

Methyl balance and transmethylation fluxes in humans¹⁻⁴

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ABSTRACT

Various questions have been raised about labile methyl balance and total transmethylation fluxes, and further discussion has been encouraged. This report reviews and discusses some of the relevant evidence now available. The fact that, if needed, labile methyl balance is maintained by methylneogenesis appears to be established, but several aspects of transmethylation remain uncertain: definitive measurements of the rate of total transmethylation in humans of both sexes on various diets and at various ages; the extent to which synthesis of phosphatidylcholine has been underestimated; and the relative contributions of the 2 pathways for the formation of sarcosine (ie, *N*-methylglycine). The available evidence indicates that the quantitatively most important pathways for *S*-adenosylmethionine-dependent transmethylation in mammals are the syntheses of creatine by guanidinoacetate methyltransferase, of phosphatidylcholine by phosphatidylethanolamine methyltransferase, and of sarcosine by glycine *N*-methyltransferase. Data presented in this report show that *S*-adenosylmethionine and methionine accumulate abnormally in the plasma of humans with glycine *N*-methyltransferase deficiency but not of those with guanidinoacetate *N*-methyltransferase deficiency or in the plasma or livers of mice devoid of phosphatidylethanolamine *N*-methyltransferase activity. The absence of such accumulations in the latter 2 conditions may be due to removal of *S*-adenosylmethionine by synthesis of sarcosine. Steps that may help clarify the remaining issues include the determination of the relative rates of synthesis of sarcosine, creatine, and phosphatidylcholine by rapid measurement of the rates of radiolabel incorporation into these compounds from L-[methyl-³H]methionine administered intraperitoneally to an experimental animal; clarification of the intracellular hepatic isotope enrichment value during stable-isotope infusion studies to enhance the certainty of methyl flux estimates during such studies; and definitive measurement of the dietary betaine intake from various diets. *Am J Clin Nutr* 2007;85:19-25.

KEY WORDS Transmethylation, labile methyl balance, phosphatidylcholine, creatine, sarcosine

INTRODUCTION

In a recent issue of this Journal, Stead et al (1) asked, "Is it time to reevaluate methyl balance in humans?" Their article, also a commentary, raised questions about existing views on this subject and expressed the hope that their comments would generate a discussion on labile methyl balance in humans. Here, we attempt to respond to that hope by further discussion of some of the questions raised by Stead et al and by presentation of additional

data bearing on labile methyl balances. The available evidence leads us to conclude that, if needed, labile methyl balance is maintained by methylneogenesis, but that several aspects of transmethylation rates remain uncertain. Among the most serious of these aspects are the possibility that the rate of synthesis of phosphatidylcholine has been underestimated and the uncertainty about the rates and relative contributions of the 2 pathways for formation of sarcosine (*N*-methylglycine). We present relevant new data on the concentrations of many of the metabolites of the transsulfuration and transmethylation pathways in plasma and livers of mice devoid of phosphatidylethanolamine *N*-methyltransferase (PEMT) activity or in plasmas of humans with severe deficiencies of either glycine *N*-methyltransferase (GNMT) or guanidinoacetate *N*-methyltransferase (GAMT). Steps that may help clarify the remaining issues are suggested.

THE OCCURRENCE OF METHYL BALANCE AND ESTIMATES OF MINIMAL TRANSMETHYLATION RATES

Initial attempts to estimate methyl balances in humans and the total fluxes of labile methyl groups originating either in methionine [proceeding through *S*-adenosylmethionine (AdoMet)] or in choline (proceeding through betaine, methionine, and

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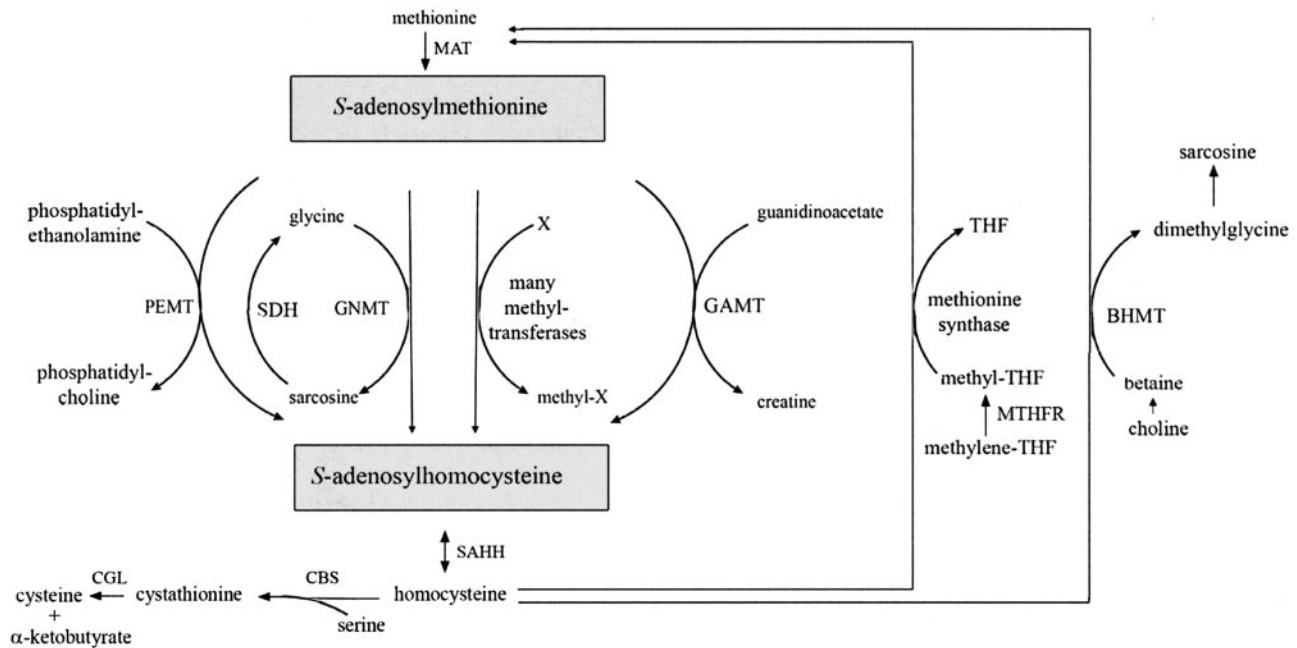


FIGURE 1. Major reactions involved in transmethylation flux and methylneogenesis. The total transmethylation flux is equivalent to the total flux occurring through reactions that convert *S*-adenosylmethionine to *S*-adenosylhomocysteine. The 3 *S*-adenosylmethionine-dependent reactions thought to contribute quantitatively most to this flux are methylation of guanidinoacetate by guanidinoacetate methyltransferase (GAMT) to form creatine; methylation of phosphatidylethanolamine by phosphatidylethanolamine methyltransferase (PEMT) to form phosphatidylcholine; and methylation of glycine by glycine *N*-methyltransferase (GNMT) to form sarcosine (*N*-methylglycine). A large number of additional *S*-adenosylmethionine-dependent methyltransferases also occur in mammals [see Katz et al (3)], but their collective quantitative contribution to transmethylation flux may be small compared with those mentioned above. The final steps in methylneogenesis are the reduction of a methylene group of 5,10-methylenetetrahydrofolate (methylene-THF) by methylenetetrahydrofolate reductase (MTHFR) to form 5-methyltetrahydrofolate (methyl-THF), followed by transfer by methionine synthase of the newly formed methyl moiety to homocysteine, forming methionine and tetrahydrofolate (THF). Sarcosine is formed not only by GNMT, but also by oxidation of choline to betaine, formation of dimethylglycine by betaine homocysteine methyltransferase (BHMT), and oxidation of dimethylglycine to sarcosine. Sarcosine is oxidized by sarcosine dehydrogenase (SDH). During the reaction, glycine is produced, and a 1-carbon unit is transferred to THF, forming methylene-THF. MAT, methionine adenosyltransferase; CBS, cystathionine β -synthase; CGL, cystathionine γ -lyase; SAHH, *S*-adenosylhomocysteine hydrolase.

AdoMet) to various methylated products were reported by Mudd and Poole (2). The relevant pathways are summarized in **Figure 1**. Young adult volunteers adapted to normal diets providing ≈ 9.9 (males) or ≈ 9.2 mmol (females) methionine/d and ≈ 3.6 (males) and ≈ 2.5 mmol (females) choline/d were found to excrete in the urine 17.5 and 12.9 mmol, respectively, methylated products/d, chiefly in the form of creatinine, but including 1.9 (males) or 2.6 (females) mmol creatine/d plus 31 additional methylated compounds. The excretions of the latter 31 compounds (all relatively minor) were estimated from published values. When the subjects were adapted to experimental diets containing 4.7 (males) or 4.5 (females) mmol methionine/d, ≈ 0.2 mmol choline/d, and negligible creatine plus creatinine, they remained in slightly positive nitrogen balance or within the zone of nitrogen equilibrium and continued to excrete in the urine 17.6 (males) and 12.7 (females) mmol methylated products/d, again chiefly in the form of creatinine. Thus, when following the latter diets, all subjects put out far more methylated products than they took in as labile methyls in their diets.

These observations provide very strong evidence that, when labile methyl intake is less than methyl output, the difference is balanced by the formation of new methyl groups. This conclusion is now amply supported by the stable-isotope infusion studies reviewed in this article and by observation of patients with genetic defects in the pathway that carries out such methylneogenesis—namely, formation of methyltetrahydrofolate (methyl-THF) from methylene-THF followed by transfer of the methyl

group of methyl-THF to homocysteine to form methionine. For example, such patients tend to have abnormally low or borderline low plasma methionine and to have elevated plasma total homocysteine (tHcy) (4). Thus, the fact is well established that, when dietary intake of labile methyls is less than that required for ongoing methylation reactions, methyl balance is normally maintained by de novo formation of methyl groups.

What remains less clear is the total amount of transmethylation flux and the constituents of that flux. Mudd and Poole (2) pointed out that their estimates of total transmethylation were preliminary and probably minimal, because methylated products in addition to those specified may be found, and, as is equally important, because no account had been taken of the utilization of methyl groups to form products that were subsequently demethylated [eg, sarcosine (*N*-methylglycine)], the use of choline to form sarcosine, and the use of methyl esters such as the protein methyl ester that may be the chief consumer of AdoMet in human red blood cells (5). Furthermore, excretion of methylated compounds other than in the urine was not taken into account. Stead et al pointed out that, according to current estimates, a typical human secretes into the bile an amount of phosphatidylcholine the synthesis of which would consume some 5 mmol AdoMet/d. Although a substantial, but uncertain, amount of this phosphatidylcholine is reabsorbed (1), biliary secretion may contribute significantly to the total output of compounds formed from AdoMet.



In an effort to address one of these problems, Mudd et al (6) measured sarcosine excretion by a 16-y-old sarcosinemic girl with a genetic deficiency in her ability to metabolize sarcosine. Weighing 50 kg and following a normal diet containing 7.1 mmol methionine/d and 2.9 mmol choline/d (as lecithin), she had a urinary sarcosine excretion of 4.3 mmol/d, excretion of creatinine plus creatine of 10.2 mmol/d, and that of other methylated compounds [those included in the earlier study (2)] of 1.4 mmol/d, for a total excretion of 15.9 mmol methyl groups/d. When labile methyl group intake from methionine plus choline was increased beyond that required for formation of creatinine and the specified methylated compounds, sarcosine excretion rose to balance the excess intake. A limitation was that it was not possible to be sure what portion of the sarcosine excreted was formed by each of 2 alternative pathways: AdoMet-dependent methylation of glycine or oxidation of choline to betaine, which was followed by conversion of betaine to dimethylglycine and then to sarcosine (Figure 1). To the extent that the latter pathway contributed, in a steady state, the utilization of a choline moiety may be balanced at least to some extent by AdoMet-dependent conversion of phosphatidylethanolamine to phosphatidylcholine, a process using 3 methyl groups, only 1 of which would be regained from the betaine methyl. Such phosphatidylcholine synthesis would require further upward revision of the labile methyl flux, as would also, of course, the other factors discussed above. Two additional adjustments of the results for this patient are pointed out by Stead et al (1). First, the recovery of sarcosine in her urine was only 60–80% of an administered dose, which suggested that she retained some capacity to metabolize sarcosine. The value for sarcosine formation might then be adjusted to $4.3/0.7 = 6.1$ mmol/d. Second, customary diets in the United States, such as the basal diet of the subject, provide ≈ 5.0 mmol creatine/d to young adult females (1). On the basis of data reported for the absorption of creatine (7–9), we estimate that $\approx 80\%$ of dietary creatine will be absorbed. The uptake from a dietary intake of 5.0 mmol/d will then be 4.0 mmol/d. Subtraction of this uptake from the observed creatinine-plus-creatine excretion yields a corrected value for creatine synthesis of $10.2 - 4.0 = 6.2$ mmol/d, and the minimal total adjusted labile methyl flux becomes $6.2 + 1.4 + 6.1 = 13.7$ mmol/d. According to these values, the contribution of creatine synthesis to the total labile methyl flux would be $(6.2/13.7) \times 100\% = 45\%$.

TOTAL LABILE METHYL FLUXES ESTIMATED BY STABLE-ISOTOPE INFUSION STUDIES

An alternative experimental approach that overcomes some of the limitations inherent in the above balance studies was devised in the Laboratory of Human Nutrition, School of Science and Clinical Research Center at the Massachusetts Institute of Technology. Constant intravenous infusion of [methyl- $^2\text{H}_3$] and [1- ^{13}C]methionine permitted calculation of total methyl fluxes, ie, transmethylation rates. In an initial study, Storch et al (10) found that, for healthy males following diets that were adequate in methionine and cystine and receiving supplementation with 500 mg choline/d, transmethylation rates were $14 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the fed state and $5.85 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the postabsorptive state. These values converted to an estimated daily flux of $238 \pm 22 \mu\text{mol/kg}$ or, for a 70-kg person, 16.7 mmol/d (10). Using the same methodology, Fukagawa et al (11) found somewhat similar postabsorptive rates: $7.61 \pm$

$1.02 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for men and $8.43 \pm 1.15 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for women. Total daily fluxes were not estimated because studies were not done when subjects were in the fed state. MacCoss et al (12) did further similar studies of postabsorptive subjects. A still unresolved difficulty with this approach is the need to estimate the isotopic enrichment of methionine in liver. For this purpose, Storch et al (10) used the enrichment of plasma methionine, but MacCoss et al (12) measured the enrichments of plasma methionine as well as those of homocysteine and cystathionine and found the enrichments of the latter compounds to be less than the enrichment of methionine. Using the homocysteine and cystathionine enrichments, MacCoss et al calculated transmethylation rates $\approx 40\%$ higher than those reported by Storch et al—ie, $16.7 \times 1.4 = 23.4$ mmol/d. However, Davis et al (13) subsequently found that the enrichment value for the M + 1 ion used by MacCoss et al is difficult to determine accurately because of its high background abundance. Using instead the M + 4 ion (which is far less subject to such inaccuracy), Davis et al found the isotopic enrichments of plasma homocysteine and methionine to be similar. On the basis of the methionine enrichment, they estimated that the correct value of MacCoss et al would have agreed closely (93%) with the earlier value of Storch et al. Most recently, Mercier et al (14) found fed and postabsorptive rates of 13.5 ± 0.8 and $8.2 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, respectively, in young subjects and of 12.5 ± 0.4 and $6.0 \pm 0.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, respectively, in subjects aged 66–76 y (again calculated by using methionine isotopic enrichment values). Converted to daily rates in the manner used by Storch et al (10), these rates indicate daily fluxes of 260 and 222 $\mu\text{mol/kg}$, or 18.2 and 15.5 mmol for a 70-kg person. Thus, after adjustment for uncertainty as to the correct intracellular hepatic isotopic enrichment value, a reasonable range for the transmethylation rate in young persons based on the stable-isotope infusion studies is 16.7–23.4 mmol/d, and that in elderly subjects is somewhat less: 15.5–21.7 mmol/d.

This rate of transmethylation in young persons may be compared with that calculated by Mudd et al (6) in the sarcosinemic girl. If the transmethylation rate is directly proportional to body weight, that subject's final weight-adjusted rate would become $12.7 \times (70/50) = 17.8$ mmol/d. Because (as discussed above) this is a minimal rate, it appears that the major portion of methylated compounds have been accounted for, although factors causing the utilization of as much as 5.6 mmol AdoMet/d (ie, $23.4 - 17.8$ mmol/d) may remain to be identified. Data are available to indicate that, although it may account for at least 50% of the AdoMet consumption of erythrocytes, the rate of AdoMet utilization in one such reaction, the isoaspartyl methyltransferase of red blood cells, is lower relative to overall transmethylation (5). By using the values in Table 6 in the report by Barber et al (5), one may calculate an AdoMet consumption rate between 0.06 and somewhat more than 0.12 mmol/d by fresh human red blood cells.

IMPORTANT REMAINING UNCERTAINTIES

Among the more important remaining uncertainties in the above analysis are 1) the correct values for total transmethylation fluxes in humans of both sexes following various diets and at various ages; 2) the distribution between the metabolic pathways leading to sarcosine—either methylation of glycine or catabolism of choline, the latter of which will require compensatory

TABLE 1

Metabolite concentrations in phosphatidylethanolamine *N*-methyltransferase knockout (*PEMT*^{-/-}) and wild-type mice and in glycine *N*-methyltransferase (GNMT)- or guanidinoacetate *N*-methyltransferase (*GAMT*)-deficient humans¹

Tissue and condition	Met	AdoMet	AdoHcy	tHcy	Cysta	tCys	MG	DMG
Liver samples								
Mouse liver (nmol/g)								
Wild-type (<i>n</i> = 5)	420 ± 35	34 ± 3	53 ± 12	14.6 ± 2.2	40 ± 12	458 ± 119	62 ± 21	88 ± 13
<i>PEMT</i> ^{-/-} (<i>n</i> = 5)	441 ± 63	32 ± 13	35 ± 19	19.3 ± 6.8	57 ± 21	512 ± 155	63 ± 27	58 ± 23
Plasma samples ²								
Mouse plasma								
Wild-type (<i>n</i> = 9)	119 ± 35	146 ± 39 ³	33 ± 8	7.6 ± 2.4	613 ± 153	257 ± 33	2.7 ± 1.1	7.6 ± 0.6
<i>PEMT</i> ^{-/-} (<i>n</i> = 9)	106 ± 26	156 ± 64 ³	42 ± 19	4.5 ± 1.4 ⁴	877 ± 322	197 ± 24	4.0 ± 0.8 ⁵	5.7 ± 1.4
Human plasma								
GNMT-deficient (<i>n</i> = 3)	668 ± 31	2213 ± 36	50 ± 19	11.6 ± 1.4	609 ± 184	222 ± 11	1.8 ± 0.3	9.1 ± 2.5
<i>GAMT</i> -deficient (<i>n</i> = 7)	19 ± 9	65 ± 41	13 ± 8	4.5 ± 1.7	162 ± 43	272 ± 23	3.0 ± 1 ⁶	5.2 ± 3.2
Reference range	13–45 ⁷	93 ± 16	15–45	5.5 ± 2.1– 6.4 ± 0.2 ⁸	44–342 ⁷	203–369 ⁷	1.36 ± 0.55 ^{7,9}	1.4–5.3 ⁷

¹ All values are $\bar{x} \pm$ SD. Met, methionine; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; tHcy, total homocysteine; Cysta, cystathionine; tCys, total cysteine; MG, methylglycine (sarcosine); DMG, dimethylglycine. *PEMT*^{-/-} and wild-type mice were maintained on stock laboratory diet as previously described (15). The patients with *GAMT* deficiency had a mean age of 6.5 y (range: 6 mo–9 y) when the samples were obtained. All were receiving supplemental dietary creatine as part of the treatment for their enzyme deficiency.

² Met, tHcy, tCys, MG, and DMG are reported in μ mol/L; AdoMet, AdoHcy, and Cysta are reported in nmol/L.

³ *n* = 7.

⁴ Significantly lower than in wild-type mice (*P* = 0.004).

⁵ Significantly higher than in wild-type mice (*P* = 0.01).

⁶ Significantly higher than reference range (*P* < 0.05).

⁷ Reference range determined in adults aged 18–65 y.

⁸ The reference value of 5.5 ± 2.1 (*n* = 150) is that for Italian children aged 7.24 ± 4.9 y (20); that of 6.4 ± 0.25 (*n* = 28) is for Spanish children aged 4–9 y (21).

⁹ *n* = 60.

synthesis; and 3) the actual rate of phosphatidylcholine synthesis, especially in humans. Stead et al (1) drew attention to the observation that plasma tHcy is decreased by 50% in mice devoid of the activity of *PEMT*, the enzyme that catalyzes AdoMet-dependent formation of phosphatidylcholine (15), and they interpreted this observation to suggest a major role for phosphatidylcholine synthesis in determining plasma tHcy in mice (1). This suggestion is supported by the observations that hepatocytes isolated from *PEMT*^{-/-} mice have less Hcy secretion than do those from their wild-type counterparts (16) and that, in CTP: phosphocholine cytidyltransferase- α (CT α) knockout mice, *PEMT* flux is increased and plasma tHcy is raised by 20–40% (17). Furthermore, the estimated rate of hepatic synthesis of *PEMT*-derived phosphatidylcholine in rats (18), converted to the rate expected for a 250-g rat (17), would be 108–150 μ mol/d, which exceeds the rate of creatine formation [based on the measured rate of creatinine excretion (19)] of 58 μ mol/d for a 250-g rat. The next section provides further data relevant to the roles in AdoMet consumption of *PEMT*, GNMT (the enzyme that catalyzes methylation of glycine to form sarcosine), and *GAMT* (the enzyme that catalyzes methylation of guanidinoacetate, the last step in creatine synthesis).

METABOLITES IN SUBJECTS DEFICIENT IN A TRANSMETHYLATION ENZYME

Values for many of the metabolites most directly involved in the transmethylation pathway under circumstances in which the enzyme activities in question are severely decreased are shown in **Table 1**. AdoMet and *S*-adenosylhomocysteine (AdoHcy) were

assayed as described by Capdevila and Wagner (22). Other metabolites were quantitated by capillary gas chromatography–mass spectrometry (23–25).

PEMT^{-/-} mice

The data in Table 1 confirm the earlier observation of Noga et al (15) that, in *PEMT*^{-/-} mice, plasma homocysteine is significantly lower, with a mean tHcy 59% of that of the wild-type mice. The plasma sarcosine (methylglycine), which was not reported by Noga et al (15), was significantly increased from 2.7 to 4.0 μ mol/L. GNMT activity did not differ significantly between the livers of the *PEMT*^{-/-} mice and those of the wild-type mice (data not shown). Although sarcosine is a poor indicator because of its rapid metabolic turnover, the rise in question is consistent with an increased overflow of AdoMet methyls to methylate glycine when methylation of phosphatidylethanolamine cannot occur. In the *PEMT*^{-/-} mice plasmas, no significant changes were seen in methionine, AdoMet, or AdoHcy. Marginal changes were seen in plasma cystathionine and dimethylglycine. In the livers of the *PEMT*^{-/-} mice, however, AdoMet, AdoHcy, and tHcy were not changed (Table 1). These observations are consistent with the studies of liver-specific CT α knockout mice (17) in which *PEMT* flux increased and plasma tHcy was raised by 20–40%. However, hepatic AdoMet or AdoHcy concentrations did not change (17); tHcy was not measured. Taking these observations together, it is evident that altered flux through hepatic *PEMT* may influence plasma tHcy independently of liver AdoMet, AdoHcy, or tHcy concentrations. This finding raises the issue of total transmethylation flux in these animals and the question of the extent to which the decreased *PEMT* flux in the *PEMT*^{-/-} mice



and the increased PEMT flux in the CT α knockout mice are compensated by, respectively, increased and decreased fluxes through GNMT. In liver of rats, the ratio between free and protein-bound Hcy is close to 1.5:1 if care is taken to freeze the liver in vivo, and the half-life for Hcy (free and bound regarded as a single pool) is 3 s (26). However, we draw attention to the lack of information as to the mechanism(s) whereby homocysteine is transported from liver to plasma.

Humans deficient in glycine *N*-methyltransferase activity

The plasma changes in GNMT-deficient humans (27, 28) differ quite strikingly from the pattern in *PEMT*^{-/-} mice. These differences reflect an important distinction between GNMT and the other methyltransferases: fluxes through methyltransferases such as PEMT are limited by the physiologic need for their products, but GNMT is not constrained in this way. In such humans, marked rises are seen in plasma AdoMet and methionine (Table 1). The same pattern is observed in liver of *GNMT*^{-/-} mice (29). The rise in AdoMet has previously been interpreted as due to a lack of consumption of AdoMet to methylate glycine and the rise of methionine attributed to one or more feedback mechanisms whereby accumulated AdoMet down-regulates the flux from methionine to AdoMet (27). The GNMT-deficient patients were following normal diets when the samples reported in Table 1 were obtained. Thus, the changes observed were not due to methionine intakes that would be considered excessive by current standards. Even under these circumstances, the methylation of glycine may contribute substantially to the transmethylation flux.

Plasma tHcy is known to rise somewhat during childhood. Therefore, the mean plasma tHcy value for the GNMT-deficient children was compared with the most relevant reference ranges we could find in the published literature (20, 21). The mean was found to be significantly higher than the means in these reference series ($P = 0.001$) and was also higher than the mean of 6.9 ± 2.2 $\mu\text{mol/L}$ ($n = 96$) for boys aged 12.9 ± 0.3 y ($P < 0.001$) reported by Thomas et al (30). The reason for this elevation is not clear.

Patients with severe, genetically determined deficiencies of guanidinoacetate *N*-methyltransferase

In the patients in question, the diagnosis of GAMT deficiency was established by the presence of elevated guanidinoacetate concentrations. In those for whom the *GAMT* mutations have been identified (4 of 7), truncating changes were found, so their GAMT deficiencies were probably very severe (SH Mudd et al, unpublished observations, 2006). At the time the samples reported in Table 1 were obtained, all the patients were receiving supplemental creatine, a treatment that was beneficial with respect to their genetic abnormality (31). Because creatine down-regulates L-arginine:glycine amidinotransferase, the enzyme that forms guanidinoacetate (32, 33), this treatment should accentuate, not diminish, any effect or effects of the existing GAMT deficiency on AdoMet utilization for creatine synthesis. Indeed, creatine administration has been shown to decrease plasma tHcy by $\approx 25\%$ in rats (33) and by 10% in humans, according to one report (34); in another report, this reduction in humans was not significant (35). The metabolic data in Table 1 show that the plasmas of these severely GAMT-deficient humans follow a pattern not unlike that in the *PEMT*^{-/-} mice: a rise in sarcosine but no elevations of AdoMet or methionine. The GAMT-deficient children for whom values are reported in Table 1 were

young, European, and mostly boys (6 of 7). The mean plasma tHcy value in these patients was lower, but not significantly so, than the mean in either of the relevant reference series listed in Table 1. It was significantly ($P < 0.05$) lower than the mean of 6.9 ± 2.2 $\mu\text{mol/L}$ found by Thomas et al (30) in boys aged 12.9 ± 0.3 y.

One interpretation of the overall results in Table 1 is that, when GNMT activity is severely diminished or absent, increases in plasma AdoMet and methionine indicate disruption of a pathway that is important to AdoMet disposal; in contrast, in the presence of intact GNMT activity, such a disruption is indicated by a rise in sarcosine with a decrease in plasma tHcy (as in the *PEMT*^{-/-} mice and, perhaps, the GAMT-deficient humans). These results do not clearly establish the relative contributions to the overall transmethylation flux of the reactions forming phosphatidylcholine, creatine, or sarcosine.


FOR THE FUTURE

Clearly, more evidence is necessary to answer the remaining questions about total transmethylation in humans and the roles therein of various reactions using AdoMet. A definitive determination of the use of AdoMet for phosphatidylcholine synthesis in humans would be an important step forward. Resolution of the uncertainty about hepatic isotopic enrichment in stable-isotope infusion experiments would be another important step, and more precise data on the absorption of dietary creatine would be useful. An approach that may empirically settle the question of the relative contributions of phosphatidylcholine, creatine, and sarcosine syntheses (at least in an experimental animal) would be the use of the procedure (or a modification thereof) described by Datko et al (36). They administered L-[methyl-³H]methionine intraperitoneally to a rat for 25 s. At 30 s, the liver was rapidly removed and freeze-clamped. It was then possible to quantitate in the liver the amounts of radiolabel in phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine, and phosphatidylcholine, whereas at longer times of labeling, the radioactivity in phosphatidylcholine far exceeded that in the monophosphatidylethanolamine and dimethylphosphatidylethanolamine derivatives. If labeling of creatine and sarcosine were also quantitated in such an experiment, it should be possible to judge the relative importance of these pathways. The results of this experiment will be most readily interpretable if the enzymes in question share the same pool of substrate AdoMet. This is likely to be so because both GNMT and GAMT use cytosolic AdoMet. Furthermore, although PEMT is located chiefly in liver on the endoplasmic reticulum (37), but also in the biliary canalicular membrane (38), Shields et al (39) proposed a model in which the AdoMet-binding portions of PEMT extend into the cytosol and would thus share the same pool of AdoMet as the other 2 enzymes.

Additional worries would be that ≥ 1 of the methylated products is exported from the liver so rapidly that, even in the short time of 30 s, a significant amount escapes, or that sarcosine is turning over so rapidly as to lose significant label. To allay these problems, it may be necessary to check the blood for labeled creatine and sarcosine and to determine the labeling of methylene-THF, the first product of sarcosine degradation, or to carry out the labeling even more rapidly. Although difficult, such an experiment could go a long way toward establishing the relative roles of the major transmethylation reactions. Ideally, this

experiment should be carried out in the context of different intakes of methionine, from deficient to generous, which would permit investigators to determine whether there is a constitutive, physiologic flux through GNMT or whether this enzyme functions chiefly to catabolize excess methionine. Finally, Stead et al (1) mentioned that clarification of the role of betaine as a dietary source of labile methyls is also needed. On the basis of unpublished observations, Craig (40) estimated that the dietary intake of betaine for humans may range from 8.5 to 21.3 mmol/d, amounts that are very significant with respect to the intakes of methionine and choline. However, more recent assessments of betaine dietary intakes have been 1.8 ± 0.8 and 2.54 ± 0.3 mmol/d in New Zealand (41) and the United States (42), respectively. If the latter values turn out to be correct, dietary betaine will make a significant but relatively small contribution to the normal intake of labile methyl groups.

SUMMARY

The following are the major conclusions reached in this discussion. First, methyl balance is maintained in normal humans, if necessary, by the process of methylneogenesis in which a methyl group newly formed on methyl-THF is transferred to homocysteine, which forms methionine (Figure 1). Second, early (and minimal) estimates of total rates of human transmethylation, based on balance studies, are in reasonable agreement with total rates calculated from more recent stable isotope infusion studies, although uncertainties exist as to the upper limit established by the latter approach and as to the effects of sex and age on these rates. Third, of the probable major contributors to total transmethylation (if dietary intakes of creatine in normal diets in the United States are taken into account), the estimated rate of creatine synthesis is significantly reduced from that calculated on the basis of balance studies of humans on diets virtually free of creatine. Fourth, in mice devoid of PEMT activity, plasma tHcy decreases by 40–50% without a significant change in hepatic tHcy concentration. These observations suggest that phosphatidylcholine synthesis is a relatively important contributor to total transmethylation flux, although the extent to which flux through GNMT may increase in these mice is unknown. Definitive measurement of the flux through either PEMT or GNMT in human liver remains to be achieved. We note also the paucity of information on the mechanism or mechanisms of homocysteine export from the liver to the plasma. Not only do we not know the specific transporter or transporters involved, we also require further information on the form of homocysteine that is exported. Fifth, *N*-methylglycine (sarcosine) is formed by 2 pathways, whose relative contributions are still unclear (Figure 1). However, the important contribution of AdoMet-dependent glycine methylation, especially under circumstances of increased dietary methionine intake, is indicated not only by balance studies of suitable subjects but also by the fact that, in humans deficient in glycine *N*-methyltransferase, abnormally elevated concentrations of plasma AdoMet are seen. The lack of elevated plasma AdoMet in mice devoid of activity of phosphatidylethanolamine methyltransferase or in humans severely deficient in guanidinoacetate *N*-methyltransferase activity may possibly be explained by disposition of AdoMet under both circumstances by increased methylation of glycine. 

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