdoMet utilization and, thus, for whole-body homocysteine production.

Recently, Mudd et al (37) described the first proven case of AdoHcy hydrolase deficiency in a human. This patient had hypermethioninemia without marked hyperhomocysteinemia, as well as plasma AdoMet and AdoHcy values that were 30- and 150-fold above normal. Of particular interest to the present discussion, the patient’s plasma phosphatidylcholine was ≈50% of normal. PEMT is inhibited by high concentrations of AdoHcy (38), as are most other methyltransferases, and phosphatidylcholine synthesized via PEMT was likely severely limited in that patient. However, phosphatidylcholine synthesized via the Kennedy pathway, which is not AdoMet-dependent, is not subject to this kind of inhibition. We suggest that the ≈50% reduction in plasma phosphatidylcholine that was observed in this patient provides additional support for a significant role of PEMT in lipoprotein secretion in humans (39). Recently, Davis et al (40) showed that folate-depleted women have increased plasma homocysteine despite having normal remethylation rates. They hypothesized that increased homocysteine synthesis, possibly PEMT-dependent, could be responsible for this observation. Furthermore, mild hyperhomocysteinemia in patients with cystic fibrosis has been linked to increased phospholipid methylation (41, 42). These observations are consistent with our findings in the liver-specific CTP-phosphocholine cytidylyltransferase-α knockout mouse (43, 44). In these mice, PEMT flux was increased 2-fold to meet hepatic demand for phosphatidylcholine and plasma homocysteine concentrations were elevated 20–40%.

Because both PEMT and guanidinoacetate methyltransferase use the same hepatic AdoMet pool, the question arises as to whether they compete for this pool. The hepatic AdoMet concentration in rats fed a normal diet was reported to be 141 nmol/g (4). This corresponds to an intracellular concentration of ≈0.25 mmol/L. The Km values of PEMT and guanidinoacetate methyltransferase for AdoMet have been reported to be 29 and 49 μmol/L, respectively (38, 45). Therefore, regulation of the relative fluxes through these enzymes as a result of competition for AdoMet is unlikely.

CONCLUSIONS

The rate of AdoMet formation that was estimated by MacCoss et al (17) and the calculations we described above have several implications. We argued that the PEMT reaction may play an important role in determining methylation demand in humans (46). We also argued that creatine synthesis may not be the major consumer of methyl groups. Finally, our calculations suggest that the homocysteine remethylation pathway may be substantially more active than previously thought, considering that the rate of AdoMet formation described by MacCoss et al (17) necessitates a much higher level of methylneogenesis for a given level of methionine intake.

Clearly, the arguments advanced here are the result of estimates, first approximations, and direct evidence from animal models. In their classic study of methyl balance, Mudd et al (8) noted that their estimates may need to be modified as more information became available. It is in this spirit that we make our arguments. It is our hope that this article will generate a discussion on labile methyl balance in humans, because debate on the matter has been largely lacking in recent years. In addition to the dietary intakes of methionine and creatine, we suggest that dietary betaine may need to be factored into the dietary sources of labile methyl groups. Certainly, clarification of the role of betaine in methyl balance is needed. Craig (47) reported that dietary intake of betaine ranged from 1–2.5 g/d, which amounts to between 8.5 and 25 nmol labile methyl groups/d. Administration of betaine lowered plasma homocysteine in homocystinuric patients (48). Yet, Davis et al (49), using a dual tracer procedure to simultaneously measure total and folate-dependent homocysteine remethylation, found that serine, which feeds one carbon unit into the folate pool, contributed 100% of the methyl groups required for remethylation. It is clear that we must find innovative methodologies by which the questions we raised and others relating to methyl balance may be addressed in humans.

All authors contributed ideas to this commentary. LMS and RLJ wrote the first draft of the manuscript. JTB is a senior investigator of the Canadian Institutes for Health Research. DEV is the Canada Research Chair in Molecular and Cell Biology of Lipids and a Medical Scientist of the Alberta Heritage Foundation of Medical Research. None of the authors had any conflicts of interest.

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